SI ADVANCES IN PHOTOSYNTHESIS

Linking water relations and hydraulics with photosynthesis

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SUMMARY

For land plants, water is the principal governor of growth. Photosynthetic performance is highly dependent on the stable and suitable water status of leaves, which is balanced by the water transport capacity, the water loss rate as well as the water capacitance of the plant. This review discusses the links between leaf water status and photosynthesis, specifically focussing on the coordination of CO_2 and water transport within leaves, and the potential role of leaf capacitance and elasticity on CO_2 and water transport.

Keywords: photosynthesis, leaf hydraulic conductance, leaf capacitance, mesophyll conductance, outside xylem hydraulic conductance, modulus of elasticity.

INTRODUCTION

Water is the most abundant compound in all active plant cells, and maintaining a stable aqueous environment in cells is crucial for virtually every physiological process in terrestrial plants (Chaves, 1991). The atmosphere is the source of CO_2, which is needed for photosynthesis; however, the atmosphere is usually quite dry, leading to a net loss of water through evaporation. To meet the contradictory demands of maximizing CO_2 uptake while minimizing water loss, plants face the challenge of balancing water loss from leaves and replacing the water lost to the atmosphere with water transport mainly from the soil. The water loss rate from leaves or transpiration (E) is mainly determined by leaf vapor diffusion and the vapor pressure deficit between leaf and atmosphere (VPD), and the water transport efficiency through the whole plant is defined as plant hydraulic conductance (K_{plant}). Although the distances that water must traverse within leaves are small relative to the entire soil−plant−atmosphere pathway, leaves constitute a bottleneck for water transport (Sack and Holbrook, 2006), significantly contributing to the plant hydraulic resistance (R_{plant}, the inverse of K_{plant}). Therefore, a large number of studies in the past decades focused on

water transport through the leaf and its influences on photosynthetic performance. The link between photosynthesis and leaf hydraulic conductance (K_{leaf}) was generally established on the common pathway for water loss and CO_2 capture via stomata and cuticle (Boyer, 2015). However, several recent studies indicated that the water transport inside leaves may also share a common pathway with CO_2 movement, providing new insights into the coordination between K_{leaf} and photosynthesis (Flexas et al., 2013; Loucos et al., 2017; Xiong et al., 2017).

For a plant under field conditions, the water flux cannot be considered to be constant in most cases. Some non-steady-state water flux occurs due to the expansion of growing cells of meristematic and young tissue and, in other cases, a net flux of water can occur out of or into non-growing tissues (i.e. storage parenchyma tissues) not normally viewed as part of the transpirational path. Water storage in these tissues can buffer against rapid water status fluctuations caused by environmental changes such as air and soil drought. Capacitance (C) is defined as the change in water content (W) per water potential (Ψ) change at a given water status range. Capacitance in stems has been acknowledged to play a significant role in water transport efficiency and safety (Pratt and Jacobsen, 2017). However, leaf capacitance

(C_{leaf}) in non-succulent species has received less attention, despite being considered a key trait for defining leaf water status (Schulte, 1992). Nonetheless, the available studies point towards the correlation between C_{leaf} and K_{leaf}, and also suggest a potential role of C_{leaf} in dehydration resistance and photosynthetic performance. C_{leaf} is partly driven by bulk modulus of elasticity (ε), defined as the change in turgor pressure per volume decrease, which relates to both water relations and cell and tissue structure (Tyree and Jarvis, 1982). The recently described relationship between ε and CO_2 assimilation, possibly through internal CO_2 diffusion (Nadal *et al.*, 2018), highlights the complexity of the link between water and photosynthesis in leaves, which may occur at several different levels. The coordination of K_{leaf} and CO_2 capture through stomata has been reviewed frequently, and it is somehow clear (Sack *et al.*, 2016; Venturas *et al.*, 2018). Therefore, in the following sections, we highlight some of the classical and recent advances on: (1) potential coordination of CO_2 and water transport inside leaf and its impacts on carbon assimilation, and (2) the influences of C_{leaf} and ε on K_{leaf} and photosynthetic performance; and (3) re-visit the network of leaf traits coordination to incorporate water relations, and point to some questions for continuing research.

BACKGROUND: WATER TRANSPORT PATHWAYS

Components of K_{leaf}

In vascular plants, water moves from the petiole to stomata mainly through a complex micro-hydrological system. The prevailing viewpoint is that liquid water moves within xylem conduits through the petiole and throughout the leaf venation network, then across the bundle sheath (BS), mesophyll and/or epidermis cells before evaporating into intercellular airspace, and finally diffusing through stomata out of the leaf (Sack and Holbrook, 2006; Buckley, 2015). In practice, K_{leaf}, representing the efficiency of liquid water transport through the leaf, is calculated as the ratio of water flow to a difference in water potential between the petiole water source and bulk leaf tissues

(for a summary of approaches for the K_{leaf} measurement, see Sack *et al.*, 2002; Flexas *et al.*, 2013). As the xylem provides a pathway of low resistance for water movement, the water movement efficiency within the xylem (K_x) is somewhat well characterized; however, the leaf hydraulic conductance outside the xylem (K_{ox}) is poorly understood, although it has received increasing attention (Rockwell *et al.*, 2014; Buckley, 2015; Buckley *et al.*, 2017). Generally, variation in K_{leaf} is dominated by variation in K_{ox} with K_x being correlated to K_{leaf} with a small R^2 (Figure 1). Notably, because no direct method is available for estimating K_{ox} nowadays, K_{ox} is usually calculated using the values of K_{leaf} and K_x ($K_{leaf}^{-1} = K_x^{-1} + K_{ox}^{-1}$) and, therefore, the correlation between K_{ox} and K_{leaf} may arise in part from mathematical circularity. As gradients in liquid water isotope composition vary between apoplastic and transcellular pathways (Barbour and Farquhar, 2004), applying water isotope (deuterium and ^{18}O) analysis of bulk leaf water to estimate the transport efficiency of different pathways has been suggested (recently reviewed by Barbour *et al.*, 2017). Hopefully, the development of more stable isotopes technologies in the near future may help discern water transport pathways in leaves and, therefore, estimate the K_{ox} directly (Song and Barbour, 2016; Barbour, 2017; Barbour *et al.*, 2017).

Xylem water transport (K_x)

Vascular plants have evolved a highly specialized vascular tissue, the xylem, for long-distance water transport. In contrast to the diffusion of water across semi-permeable membranes, water moves under negative pressure inside the xylem, via the cohesion–tension mechanism, and the pressure-driven bulk flow inside the xylem is a metabolic energy-free process, and independent of solute concentration gradients (Dixon Henry and Joly, 1895). Under non-stressed conditions, the K_x is suggested to be mainly determined by vein architecture traits such as vein density, arrangement, geometry and the radius and length of xylem conduit within veins (Venturas *et al.*, 2017). Indeed, K_x calculated using Poiseuille's equation assuming xylem

Figure 1. Drivers of leaf hydraulic conductance (K_{leaf}) across species.
K_x, the hydraulic conductance inside xylem; and K_{ox}, outside-xylem hydraulic conductance. Data collected from Sack *et al.* (2004, 2005); Scoffoni *et al.* (2016); Xiong *et al.* (2017); Ohtsuka *et al.* (2018) and Lu *et al.* (2019).
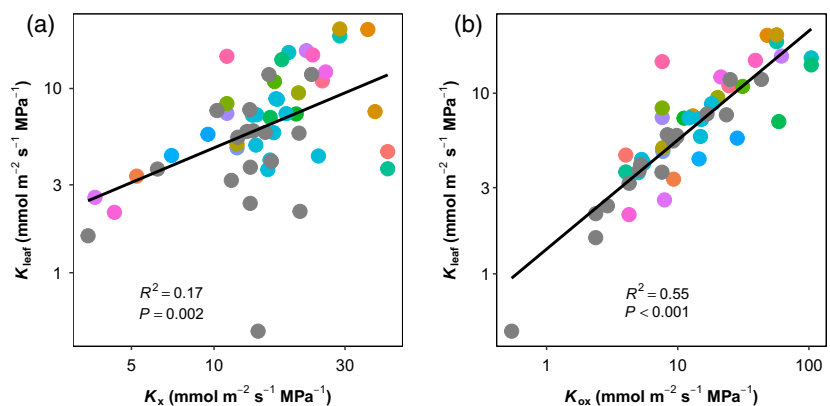
conduits as tubes is usually in agreement with the measured K_x using the minor vein cutting method (Scoffoni *et al.*, 2016; Xiong *et al.*, 2017). It is important to emphasize that the 'negative pressure' in the cohesion−tension hypothesis refers to the liquid phase, as a negative gas phase pressure is physically impossible. When tension is too high, i.e. under drought conditions, it can cause air to aspirate through a xylem conduit, a process known as cavitation, which can lead to more severe embolism events. The appearance of cavitation in the liquid-filled conduit immediately relaxes the negative sap pressure to near zero and breaks the bulk flow inside the xylem, thus possibly contributing to K_{leaf} decline under dehydration (Brodribb and Holbrook, 2003b).

Outside-xylem water transport (K_{ox})

The outside-xylem pathway is extremely complex, as water travels via apoplastic and/or symplastic (includes transmembrane flow through the cell membranes and transcellular flow through plasmodesmata) routes through living cells. As much less specialized tissues exist for water movement outside the xylem, the water flow should be strongly limited in this part both for apoplastic and symplastic pathways. This was further advanced by Brodribb *et al.* (2007), who observed a strong negative relationship between K_{leaf} and the path-length for water flow from xylem to stomata. The apoplastic pathway via cell walls is often implicitly assumed to be the major pathway of water transport through the outside xylem (Buckley, 2015). However, the observation of aquaporin-mediated water transport in BS using aquaporin mutants means that the symplastic pathway is in fact plausible (Sade *et al.*, 2010, 2014; Prado *et al.*, 2013; Kelly *et al.*, 2017). Other evidence for symplastic water transport comes from the diurnal rhythms in K_{leaf}, and the dynamic response of K_{leaf} to environmental changes including temperature and light intensity. Beyond liquid water transport, the important role of vapor diffusion through intercellular air space in outside-xylem water transport was suggested by Boyer (1985) and highlighted by several recent modeling studies (Rockwell *et al.*, 2014; Buckley, 2015; Buckley *et al.*, 2017; Rockwell and Holbrook, 2017). The water pathway outside the xylem involves multiple tissues, including BS, mesophyll and epidermis, the hydraulic resistances of which remain largely unknown. For instance, early studies that inferred the effect of BSs and their extensions (BSEs) on K_{leaf} from anatomy, modeling and K_{leaf} response to light have hypothesized that BSs and BSEs are major hydraulic resistance on K_{leaf} (Griffiths *et al.*, 2013; Caringella *et al.*, 2015); however, other empirical and modeling studies suggested that mesophyll tissues are the most hydraulic resistive part rather than BSs and BSEs (Zwieniecki *et al.*, 2007; Buckley *et al.*, 2015; Zsögön *et al.*, 2015).

COUPLING OF HYDRAULICS AND PHOTOSYNTHESIS

### Links between K_{leaf} and CO_2 diffusion

The coupling of K_{leaf} and photosynthesis (A) has been widely observed, and the coordination between K_{leaf} and A has been suggested to arise from stomatal conductance (g_s), a key trait in regulating water loss and carbon gain of plants (Brodribb *et al.*, 2007, Scoffoni *et al.*, 2016; Figure 2). When a stable equilibrium is achieved in the soil−plant−atmosphere continuum, from the Ohm's law analogy, the relationship between conductance and water potential is given by:

$$\frac{g}{K_{plant}} = \frac{\Psi_{soil} - \Psi_{leaf} - \Psi_g}{VPD}$$

where g is the vapor conductance from intercellular air space to atmosphere including g_s and boundary-layer conductance (g_b), Ψ_{soil} is the soil water potential, Ψ_{leaf} is the leaf water potential, Ψ_g is the gravitational potential caused by the plant height, and VPD is the vapor pressure deficit between intercellular air space and atmosphere. Notably, g is primarily driven by g_s under wind of sufficient speed so g_b is not limiting. Under a given environment, i.e. a given Ψ_{soil} and a given VPD, the Ψ_{leaf} is determined by the ratio of g_s to K_{plant} (then K_{leaf}) and C_{leaf}. Moreover, stomata are very sensitive to ψ_{leaf}, and stomatal closure occurs when Ψ_{leaf} drops below a threshold with a narrow range. As shown in Figure 2, K_{leaf} is related to g_s across species and/or environmental conditions, and thus links to A due to the tight correlation between g_s and A.

Recently, the coordination of K_{leaf} and mesophyll conductance to CO_2 (g_m), another major photosynthetic limitation factor (for review, see Flexas *et al.*, 2012), across species and/or genotypes was suggested by several studies (Flexas *et al.*, 2013; Xiong *et al.*, 2017, 2018; Lu *et al.*, 2019; Figure 2c). The g_m accounts for the apparent diffusion efficiency of CO_2 from substomatal cavity through mesophyll cell components into the chloroplasts where biochemical assimilation occurs. As the g_m represents the CO_2 diffusion efficiency in mesophyll tissues, the g_m might be related only to the K_{ox} in mesophyll tissues. Although the details remain to be clarified, partly shared pathways of CO_2 and water movement in mesophyll tissues have been suggested to link g_m and K_{ox}. As described before, water moves through mesophyll tissues via apoplastic, symplastic and vapor phase pathways and, in contrast, the question of whether CO_2 moves into mesophyll cells via apoplastic and/or symplastic has never been addressed. Nonetheless, for C_3 plants, it has been widely suggested that CO_2 diffuses from the substomatal cavity to mesophyll cell wall surfaces, and then into single mesophyll cells via membranes after dissolving to solution in the cell wall (Evans *et al.*, 2009). In fact, the influences of mesophyll properties such as the intercellular airspace, mesophyll

Figure 2. The correlation between gas exchange parameters and leaf hydraulic conductance.

A, photosynthetic rate, g_s, stomatal conductance, g_m, mesophyll conductance, and K_{leaf}, leaf hydraulic conductance. Gas exchange and K_{leaf} data collected across different species (Xiong *et al.*, 2018; Lu *et al.*, 2019) and a wide range of environmental conditions, including high temperature (Huang *et al.*, 2017), light intensities (Loucos *et al.*, 2017), long-term drought (Theroux-Rancourt *et al.*, 2014) and potassium application (Lu *et al.*, 2019).
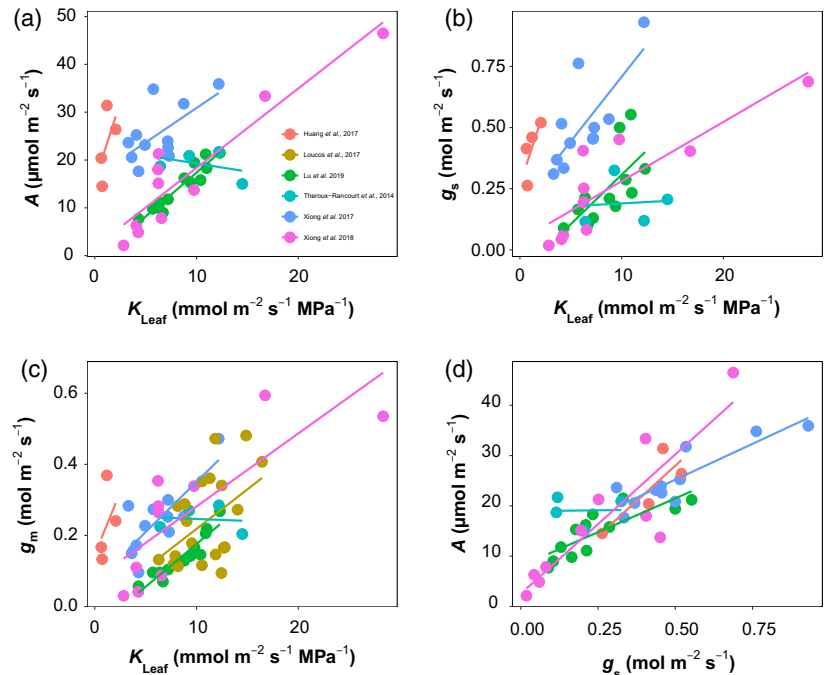
surface facing the intercellular airspace (S_m), cell wall properties (i.e. thickness, T_{cw}), aquaporin mediated lipid membranes permeability, the cytosol and chloroplasts shape and size on g_m have been widely studied, and the permeability of membranes, T_{cw} and S_m have been found to be the strongest limitations on g_m. Unlike in the case of g_m, the impact of leaf anatomical traits on K_{leaf} has been seldom investigated and the influences of leaf anatomical traits on K_{leaf} are largely unknown. Interestingly, recent modeling and experimental studies found S_m, T_{cw} and membrane permeability also influence the water transport efficiency through mesophyll tissues (Xiong *et al.*, 2017). However, Lu *et al.* (2019) did not find such relationships; in fact, they observed intraspecific relationships between the fraction of the mesophyll cell wall adjoining other cell walls (f_{cm}) and K_{ox} among four crop species subjected to different K supplies. This result would suggest a major role of cell connectivity across tissues in the apoplastic pathway, contrasting with the greater vapor phase that could be associated with S_m, which reflects cell exposure to intercellular spaces and thus increased surface for water evaporation along the water pathway. Certainly, the influence of leaf ultrastructure on K_{ox} deserves further investigation.

Leaf vein density (typically expressed as vein length per leaf area, VLA) is also an important feature in determining K_{ox}, as the high VLA can shorten the water transport pathways from xylem to stomata. Indeed, VLA has been suggested to be the key anatomical trait dominating both K_x and K_{ox}. If the VLA is the key trait determining K_{ox}, the coordination of g_m and K_{ox} cannot be explained by VLA because g_m is independent of VLA. However, beyond common pathways of liquid phase transport, the gas phase diffusion of CO_2 and H_2O via intercellular airspace also shares a common pathway. It has been suggested by two recent modeling studies that liquid water can evaporate along the liquid pathways from BS to near stomata, and diffuse as gas until reaching the stoma (Rockwell *et al.*, 2014; Buckley *et al.*, 2017). Importantly, a recent study found that vapor transport via intercellular airspace contributes up to two-thirds of K_{ox} (Buckley, 2015), which indicates the common gas phase pathway may contribute to the coordination of K_{leaf} and g_m.

Coordination under variable conditions

As g_s, g_m and K_{leaf} are sensitive to environmental changes, the coordination of g_s, g_m and K_{leaf} with short-term environmental variation has attracted increasing attention because their dynamics can influence plant performance. Due to the fact that coordination of g_s and K_{leaf} in response to environmental changes has been widely studied and recently reviewed (Scoffoni and Sack, 2017), here we mainly focus on the response pattern of g_m and K_{leaf} to environmental changes. Although a large amount of studies investigated the response of g_m and K_{leaf} to variable environments such as light intensities, drought and CO_2 concentration (Sack and Holbrook, 2006; Flexas *et al.*, 2008, 2012), nearly all of the previous studies had only measured one of the two parameters (i.e. either g_m or K_{leaf}). To the best of our knowledge, no study has measured the g_m and K_{ox} under variable environment conditions. Hence, here we consider the coordination of g_m and K_{leaf} rather than K_{ox} under dynamic conditions. The responses of g_m and K_{leaf}

to short-term environmental changes are summarized in Table 1. Surprisingly, no evidence supports the coordination of g_m and K_{leaf} in responding to dynamic environment. Although the mechanisms need to be further revealed, there are several possibilities for the disappeared correlation between g_m and K_{leaf} under variable environmental conditions. Firstly, as introduced before, the water transport and the CO_2 diffusion pathways inside leaves are complicated, and they only share a limited part of the pathways. Thus, the different responses of K_{leaf} and/or g_m to environmental changes may be caused by the changes of the independent parts: for instance, the water permeability of BSE and the CO_2 permeability of chloroplast stroma. Secondly, water moves through four parallel pathways within the leaf mesophyll: apoplastic, cell-to-cell movement through plasmodesmata, transcellular transport across membranes via aquaporin and vapor phase, and the dominating pathway may change under variable environments to maintain an adequate K_{ox} (thus K_{leaf}). On the other hand, CO_2 diffuses via transcellular pathway and it likely lacks substitution pathways when the transcellular pathway is blocked. Thirdly, the current approaches for estimating both K_{leaf} and g_m include sources of error especially under variable environmental conditions that require consideration when interpreting data (Flexas *et al.*, 2013; Gu and Sun, 2014).The complexity (and even unreliability) of short-term g_m responses (Theroux-Rancourt and Gilbert, 2016; Carriquí *et al.*, 2019) is still a major constrain for unravelling the coordination of g_m and K_{leaf} under CO_2 and light variations.

Water status: the role of K_{leaf}

Water stress is one of the major constraints to water transport and photosynthesis, as Ψ_{leaf} decline strongly induces reduction in K_{leaf}, g_s and g_m. Although the responses of these variables have been extensively studied separately, very few studies have explored their coordination upon water stress, especially considering g_m (Flexas *et al.*, 2018). The response of K_{leaf} to declining Ψ_{leaf} was thought to be only due to xylem cavitation events affecting K_x (Brodribb and Holbrook, 2003a). However, in recent years, leaf shrinkage and changes in membrane permeability with dehydration have been related to K_{ox} reduction, which nowadays is suspected to be the main driver of K_{leaf} decline under water stress (Trifilo *et al.*, 2016; Scoffoni *et al.*, 2017, 2018). The coordination between K_{leaf} and g_s has been widely studied in order to establish if hydraulic decline triggers stomatal closure or stomata close in order to prevent further potentially damaging cavitation events (Brodribb and Holbrook, 2004; Theroux-Rancourt *et al.*, 2014; Flexas *et al.*, 2018), i.e. extending the safety margins, defined as the difference between Ψ at stomatal closure and Ψ at embolism formation (Martin-StPaul *et al.*, 2017; Skelton *et al.*, 2017). On the other hand, g_m also declines under water stress, imposing a similar limitation to A compared with g_s especially at moderate and severe drought (Nadal and Flexas, 2019). Although the mechanisms that underly g_m decline under drought are yet to be elucidated (for details, see Nadal and Flexas, 2018), some shared mechanisms with g_s are thought to play a big role, such as abscisic acid-triggered decline (Mizokami *et al.*, 2015; Sorrentino *et al.*, 2016). The study performed by Wang *et al.* (2018) reports simultaneously the dynamics of K_{leaf}, g_s and g_m during a drought event, showing that K_{leaf} is the main driver of g_s and g_m decline under water stress in rice. K_{leaf} decline with water stress compromises water availability in leaves, thus contributing to the hydraulic-driven decline in g_s (Theroux-Rancourt *et al.*, 2014) and possibly to reduced

Table 1 . Responses of mesophyll conductance to CO_2 (g_m), and leaf hydraulic conductance (K_{leaf}) to short-term environmental changes

Environment factor	Species	g_m	K_{leaf}	Coupling	Reference
Low light	*Gossypium hirsutum*	No response	Slight decline	No	Loucos *et al.* (2017)
	Phlebodium aureum	No response	No response	–	Xiong *et al.* (2018)
	Nephrolepis cordifolia	No response	No response	–	
	Taxus baccata	Decline	No response	No	
	Ginkgo biloba	Decline	No response	No	
	Nerium oleander	Decline	Slight decline	No	
	Populus nigra	Decline	Slight decline	No	
	Gossypium hirsutum	Decline	Slight decline	No	
	Helianthus annuus	Decline	Decline	Yes	
	Centella asiatica	Decline	No response	No	
	Oryza sativa	Decline	Decline	Yes	
High temperature	*Oryza sativa*	Decline	Increase	No	Huang *et al.* (2017)
Elevated CO_2	*Glycine max*	Decline	No response	No	Locke *et al.* (2013)
	Gossypium hirsutum	Decline	No response	No	Loucos *et al.* (2017)
	Populus	Decline	No response	No	Theroux-Rancourt *et al.* (2014)
Drought	*Oryza sativa*	Decline	Decline, faster than g_m	No	Wang *et al.* (2018)
	Populus	Threshold response	Decline	No	Theroux-Rancourt *et al.* (2014)

g_m as well. However, the interplay between K_{leaf}, g_s and g_m may be species-dependent; in contrast to rice, poplar clones displayed a delayed response of g_m relative to g_s and K_{leaf} declines (Theroux-Rancourt *et al.*, 2014). This differential g_m response may be related to reduction in aquaporin and carbonic anhydrase expression, which are also thought to play a key role in g_m decline under drought (Perez-Martin *et al.*, 2014). On the other hand, other photosynthesis-related processes may not be exclusively related to Ψ_{leaf} under water stress: early studies indicated that the reduction in symplast volume due to reduced water content (*W*) was responsible of *A* decline (Gupta and Berkowitz, 1987; Meinzer *et al.*, 1990), possibly due to the negative effects associated with increased solute concentration in the symplast. Hence, although K_{leaf} emerges as a key parameter to understand the responses of g_s and g_m under variable Ψ_{leaf} conditions, because of the strong interplay between K_{leaf} and Ψ_{leaf} (Scoffoni and Sack, 2017; Cardoso *et al.*, 2018), the role of water relations cannot be disregarded, as Ψ_{leaf} also depends on the availability of the water stored in the tissue, which relates to leaf capacitance (C_{leaf}). Thus, the interplay between Ψ and *W* is key to understand the potential relationships between C_{leaf} and ε with water status and photosynthesis (Schulte, 1992). The potential role and mechanisms of these parameters are discussed in the next section.

LEAF CAPACITANCE AND WATER TRANSPORT

Leaf water relations: capacitance

Leaf water relations, i.e. the interplay between water volume, storage and water potential, have been strongly related to water stress tolerance, being a key aspect shaping plant strategies to cope with the environment (Philip, 1966; Meinzer *et al.*, 1990; Bartlett *et al.*, 2012; Blackman, 2018). The main parameters that describe water relations are turgor loss point, expressed in either water potential or relative water content terms (Ψ_{tlp} and RWC_{tlp}, respectively), osmotic potential at full turgor (π_o), bulk modulus of elasticity (ε), and the fraction of apoplastic water (a_f). These parameters are usually derived from pressure–volume (PV) curves, which combine Ψ and RWC measurements as the tissue dehydrates (Tyree and Hammel, 1972; Tyree and Jarvis, 1982; Bartlett *et al.*, 2012). In addition, from PV measurements tissue capacitance can also be obtained. Capacitance is defined as the ratio of change in water content for a given water potential interval ($\Delta W/\Delta \Psi$), and it is usually normalized by volume in stems and either area or dried mass in leaves (Nobel and Jordan, 1983; Tyree and Ewers, 1991; Sperry *et al.*, 2008). Thus, capacitance describes the amount of water (mol or kg) mobilized within a given tissue (m^{-3}, m^{-2} or g^{-1}) under the application of a pressure of 1 MPa at the referred hydration state. The role and structural determinants of *C* have been profusely described

in stems (Scholz *et al.*, 2011; Pratt and Jacobsen, 2017): stem capacitance (C_{stem}) ranges from 20 to 500 kg m^{-3} MPa^{-1}, it is mainly driven by sapwood density and water content, and reflects the source of available water for transpiration when xylem transport is hindered (Stratton *et al.*, 2000; Scholz *et al.*, 2007; McCulloh *et al.*, 2014; Savi *et al.*, 2017). Moreover, C_{stem} is thought to play a buffering role to avoid potentially damaging Ψ fluctuations (Scholz *et al.*, 2007; Meinzer *et al.*, 2009). Thus, C_{stem} may be a key trait related to the efficiency–safety trade-off proposed for xylem water transport (Meinzer *et al.*, 2008; Sperry *et al.*, 2008; McCulloh *et al.*, 2014; Pratt and Jacobsen, 2017). On the other hand, C_{leaf} has received much less attention, despite its potential relationships with other aspects of leaf physiology such as water transport and carbon assimilation. The following sections discuss the potential role and structural determinants of C_{leaf} as well as its position among other leaf traits.

A 'buffering' role under high transpiration

The correlation between leaf capacitance and K_{leaf} was reported by Sack *et al.* (2003), who described a positive relationship between lamina hydraulic conductance and leaf capacitance across species under well-watered conditions. Indeed, this report on a possible correlation between K_{leaf} and C_{leaf} is still well supported when including more species (Figure 3a). In addition, C_{leaf} is also positively related to *A* (Figure 3b), possibly due to the *A* and K_{leaf} link discussed above. Interestingly, K_{leaf} shows a negative relationship with ε when data from different studies are pooled together (Figure 3c). These relationships imply that leaves with a high water transport efficiency (high K_{leaf}) show, in turn, high capacitance and elasticity. Using an electric circuit analogy, capacitance behaves as a capacitor, i.e. a dynamic storage compartment that can be recharged and serves as a buffer to prevent power surge fluctuations (Hunt *et al.*, 1991; Tyree and Ewers, 1991). In plant tissues, its buffer function is displayed in order to avoid extreme Ψ changes experienced under the transpiration stream (Scholz *et al.*, 2011). Thus, C_{leaf} is thought to be the third key feature determining the overall water potential of the leaf, besides water loss (transpiration via stomata) and water entrance into the leaf (quantified as K_{leaf}). Using the modeling approach described in Cardoso *et al.* (2018), Figure 4a shows the interplay between C_{leaf} and K_{leaf} in determining Ψ_{leaf} under a sudden increase in *E*. From the simulation, C_{leaf} emerges as the main parameter driving the very first Ψ_{leaf} response, as the effect of water loss due to high *E* on Ψ_{leaf} is diminished when C_{leaf} is high. The buffering role of capacitance has been clearly described in stems (Scholz *et al.*, 2011; Pratt and Jacobsen, 2017). However, in the case of leaves, its buffering role may be reflected at a different level: Martins *et al.* (2016) showed a clear trend where low C_{leaf} values were related to faster

stomatal closure upon VPD increases in conifers and ferns. Those plant groups generally display an hydropassive stomatal control (Brodribb and McAdam, 2017), where stomata respond directly to the water status of the leaf. Hence, low C_{leaf} would be reflected in lower Ψ_{leaf} per transpiration (water loss) increase, thus triggering lower pressure and stomatal closure. This same mechanism is described even within a single fern species (*Athyrium filix-femina*) that displays different fronds with contrasting C_{leaf} (Cardoso *et al.*, 2019). Interestingly, the relationship between T_{50} (the time at which g_s is at 50%) and C_{leaf} has also been observed in angiosperm tree species differing in their degree of iso- and anisohydry (Fu *et al.*, 2019), although they presented higher overall T_{50} values than those reported for ferns and conifers. Figure 4b shows that stomatal closure, even at the fastest rate reported in Martins *et al.* (2016), does not prevent Ψ_{leaf} decrease when C_{leaf} is low. Hence, the simulation presented here implies that the stomatal response reported in Martins *et al.* (2016) and Fu *et al.* (2019) possibly reflects a passive hydraulic response to a sudden drop in Ψ_{leaf} –driven by the low buffering capacity of species with low C_{leaf}. The active stomatal control in angiosperms in response to water deficits (Brodribb and McAdam, 2011), together with the so-called 'wrong way response' of transient stomatal opening with increased transpiration (Powles *et al.*, 2006; Buckley *et al.*, 2011), lead to a higher sensitivity of angiosperm leaves to sudden Ψ_{leaf} drops (Zhang *et al.*, 2016). High C_{leaf} would confer a protection mechanism to avoid these decreases in water potential; however, a similar result could be obtained by haltering further water loss by dropping of K_{leaf} due to terminal vein collapse (Zhang *et al.*, 2016). Nonetheless, the collapse-driven model of Zhang *et al.* (2016) only applies at a lower Ψ range (< -2.5 MPa), when the potential role of C_{leaf} would be overshadowed by other mechanisms (such as stomatal closure) preventing further dehydration.

The modeling described here only shows a C_{leaf} effect on Ψ_{leaf} at a very narrow timescale (0–60 s); however, Fu *et al.* (2019) reported T_{50} as high as 2500 s and still driven by C_{leaf}. Moreover, higher C_{leaf} has been related to the maintenance of higher midday Ψ_{leaf} in savanna tree species (Hao *et al.*, 2008) and to the maintenance of transpiration for extended periods of time (~hours) in a mangrove species (Nguyen *et al.*, 2017). These effects of C_{leaf} on water status at a larger timescale are not reflected in the model of Cardoso *et al.* (2018) as depicted in Figure 4. This is because C_{leaf} derived from PV measurements reflects the 'bulk capacitance' (C_{bulk}), that is, the overall capacitance of a tissue, without distinguishing potential multiple water compartments that contribute differently to water transport and storage. Indeed, Blackman and Brodribb (2011) distinguished leaf C_{bulk} and dynamic capacitance (C_{dyn}), i.e. the capacitance that actively contributes to the buffering of the
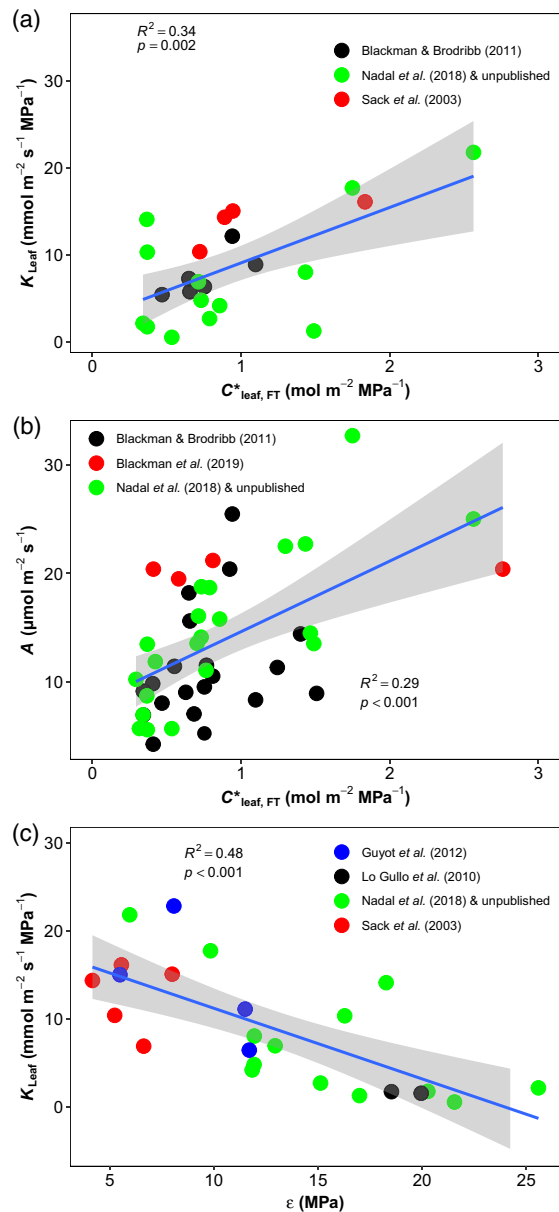
Figure 3. Relationships between photosynthesis and water-related traits from published studies: leaf-specific capacitance at full turgor (C^*_{FT}) with hydraulic conductance (K_{leaf}; a) and photosynthetic rate (A; b), and K_{leaf} with bulk modulus of elasticity (ε; c). Lines represent linear fittings and shaded areas are the 95% confidence intervals. K_{leaf} data were measured in four–six leaves from the same individuals in 13 of the 20 species used in Nadal *et al.* (2018) using the evaporative flux method under saturating light conditions at 25–30°C as described in Sack and Scoffoni (2012). Only 'sun' values are shown from Sack *et al.* (2003), where sun and shade leaves were measured for the same species. The fern *Polystichum setiferum* from Lo Gullo *et al.* (2010) is not shown due to its extremely high ε (49 MPa); nonetheless, it also displayed low K_{leaf} (2 mmol m^{-2} MPa^{-1}).

transpiration stream, and found that only C_{dyn} was positively related with K_{leaf} across different species. On the other hand, water storage is also reflected in C_{bulk}. Water
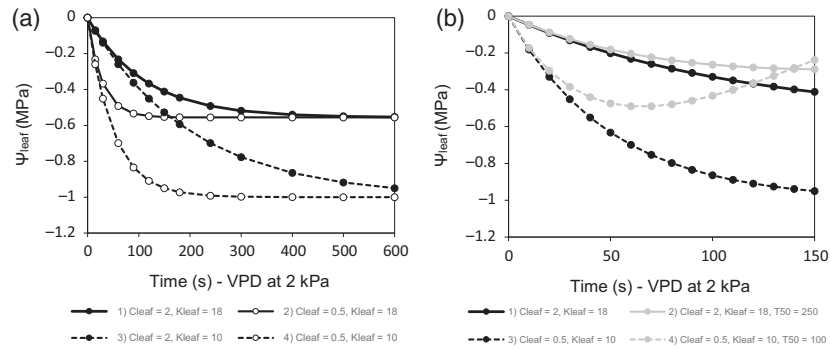
Figure 4. Modeling of leaf water potential (Ψ_{leaf}) dynamics in response to a sudden vapor pressure deficit (VPD) increase following the equation described in Cardoso *et al.* (2018): $\Psi_{leaf} = -[\Psi_{min} \times \exp(-t \times K_{leaf}/C_{leaf})]$, where t is time and Ψ_{min} is the minimum Ψ_{leaf} that would be reached at steady-state conditions under a VPD of 2 kPa and maximum transpiration (E_{max}) of 10 mmol m^{-2} s^{-1}, and it is calculated as $\Psi_{min} = E_{max}/K_{leaf}$.
(a) Different combinations of C_{leaf} (0.5–2 mol m^{-2} MPa^{-1}) and K_{leaf} (10–18 mmol m^{-2} s^{-1} MPa^{-1}) under no variation in E_{max}. Notice how K_{leaf} sets Ψ_{min} whereas C_{leaf} determines the slope of Ψ_{leaf} decrease per time: the combination of high transpiration and low C_{leaf} implies a very fast (0–30 s) drop in Ψ_{leaf} independently of K_{leaf}. However, this initial simulation does not account for stomatal closure in response to high VPD, which occurs in most plant species (McAdam and Brodribb, 2015; Brodribb and McAdam, 2017).
(b) Realistic values of stomatal conductance (g_s) decrease are incorporated as reported in Martins *et al.* (2016) in a survey of conifer and fern species, where half-times for stomatal closure (T_{50}) ranged from 100 to 250 s across C_{leaf} values from 0.2 to 2 mol m^{-2} MPa^{-1}. Despite the significant effect of g_s decrease over time in both scenarios, resulting in a compensatory effect on Ψ_{leaf}, C_{leaf} still strongly determines the Ψ_{leaf} response in the first stages under high transpiration, even at the highest stomatal closure times reported ($T_{50} = 100$ s). Although possible direct effects of Ψ_{leaf} on g_s and K_{leaf} are not considered, this simple modeling approach illustrates the key role of C_{leaf} in buffering Ψ_{leaf} under high evaporative demands.

storage within a tissue is often described as the water content per dry mass (g g^{-1}), and is clearly related with sapwood density and capacitance in stems (Stratton *et al.*, 2000; Scholz *et al.*, 2011; Pratt and Jacobsen, 2017). The role and mechanisms of water storage have been extensively described in succulent species (Lamont and Lamont, 2000; Males and Griffiths, 2017), although its contribution to the water transport buffering is doubted (Males, 2017). In stems, stored water in the xylem can significantly contribute to the total amount of daily transpired water (Scholz *et al.*, 2011). On the other hand, the contribution of the water stored in leaves to the transpiration in non-succulent species is relatively small compared to stems: only 1–5.5% of total daily transpired water comes from water stored in leaves (Tyree *et al.*, 1991), although other studies point towards a greater contribution of leaves in small-sized plants (Gleason *et al.*, 2014). Hence, although C_{leaf} is relatively small compared with C_{stem} (Scholz *et al.*, 2011), it is important in determining Ψ_{leaf} and subsequently the driving force for water transport through the soil−plant−atmosphere system (Hunt *et al.*, 1991).

Other roles of C_{leaf}

The role of capacitance in maintaining high Ψ can prevent against damaging cavitation and embolism events that hinder hydraulic efficiency (Scholz *et al.*, 2011). In stems, high C_{stem} is related to higher $P50$ (the Ψ at which 50% of the hydraulic conductance is lost) and minimum Ψ_{branch}, suggesting a prominent role of C_{stem} in preventing extreme pressures and a need for higher embolism resistance in trees with low C_{stem} (Pratt *et al.*, 2007; Meinzer *et al.*, 2008, 2009). Thus, greater branch capacitance

and water storage capacity extends the time before damaging dehydration occurs (Blackman *et al.*, 2016). Surprisingly, no clear correlation between C_{stem} and C_{leaf} has been described (Savi *et al.*, 2017; Fu *et al.*, 2019). Zhang *et al.* (2013) found that high C_{stem} contributed to maintain high midday Ψ_{branch} but with simultaneous decreased Ψ_{leaf}. The lack of correlation between the two capacitances may reflect the need for greater Ψ gradients from stem to leaf in species with strong reliance on stem water storage. Nonetheless, leaf capacitance can prevent hydraulic loss in a similar manner as stems, contributing to higher Ψ_{leaf} and wider safety margins, and thus preserving K_{leaf} for extended periods under drought (Scholz *et al.*, 2014; Blackman *et al.*, 2019). Resistance to hydraulic failure is also attributed to tissue rigidity by preventing leaf shrinkage (Trifilo *et al.*, 2016) and conduit collapse (Blackman *et al.*, 2010). An extreme case of dependence on leaf water storage for survival are epiphyte and desiccation-tolerant fern species, where C_{leaf} determines the time to leaf death (McAdam and Brodribb, 2013). In addition to its role in preventing hydraulic stress, C_{leaf} also affects the dynamics of foliar water uptake (FWU): species with high capacitance can maintain the needed Ψ_{leaf} for FWU during longer timespans, thus resulting in greater amounts of water incorporated into leaves (needed for full refilling of water storage; Berry *et al.*, 2018; Boanares *et al.*, 2018).

Causes of C_{leaf} variation

The structural determinants of capacitance in leaves are yet poorly understood. Area-based C_{leaf} has been weakly related to leaf mass per area (LMA; Blackman *et al.*, 2010),

possibly through the thickness component of LMA (Sack *et al.*, 2003), as this would imply a higher amount of tissue and water per unit area. However, this relationship was not found in another set of species (Nadal *et al.*, 2018). Leaf composition could also affect C_{leaf}: Blackman and Brodribb (2011) showed that higher lignin content reduces C_{leaf}. Nonetheless, the main driver of C_{leaf} appears to be water content: a strong positive relationship emerges between C_{leaf} at full turgor on a mass basis and saturated water content (SWC) across non-succulent species (Figure 5a). Interestingly, the link between A and C_{leaf} shown above results in a tight relationship between mass-based assimilation (A_{mass}) and SWC. On the other hand, area-based C_{leaf} is related to leaf modulus of elasticity (ε), where more elastic

leaves present, in turn, higher C_{leaf} (Figure 5b). The fact that these parameters can influence capacitance depending on its expression (area- or mass-based) still points to LMA as a driver of C_{leaf} due to its relationship to the amount of water per leaf area (Sack *et al.*, 2003; Buckley *et al.*, 2017). Leaf water content can be attributed to the presence of specialized storage tissue in succulent species (Nobel, 2006; Males and Griffiths, 2017). On the other hand, some extracellular features such as large cavities filled with a polysaccharide matrix (mainly constituted by pectins) can contribute to water storage and capacitance (Robichaux and Morse, 1990). In addition, cell dimensions could reflect the amount of water stored in the leaf (Nobel, 2006). Figures 5c,d compare the structure of *Populus nigra* and
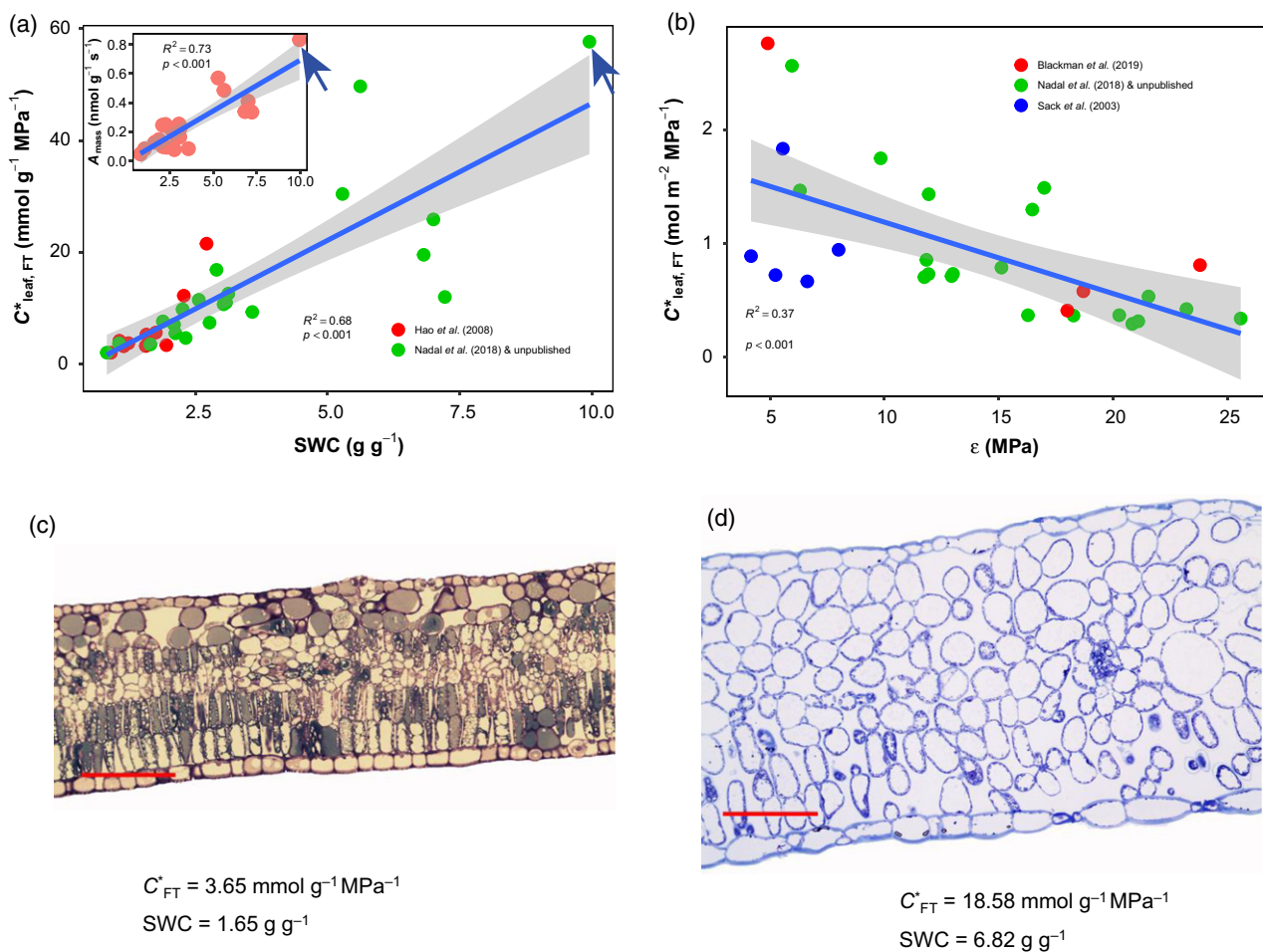

$C^*_{FT} = 3.65$ mmol g^{-1} MPa^{-1}

SWC = 1.65 g g^{-1}

$C^*_{FT} = 18.58$ mmol g^{-1} MPa^{-1}

SWC = 6.82 g g^{-1}

Figure 5. The determinants of leaf capacitance from published studies: relationships of the saturated water content (SWC) and mass-based leaf-specific capacitance at full turgor (C^*_{FT}; a) and mass-based light-saturated net assimilation (A_{mass}; inset) across species. The arrows in (a) point to *Phaseolus vulgaris*, which was grown in a growth chamber at a light intensity of 200–300 μmol m^{-2} sec^{-1} and measured as described in Nadal *et al.* (2018). Relationship between area-based C^*_{FT} and bulk modulus of elasticity (ε; b). Only 'sun' values are shown from Sack *et al.* (2003), where sun and shade leaves were measured for the same species. Lines represent linear fittings and shaded areas are the 95% confidence intervals. The range in SWC and C^*_{FT} may be a result of leaf anatomy. Microscopic images are shown of representative leaves of *Populus nigra* (c) and *Spinacia oleracea* (d) from the same plants used in Nadal *et al.* (2018). The red bars in both images correspond to 100 μm. Sample fixation and processing was done following the procedures described in Tomàs *et al.* (2013). Photographs were taken at × 200 magnifications with a digital camera (U-TVO.5XC; Olympus, Tokyo, Japan). Leaf-specific capacitance at full turgor (C^*_{FT}) and the saturated water content (SWC) values are from Nadal *et al.* (2018). Note the increased leaf thickness and cell area in *S. oleracea*, possibly accounting for the increased water storage and capacitance.

Spinacia oleracea leaves differing in SWC; the greater cell dimensions of *S. oleracea* could account for the higher SWC and leaf-specific capacitance at full turgor (C^*_{FT}) of this species. The potential role of leaf structure and anatomy in determining C_{leaf}, as well as its possible link with aquaporin expression (Vitali *et al.*, 2016), deserve further investigation.

Hydraulic compartmentalization

Although leaf water relations are related to traits reflecting the bulk of leaf structure, it is very likely that the tissue differentiation is reflected in different contributions to water transport and dynamics within the leaf, i.e. the so-called 'hydraulic compartmentalization' (Sack and Tyree, 2005; Blackman and Brodribb, 2011). Hydraulic compartmentalization may be key to explain the interplay between K_{leaf}, C_{leaf} and ε. Tissue capacitance is defined for a given $\Delta\Psi$ range, and it may vary depending on Ψ_{tlp} (Scholz *et al.*, 2014). Three phases, distinguished by different slopes of the $\Delta W/\Delta\Psi$ relationship, have been identified for C_{stem}: capillary and elastic water release, and the contribution of water from the lumen of vessels once cavitation occurs (Tyree and Yang, 1990; Hunt *et al.*, 1991; Vergeynst *et al.*, 2014; Pratt and Jacobsen, 2017). In stems, tissue structure accounts for the relative contribution of each of these compartments: capillary storage is related to the abundance of dead tissue and fibers, whereas elastic storage is driven by the cell wall thickness of parenchyma cells (Jupa *et al.*, 2016; Knipfer *et al.*, 2017). In the case of leaves, it could be expected a far greater contribution of elastic water storage as this is mainly associated with living tissue (Tyree and Ewers, 1991; Jupa *et al.*, 2016), which presents thinner cell walls compared with vessels. Capacitance is mechanistically linked with modulus of elasticity for the Ψ range above the turgor loss point (Tyree and Jarvis, 1982; Hunt *et al.*, 1991; Sack and Tyree, 2005); indeed, this is reflected in the negative relationship between C_{leaf} and ε (Figure 5b). In turn, elasticity within the leaf differs across tissues, being the stiffness of the epidermis and cuticle compared with the relatively elastic mesophyll the most striking example of this phenomenon (Onoda *et al.*, 2015). This differentiation is also reflected under dehydration, where mesophyll shows a higher level of shrinkage than the more rigid epidermis (Zhang *et al.*, 2016). The link between K_{leaf} and both C_{leaf} and ε points towards a tight coordination between the need for buffering capacity provided by C_{leaf} and the ability to sustain deformation and shrinkage under tension, which is related to elasticity in the case of stems (Pratt *et al.*, 2007; Pratt and Jacobsen, 2017) and cells (Canny *et al.*, 2012). Moreover, even within the leaf mesophyll cells could play a different role depending on their contribution to the water flow. Experimental data and modeling indicate a significant role of spongy mesophyll in sustaining the bulk of

water flow within hipostomatous leaves and determining K_{ox} (Buckley, 2015; Buckley *et al.*, 2015). In turn, bulk C_{leaf} has been related to a higher proportion of spongy mesophyll, whereas no such relationship has been observed for palissade mesophyll (Binks *et al.*, 2016). This separate contribution to C_{leaf} is well reflected in their different elasticity: using ultrasonic resonant spectroscopy, Alvarez-Arenas *et al.* (2018) concluded that spongy mesophyll significantly contributes to lower ε compared with palisade mesophyll. The more elastic properties of spongy cells are also reflected in its greater shrinkage upon dehydration (Muries *et al.*, 2019). Leaf rehydration kinetics also point towards hydraulic compartmentalization within leaves, with a prominent role of spongy mesophyll during the fast phase of rehydration in angiosperms (Zwieniecki *et al.*, 2007). Both reduced elasticity (Alvarez-Arenas *et al.*, 2018) and, possibly, C_{leaf} of palisade mesophyll could relate to its behavior as a rather 'static' water compartment (Zwieniecki *et al.*, 2007; Guzmán-Delgado *et al.*, 2018), isolated from the bulk of water transport within the leaf but nonetheless contributing to the buffering of water status (Nardini *et al.*, 2010; Rockwell *et al.*, 2014). However, even within the 'static' palisade mesophyll some degree of hydraulic compartmentalization could occur: Canny *et al.* (2012) found that isolated palisade cells experienced a greater degree of shrinkage compared with more closely packed ('matrix') cells.

Extreme cases of hydraulic compartmentalization have been described in plants thriving in dry forests and marine water, two particularly harsh environments where the water (and osmotic) stress conditions shape interesting water-usage and conservation strategies (Nobel, 2006; Nguyen *et al.*, 2017). Trichomes can serve for water storage and uptake, for instance, significantly contributing to leaf capacitance at relatively high Ψ_{leaf} ranges, as described in the mangrove species *Avicennia marina* (−0.10 to −0.85 MPa; Nguyen *et al.*, 2017). These 'outside-mesophyll' leaf structures are able to contribute to leaf water relations despite being completely disconnected from the xylem and apoplast water pathway, possibly contributing to the symplast water transport (Nguyen *et al.*, 2017) through FWU (Berry *et al.*, 2018). On the other hand, the water storage in the parenchyma tissue supported the chlorenchyma (photosynthetic tissue) under drought in the hemiepiphytic cactus *Hylocereus undatus*, where parenchyma cells underwent length reduction due to changes in water content, thus helping to sustain net CO_2 uptake over a 6-week drought period (Nobel, 2006). These volume changes, associated with water dynamics, are possible due to highly elastic cells, as described for other succulent species (Steudle *et al.*, 1980; Goldstein *et al.*, 1991).

The high degree of hydraulic compartmentalization within leaves, possibly driven by different capacitance

and elastic tissues of cells, is illustrated in Figure 6. Notably, the figure shows the potential coupling between K_{ox} and C_{leaf} and ε at the tissue and cell levels, as well as the contribution to water status maintenance by structures not directly related to xylem water transport, such as trichomes and extracellular polysaccharides. Overall, the available literature suggests a complex relationship across leaf tissues regarding water transport and the subsidiary needs (elasticity, water storage) to sustain water flow and evaporation (Rockwell *et al.*, 2014; Buckley *et al.*, 2015). Although capacitance and elasticity are difficult to characterize in complex tissues where elastic properties of individual cells are 'diluted' in a complex matrix of cell-to-cell interactions (Zhang *et al.*, 2016), the studies of Alvarez-Arenas *et al.* (2018) and Nguyen *et al.* (2017) illustrate how layered water relations can still be inferred from complex structures. Moreover, the use of techniques such as the pressure probe (Hüsken *et al.*, 1978; Murphy and Ortega, 1995) can provide insights into interesting cell properties that affect the whole tissue water relations and thus help our understanding of individual cell properties.

CONCLUDING REMARKS

A growing network of leaf traits

In previous work, leaf traits relationships were established highlighting the relationships among flux-based parameters (Flexas *et al.*, 2013; Sack *et al.*, 2013). Here, these relationships are extended to include leaf water relations. Despite early work describing the link between K_{leaf} and C_{leaf} across species (Sack *et al.*, 2003), the potential coordination between water relation traits and photosynthesis and carbon economy traits has only received attention in the recent years (Peguero-Pina *et al.*, 2017; Nadal *et al.*, 2018; Zhu *et al.*, 2018). The expanded network of leaf traits is shown in Figure 7. The network proposed here displays the significant relationships described among photosynthesis and water transport, expanded on the basis of the described g_m and K_{ox} relationship, although the mechanisms of such relationship still need to be elucidated. The coupling of K_{leaf} with both g_s and g_m results in the overall effect on A, thus clearly relating to water and CO_2 transport in leaves. On the other hand, C_{leaf} emerges as a subsidiary aspect related to K_{leaf} and A due to the need for high
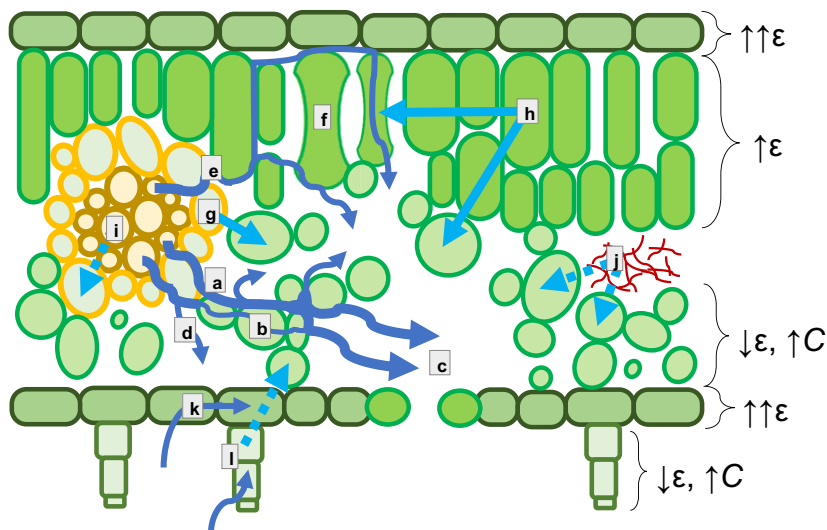

Figure 6. Scheme of the possible water movements and storage dynamics within a representative hipostomatous leaf.
The figure highlights the different pathways of water transport outside the xylem as well as the potential compartmentalization of water relations across the different leaf tissues and their roles in capacitance. Emerging from the vein, the bulk of the transpiration stream moves through the spongy mesophyll mainly via apoplast (a), although some portion of the water flow may move via symplast (b) to the sites of evaporation in the substomatal chamber (c), possibly evaporating at the bundle sheath (d), along the water pathway and near the stomata (Buckley, 2015; Buckley *et al.*, 2015; Scoffoni, 2015). The low modulus of elasticity (ε) and high capacitance (C) of the spongy tissue (Binks *et al.*, 2016; Alvarez-Arenas *et al.*, 2018) could allow the cells to sustain shrinkage and tension under the water flow and provide dynamic capacitance to buffer transpiration (Zwieniecki *et al.*, 2007). Water flow also occurs in the palissade tissue (e), although palissade is thought to play a more 'static' role, being regarded as a rather subsidiary compartment (Zwieniecki *et al.*, 2007; Canny *et al.*, 2012). The transpiration stream may be higher in isolated cells (f), where shrinkage under transpiration tension has been observed (Canny *et al.*, 2012). Bundle sheath cells or, in the case of some gymnosperms, the so-called 'transfusion tissue' (Johnson *et al.*, 2009) are thought to also provide a dynamic pool of water (g) under transpiration (Muries *et al.*, 2019). Palissade mesophyll also constitutes a source of water (h) to buffer against water loss in transpiring cells (Zwieniecki *et al.*, 2007; Blackman and Brodribb, 2011). The stored water in the leaf can be replenished through the vein conduits (i) during night (Zeppel *et al.*, 2014) or liberated from extracellular water-embedding polysaccharides (j) such as pectins, which are present in some species (Morse, 1990; Robichaux and Morse, 1990). Foliar water uptake (k), which has been related to high capacitance (Berry *et al.*, 2018), occurs mainly through epidermal cells (Guzmán-Delgado *et al.*, 2018). In addition, trichomes (l) have been described in mangrove species to contribute to leaf capacitance to possibly help sustaining transpiration for longer periods (Nguyen *et al.*, 2017).
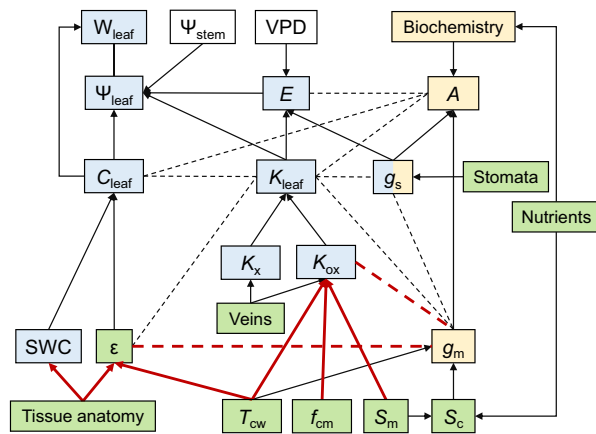
Figure 7. Network of photosynthesis and water-related leaf traits, showing their mechanistic links and their coordination. Blue-shaded variables are related to water status and/or transport; golden-shaded variables are related to carbon fluxes and photosynthesis; green-shaded variables are related to leaf structure and chemistry; finally, white-shaded variables refer to atmosphere (VPD) and plant (Ψ_{stem}) conditions. All abbreviations are already defined in the text. Stomatal conductance (g_s) displays both blue and golden colors as stomatal conductance to water vapor (g_{sw}) and to CO_2 (g_{sc}) are easily inter-converted accounting for the different molecular weights of H_2O and CO_2. Black solid arrows indicate clearly, mechanistic-based relationships among two traits, where one directly influences the other. On the other hand, black dashed lines reflect correlation rather than causation among two traits, potentially linked through the influence of other traits. Red lines indicate hypothesized or not fully demonstrated relationships; these highlight the gap of knowledge regarding the tissue and anatomical traits driving both ε and K_{ox}, which may help to clearly establish their relationships with g_m. For the sake of simplicity, the relationships presented here have been described or suggested to occur under optimum conditions; their coordination may change under variable or stressful conditions (as depicted in Table 1). For example, Ψ_{leaf} is a strong driver of photosynthesis and transpiration decline under water stress, and lowering Ψ_{leaf} negatively affects K_{leaf}, g_s and g_m (Flexas *et al.*, 2018; Wang *et al.*, 2018).

capacitance in leaves with high transpiration (and photosynthetic) capacity, providing a buffering role preventing sudden fluctuations in Ψ_{leaf}. The relationships among traits displayed in Figure 7 highlight that most traits may not necessarily be related on a 'cause−effect' basis, but rather emerge due to the influence of common, underlying variables.

Besides the traits usually related to A (g_s, g_m, K_{leaf}, among others), bulk tissue elasticity is hypothesized to be a key parameter in the network of traits, as it is a determinant of C_{leaf} (Tyree and Jarvis, 1982) but it also has been related to g_m (Nadal *et al.*, 2018). The reduced CO_2 diffusion capacity of more rigid tissues may be due to cell wall thickness, as T_{cw} in single cells is mechanistically linked to ε (Tyree and Jarvis, 1982). Although extrapolating the ε determinants in single cells to complex, 'matrix-like' organs such as leaves is not so straightforward, there are promising results relating T_{cw} of mesophyll cells to bulk leaf ε (Peguero-Pina *et al.*, 2017). On the other hand, bulk ε could also be a product of greater epidermis and cuticle investment (Onoda *et al.*, 2015; Binks *et al.*, 2016).

Furthermore, ε can experience dynamic variations (the so-called 'elastic adjustment') under variable conditions such as water and cold stress (Schulte, 1992; Bartlett *et al.*, 2012; Niinemets, 2016). These rapid changes are not likely driven by structural modifications, but rather changes in cell wall components and rearrangements of cell wall polysaccharides (Peltier and Marigo, 1999; Miranda-Apodaca *et al.*, 2018). Notably, changes in cell wall composition have recently been described to affect g_m (Ellsworth *et al.*, 2018; Clemente-Moreno *et al.*, 2019). Further research is needed in order to clearly establish the structural and chemical drivers of ε, and to explore the possible shared mechanisms with g_m.

Future opportunities

Although significant progress has been made towards understanding the influence of plant hydraulics and plant water relations on photosynthetic performance, much remains to be learned. Several research areas, in particular, are likely to yield significant insights in the near future.

1. The contributions of each of the four water transport pathways to K_{ox} should be quantified, especially under dynamic conditions.
2. As all the current methods for estimating g_m and K_{leaf} are imperfect and, worse of all, there is no method available for K_{ox} direct estimation, new theories and/or techniques are required for leaf hydraulics and g_m estimation.
3. The possible coordination of CO_2 and water transport, as well as the hydraulic compartmentalization in water storage and capacitance, should be explored using realistic leaf anatomy.
4. The molecular genetic control of g_m, K_{leaf} as well as C_{leaf} has never been revealed, and identifying the key genes in controlling those traits is required for further crop productivity, stress tolerance and water-use efficiency improvements. Aquaporins could be a potential target, as they have been related to water (Vitali *et al.*, 2016) and CO_2 dynamics (Flexas *et al.*, 2012).
5. The mechanistic determinants of leaf ε need to be explored in order to establish a potentially mechanistically driven effect of tissue elasticity on water transport and CO_2 diffusion.
6. Expanding the knowledge regarding water relations and photosynthesis in non-vascular plant groups such as mosses and bryophytes, where T_{cw} has been described to be at the highest end of land plants (Carriqui *et al.*, 2019).

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AUTHOR CONTRIBUTIONS

DX and MN planned and designed the study; analyzed the data and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data referred to are included in the manuscript and supplementary materials.

REFERENCES

Alvarez-Arenas, T.E.G., Sancho-Knapik, D., Peguero-Pina, J.J., Gomez-Arroyo, A. and Gil-Pelegrin, E. (2018) Non-contact ultrasonic resonant spectroscopy resolves the elastic properties of layered plant tissues. *Appl. Phys. Lett.* **113**, 253 704.

Barbour, M.M. (2017) Understanding regulation of leaf internal carbon and water transport using online stable isotope techniques. *New Phytol.* **213**, 83–88.

Barbour, M.M. and Farquhar, G.D. (2004) Do pathways of water movement and leaf anatomical dimensions allow development of gradients in H218O between veins and the sites of evaporation within leaves? *Plant Cell Environ.* **27**, 107–121.

Barbour, M.M., Farquhar, G.D. and Buckley, T.N. (2017) Leaf water stable isotopes and water transport outside the xylem. *Plant Cell Environ.* **40**, 914–920.

Bartlett, M.K., Scoffoni, C. and Sack, L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol. Lett.* **15**, 393–405.

Berry, Z.C., Emery, N.C., Gotsch, S.G. and Goldsmith, G.R. (2018) Foliar water uptake: Processes, pathways, and integration into plant water budgets. *Plant Cell Environ.* **42**(2), 410–423.

Binks, O., Meir, P., Rowland, L., da Costa, A.C., Vasconcelos, S.S., de Oliveira, A.A., Ferreira, L., Christoffersen, B., Nardini, A. and Mencuccini, M. (2016) Plasticity in leaf-level water relations of tropical rainforest trees in response to experimental drought. *New Phytol.* **211**, 477–488.

Blackman, C.J. (2018) Leaf turgor loss as a predictor of plant drought response strategies. *Tree Physiol.* **38**, 655–657.

Blackman, C.J. and Brodribb, T.J. (2011) Two measures of leaf capacitance: insights into the water transport pathway and hydraulic conductance in leaves. *Funct. Plant Biol.* **38**, 118–126.

Blackman, C.J., Brodribb, T.J. and Jordan, G.J. (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol.* **188**, 1113–1123.

Blackman, C.J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S.M. and Duursma, R.A. (2016) Toward an index of desiccation time to tree mortality under drought. *Plant Cell Environ.* **39**, 2342–2345.

Blackman, C.J., Creek, D., Maier, C. et al. (2019) Drought response strategies and hydraulic traits contribute to mechanistic understanding of plant dry-down to hydraulic failure. *Tree Physiol.* **39**(6), 910–924.

Boanares, D., Ferreira, B.G., Kozovits, A.R., Sousa, H.C., Isaias, R.M.S. and Franca, M.G.C. (2018) Pectin and cellulose cell wall composition enables different strategies to leaf water uptake in plants from tropical fog mountain. *Plant Physiol. Biochem.* **122**, 57–64.

Boyer, J.S. (1985) Water transport. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **36**, 473–516.

Boyer, J.S. (2015) Turgor and the transport of CO_2 and water across the cuticle (epidermis) of leaves. *J. Exp. Bot.* **66**, 2625–2633.

Brodribb, T.J. and Holbrook, N.M. (2003a) Changes in leaf hydraulic conductance during leaf shedding in seasonally dry tropical forest. *New Phytol.* **158**, 295–303.

Brodribb, T.J. and Holbrook, N.M. (2003b) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol.* **132**, 2166–2173.

Brodribb, T.J. and Holbrook, N.M. (2004) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant Cell Environ.* **27**, 820–827.

Brodribb, T.J. and McAdam, S.A.M. (2011) Passive origins of stomatal control in vascular plants. *Science* **331**, 582–585.

Brodribb, T.J. and McAdam, S.A. (2017) Evolution of the stomatal regulation of plant water content. *Plant Physiol.* **174**, 639–649.

Brodribb, T.J., Feild, T.S. and Jordan, G.J. (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol.* **144**, 1890–1898.

Buckley, T.N. (2015) The contributions of apoplastic, symplastic and gas phase pathways for water transport outside the bundle sheath in leaves. *Plant Cell Environ.* **38**, 7–22.

Buckley, T.N., Sack, L. and Gilbert, M.E. (2011) The role of bundle sheath extensions and life form in stomatal responses to leaf water status. *Plant Physiol.* **156**, 962–973.

Buckley, T.N., John, G.P., Scoffoni, C. and Sack, L. (2015) How does leaf anatomy influence water transport outside the xylem? *Plant Physiol.* **168**, 1616–1635.

Buckley, T.N., John, G.P., Scoffoni, C. and Sack, L. (2017) The sites of evaporation within leaves. *Plant Physiol.* **173**, 1763–1782.

Canny, M., Wong, S.C., Huang, C. and Miller, C. (2012) Differential shrinkage of mesophyll cells in transpiring cotton leaves: implications for static and dynamic pools of water, and for water transport pathways. *Funct. Plant Biol.* **39**, 91–102.

Cardoso, A.A., Brodribb, T.J., Lucani, C.J., DaMatta, F.M. and McAdam, S.A.M. (2018) Coordinated plasticity maintains hydraulic safety in sunflower leaves. *Plant Cell Environ.* **41**, 2567–2576.

Cardoso, A.A., Randall, J.M. and McAdam, S.A.M. (2019) Hydraulics regulate stomatal responses to changes in leaf water status in the fern *Athyrium filix femin. Plant Physiol.* **179**, 533–543.

Caringella, M.A., Bongers, F.J. and Sack, L. (2015) Leaf hydraulic conductance varies with vein anatomy across *Arabidopsis thaliana* wild-type and leaf vein mutants. *Plant Cell Environ.* **38**, 2735–2746.

Carriqui, M., Roig-Oliver, M., Brodribb, T.J. et al. (2019) Anatomical constraints to nonstomatal diffusion conductance and photosynthesis in lycophytes and bryophytes. *New Phytol.* **222**(3), 1256–1270.

Carriquí, M., Douthe, C., Molins, A. and Flexas, J. (2019) Leaf anatomy does not explain apparent short-term responses of mesophyll conductance to light and CO_2 in tobacco. *Physiol. Plant.* **165**, 604–618.

Chaves, M.M. (1991) Effects of water deficits on carbon assimilation. *J. Exp. Bot.* **42**, 1–16.

Clemente-Moreno, M.J., Gago, J., Diaz-Vivancos, P., Bernal, A., Miedes, E., Bresta, P., Liakopoulos, G., Fernie, A.R., Hernandez, J.A. and Flexas, J. (2019) The apoplastic antioxidant system and altered cell wall dynamics influence mesophyll conductance and the rate of photosynthesis. *Plant J.* **99**(6), 1031–1046.

Dixon Henry, H. and Joly, J. (1895) XII. On the ascent of sap. *Philos. Trans. R. Soc. Lond. B*, **186**, 563–576.

Ellsworth, P.V., Ellsworth, P.Z., Koteyeva, N.K. and Cousins, A.B. (2018) Cell wall properties in *Oryza sativa* influence mesophyll CO_2 conductance. *New Phytol.* **219**(1), 66–76.

Evans, J.R., Kaldenhoff, R., Genty, B. and Terashima, I. (2009) Resistances along the CO_2 diffusion pathway inside leaves. *J. Exp. Bot.* **60**, 2235–2248.

Flexas, J., Ribas-Carbo, M., Diaz-Espejo, A., Galmes, J. and Medrano, H. (2008) Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant Cell Environ.* **31**, 602–621.

Flexas, J., Barbour, M.M., Brendel, O. et al. (2012) Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. *Plant Sci.* **193–194**, 70–84.

Flexas, J., Scoffoni, C., Gago, J. and Sack, L. (2013) Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *J. Exp. Bot.* **64**, 3965–3981.

Flexas, J., Carriquí, M. and Nadal, M. (2018) Gas exchange and hydraulics during drought in crops: who drives whom? *J. Exp. Bot.* **69**, 3791–3795.

Fu, X., Meinzer, F.C., Woodruff, D.R., Liu, Y.-Y., Smith, D.D., McCulloh, K.A. and Howard, A.R. (2019) Coordination and trade-offs between leaf and

stem hydraulic traits and stomatal regulation along a spectrum of iso-hydry to anisohydry. *Plant Cell Environ.* 42, 2245–2258.

Gleason, S.M., Blackman, C.J., Cook, A.M., Laws, C.A. and Westoby, M. (2014) Whole-plant capacitance, embolism resistance and slow transpiration rates all contribute to longer desiccation times in woody angiosperms from arid and wet habitats. *Tree Physiol.* 34, 275–284.

Goldstein, G., Sharifi, M.R., Kohorn, L.U., Lighton, J.R.B., Shultz, L. and Rundel, P.W. (1991) Photosynthesis by inflated pods of a desert shrub, Isomeris arborea. *Oecologia*, 85, 396–402.

Griffiths, H., Weller, G., Toy, L.F.M. and Dennis, R.J. (2013) You're so vein: bundle sheath physiology, phylogeny and evolution in C₃ and C₄ plants. *Plant Cell Environ.* 36, 249–261.

Gu, L. and Sun, Y. (2014) Artefactual responses of mesophyll conductance to CO₂ and irradiance estimated with the variable *J* and online isotope discrimination methods. *Plant Cell Environ.* 37, 1231–1249.

Gupta, A.S. and Berkowitz, G.A. (1987) Osmotic adjustment, symplast volume, and nonstomatally mediated water stress inhibition of photosynthesis in wheat. *Plant Physiol.* 85, 1040–1047.

Guzmán-Delgado, P., Mason Earles, J. and Zwieniecki, M.A. (2018) Insight into the physiological role of water absorption via the leaf surface from a rehydration kinetics perspective. *Plant Cell Environ.* 41, 1886–1894.

Hao, G.-Y., Hoffmann, W.A., Scholz, F.G., Bucci, S.J., Meinzer, F.C., Franco, A.C., Cao, K.-F. and Goldstein, G. (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia*, 155, 405–415.

Huang, G., Zhang, Q., Wei, X., Peng, S. and Li, Y. (2017) Nitrogen can alleviate the inhibition of photosynthesis caused by high temperature stress under both steady-state and flecked irradiance. *Front. Plant Sci.* 8, 945.

Hunt, E.R., Running, S.W. and Federer, C.A. (1991) Extrapolating plant water flow resistances and capacitances to regional scales. *Agric. For. Meteorol.* 54, 169–195.

Hüsken, D., Steudle, E. and Zimmermann, U. (1978) Pressure probe technique for measuring water relations of cells in higher plants. *Plant Physiol.* 61, 158–163.

Johnson, D.M., Meinzer, F.C., Woodruff, D.R. and McCulloh, K.A. (2009) Leaf xylem embolism, detected acoustically and by cryo-SEM, corresponds to decreases in leaf hydraulic conductance in four evergreen species. *Plant Cell Environ.* 32, 828–836.

Jupa, R., Plavcová, L., Gloser, V. and Jansen, S. (2016) Linking xylem water storage with anatomical parameters in five temperate tree species. *Tree Physiol.* 36, 756–769.

Kelly, G., Sade, N., Doron-Faigenboim, A. *et al.* (2017) Sugar and hexokinase suppress expression of PIP aquaporins and reduce leaf hydraulics that preserves leaf water potential. *Plant J.* 91, 325–339.

Knipfer, T., Cuneo, I.F., Earles, J.M., Reyes, C., Brodersen, C.R. and McElrone, A.J. (2017) Storage compartments for capillary water rarely refill in an intact woody plant. *Plant Physiol.* 175, 1649–1660.

Lamont, B.B. and Lamont, H.C. (2000) Utilizable water in leaves of 8 arid species as derived from pressure-volume curves and chlorophyll fluorescence. *Physiol. Plant.* 110, 64–71.

Locke, A.M., Sack, L., Bernacchi, C.J. and Ort, D.R. (2013) Soybean leaf hydraulic conductance does not acclimate to growth at elevated CO₂ or temperature in growth chambers or in the field. *Ann. Bot.* 112, 911–918.

Lo Gullo, M.A., Raimondo, F., Crisafulli, A., Salleo, S. and Nardini, A. (2010) Leaf hydraulic architecture and water relations of three ferns from contrasting light habitats. *Funct. Plant Biol.* 37, 566–574.

Loucos, K.E., Simonin, K.A. and Barbour, M.M. (2017) Leaf hydraulic conductance and mesophyll conductance are not closely related within a single species. *Plant Cell Environ.* 40, 203–215.

Lu, Z., Xie, K., Pan, Y., Ren, T., Lu, J., Wang, M., Shen, Q. and Guo, S. (2019) Potassium mediates coordination of leaf photosynthesis and hydraulic conductance by modifications of leaf anatomy. *Plant Cell Environ.* 42, 2231–2244.

Males, J. (2017) Secrets of succulence. *J. Exp. Bot.* 68, 2121–2134.

Males, J. and Griffiths, H. (2017) Leaf economic and hydraulic divergences underpin ecological differentiation in the Bromeliaceae. *Plant Cell Environ.* 41, 64–78.

Martins, S.C., McAdam, S.A., Deans, R.M., DaMatta, F.M. and Brodribb, T.J. (2016) Stomatal dynamics are limited by leaf hydraulics in ferns and conifers: results from simultaneous measurements of liquid and vapour fluxes in leaves. *Plant Cell Environ.* 39, 694–705.

Martin-StPaul, N., Delzon, S. and Cochard, H. (2017) Plant resistance to drought depends on timely stomatal closure. *Ecol. Lett.* 20, 1437–1447.

McAdam, S.A. and Brodribb, T.J. (2013) Ancestral stomatal control results in a canalization of fern and lycophyte adaptation to drought. *New Phytol.* 198, 429–441.

McAdam, S.A.M. and Brodribb, T.J. (2015) The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiol.* 167, 833–843.

McCulloh, K.A., Johnson, D.M., Meinzer, F.C. and Woodruff, D.R. (2014) The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. *Plant Cell Environ.* 37, 1171–1183.

Meinzer, F.C., Grantz, D.A., Goldstein, G. and Saliendra, N.Z. (1990) Leaf water relations and maintenance of gas exchange in coffee cultivars grown in drying soil. *Plant Physiol.* 94, 1781–1787.

Meinzer, F.C., Woodruff, D.R., Domec, J.C., Goldstein, G., Campanello, P.I., Gatti, M.G. and Villalobos-Vega, R. (2008) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* 156, 31–41.

Meinzer, F.C., Johnson, D.M., Lachenbruch, B., McCulloh, K.A. and Woodruff, D.R. (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Funct. Ecol.* 23, 922–930.

Miranda-Apodaca, J., Perez-Lopez, U., Lacuesta, M., Mena-Petite, A. and Munoz-Rueda, A. (2018) The interaction between drought and elevated CO₂ in water relations in two grassland species is species-specific. *J. Plant Physiol.* 220, 193–202.

Mizokami, Y., Noguchi, K., Kojima, M., Sakakibara, H. and Terashima, I. (2015) Mesophyll conductance decreases in the wild type but not in an ABA-deficient mutant (*aba1*) of Nicotiana plumbaginifolia under drought conditions. *Plant Cell Environ.* 38, 388–398.

Morse, S.R. (1990) The role of extracellular polysaccharide in the water balance of the leaves of early and late flowering subspecies of Hemizonia luzulifolia DC (Compositae). *Plant Cell Environ.* 13, 39–48.

Muries, B., Mom, R., Benoit, P. *et al.* (2019) Aquaporins and water control in drought-stressed poplar leaves: A glimpse into the extraxylem vascular territories. *Environ. Exp. Bot.* 162, 25–37.

Murphy, R. and Ortega, J.K.E. (1995) A new pressure probe method to determine the average volumetric elastic modulus of cells in plant tissue. *Plant Physiol.* 107, 995–1005.

Nadal, M. and Flexas, J. (2018) Mesophyll conductance to CO₂ diffusion: Effects of drought and opportunites for improvement. In *Water scarcity and sustainable agriculture in semiarid environment* (García-Tejero, I.F. and Durán-Zuazo, V.H., eds.). London: Elsevier, pp. 403–438.

Nadal, M. and Flexas, J. (2019) Variation in photosynthetic characteristics with growth form in a water-limited scenario: Implications for assimilation rates and water use efficiency in crops. *Agric. Water Manag.* 216, 457–472.

Nadal, M., Flexas, J. and Gulías, J. (2018) Possible link between photosynthesis and leaf modulus of elasticity among vascular plants: a new player in leaf traits relationships? *Ecol. Lett.* 21, 1372–1379.

Nardini, A., Raimondo, F., Lo Gullo, M.A. and Salleo, S. (2010) Leafminers help us understand leaf hydraulic design. *Plant Cell Environ.* 33, 1091–1100.

Nguyen, H.T., Meir, P., Sack, L., Evans, J.R., Oliveira, R.S. and Ball, M.C. (2017) Leaf water storage increases with salinity and aridity in the mangrove Avicennia marina: integration of leaf structure, osmotic adjustment, and access to multiple water sources. *Plant Cell Environ.* 40, 1576–1591.

Niinemets, U. (2016) Does the touch of cold make evergreen leaves tougher? *Tree Physiol.* 36, 267–272.

Nobel, P.S. (2006) Parenchyma–chlorenchyma water movement during drought for the hemiepiphytic cactus Hylocereus undatus. *Ann. Bot.* 97, 469–474.

Nobel, P.S. and Jordan, P.W. (1983) Transpiration stream of desert species: resistances and capacitances for a C₃, a C₄, and a CAM Plant. *J. Exp. Bot.* 34, 1379–1391.

Ohtsuka, A., Sack, L. and Taneda, H. (2018) Bundle sheath lignification mediates the linkage of leaf hydraulics and venation. *Plant Cell Environ.* 41, 342–353.

Onoda, Y., Schieving, F. and Anten, N.P. (2015) A novel method of measuring leaf epidermis and mesophyll stiffness shows the ubiquitous nature

of the sandwich structure of leaf laminas in broad-leaved angiosperm species. *J. Exp. Bot.* **66**, 2487–2499.

Peguero-Pina, J.J., Sancho-Knapik, D. and Gil-Pelegrín, E. (2017) Ancient cell structural traits and photosynthesis in today's environment. *J. Exp. Bot.* **68**, 1389–1392.

Peltier, J.P. and Marigo, G. (1999) Drought adaptation in *Fraxinus excelsior* L.: physiological basis of the elastic adjustment. *J. Plant. Physiol.* **154**, 529–535.

Perez-Martin, A., Michelazzo, C., Torres-Ruiz, J.M., Flexas, J., Fernández, J.E., Sebastiani, L. and Diaz-Espejo, A. (2014) Regulation of photosynthesis and stomatal and mesophyll conductance under water stress and recovery in olive trees: correlation with gene expression of carbonic anhydrase and aquaporins. *J. Exp. Bot.* **65**, 3143–3156.

Philip, J.R. (1966) Plant water relations: some physical aspects. *Annu. Rev. Plant Physiol.* **17**, 245–268.

Powles, J.E., Buckley, T.N., Nicotra, A.B. and Farquhar, G.D. (2006) Dynamics of stomatal water relations following leaf excision. *Plant Cell Environ.* **29**, 981–992.

Prado, K., Boursiac, Y., Tournaire-Roux, C., Monneuse, J.M., Postaire, O., Da Ines, O., Schaffner, A.R., Hem, S., Santoni, V. and Maurel, C. (2013) Regulation of *Arabidopsis* leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. *Plant Cell*, **25**, 1029–1039.

Pratt, R.B. and Jacobsen, A.L. (2017) Conflicting demands on angiosperm xylem: tradeoffs among storage, transport and biomechanics. *Plant Cell Environ.* **40**, 897–913.

Pratt, R.B., Jacobsen, A.L., Ewers, F.W. and Davis, S.D. (2007) Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytol.* **174**, 787–798.

Robichaux, R.H. and Morse, S.R. (1990) Extracellular polysaccharide and leaf capacitance in a Hawaiian bog species, *Argyroxiphium-Grayanum* (Compositae, Madiinae). *Am. J. Bot.* **77**, 134–138.

Rockwell, F.E. and Holbrook, N.M. (2017) Leaf hydraulic architecture and stomatal conductance: a functional perspective. *Plant Physiol.* **174**, 1996–2007.

Rockwell, F.E., Holbrook, N.M. and Stroock, A.D. (2014) The competition between liquid and vapor transport in transpiring leaves. *Plant Physiol.* **164**, 1741–1758.

Sack, L. and Holbrook, N.M. (2006) Leaf hydraulics. *Annu. Rev. Plant Biol.* **57**, 361–381.

Sack, L. and Scoffoni, C. (2012) Measurement of leaf hydraulic conductance and stomatal conductance and their responses to irradiance and dehydration using the evaporative flux method (EFM). *J. Vis. Exp.* **70**, e4179.

Sack, L. and Tyree, M.T. (2005) Leaf hydraulics and its implications in plant structure and function. In *Vascular Transport in Plants* (Holbrook, N.M. and Zwieniecki, M.A., eds). Burlington: Academic Press, pp. 93–114.

Sack, L., Melcher, P.J., Zwieniecki, M.A. and Holbrook, N.M. (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *J. Exp. Bot.* **53**, 2177–2184.

Sack, L., Cowan, P.D., Jaikumar, N. and Holbrook, N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant Cell Environ.* **26**, 1343–1356.

Sack, L., Streeter, C.M. and Holbrook, N.M. (2004) Hydraulic analysis of water flow through leaves of sugar maple and red oak. *Plant Physiol.* **134**, 1824–1833.

Sack, L., Tyree, M.T. and Holbrook, N.M. (2005) Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytol.* **167**, 403–413.

Sack, L., Scoffoni, C., John, G.P., Poorter, H., Mason, C.M., Mendez-Alonzo, R. and Donovan, L.A. (2013) How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *J. Exp. Bot.* **64**, 4053–4080.

Sack, L., Ball, M.C., Brodersen, C. *et al.* (2016) Plant hydraulics as a central hub integrating plant and ecosystem function: meeting report for "Emerging Frontiers in Plant Hydraulics" (Washington, DC, May 2015). *Plant Cell Environ.* **39**, 2085–2094.

Sade, N., Gebretsadik, M., Seligmann, R., Schwartz, A., Wallach, R. and Moshelion, M. (2010) The role of tobacco Aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. *Plant Physiol.* **152**, 245–254.

Sade, N., Shatil-Cohen, A., Attia, Z., Maurel, C., Boursiac, Y., Kelly, G., Granot, D., Yaaran, A., Lerner, S. and Moshelion, M. (2014) The role of plasma membrane aquaporins in regulating the bundle sheath-mesophyll continuum and leaf hydraulics. *Plant Physiol.* **166**, 1609–1620.

Savi, T., Miotto, A., Petruzzellis, F., Losso, A., Pacile, S., Tromba, G., Mayr, S. and Nardini, A. (2017) Drought-induced embolism in stems of sunflower: a comparison of *in vivo* micro-CT observations and destructive hydraulic measurements. *Plant Physiol. Biochem.* **120**, 24–29.

Scholz, F.G., Bucci, S.J., Goldstein, G., Meinzer, F.C., Franco, A.C. and Miralles-Wilhelm, F. (2007) Biophysical properties and functional significance of stem water storage tissues in *Neotropical savanna* trees. *Plant Cell Environ.* **30**, 236–248.

Scholz, F.G., Phillips, N.G., Bucci, S.J., Meinzer, F.C. and Goldstein, G. (2011) Hydraulic capacitance: Biophysics and functional significance of internal water sources in relation to tree size. In *Size- and Age-Related Changes in Tree Structure and Function* (Meinzer, F.C., Lachenbruch, B. and Dawson, T.E., eds). Dordrecht: Springer, Netherlands, pp. 341–361.

Scholz, F.G., Bucci, S.J. and Goldstein, G. (2014) Strong hydraulic segmentation and leaf senescence due to dehydration may trigger die-back in *Nothofagus dombeyi* under severe droughts: a comparison with the co-occurring *Austrocedrus chilensis*. *Trees* **28**, 1475–1487.

Schulte, P.J. (1992) The units of currency for plant water status. *Plant Cell Environ.* **15**, 7–10.

Scoffoni, C. (2015) Modelling the outside-xylem hydraulic conductance: towards a new understanding of leaf water relations. *Plant Cell Environ.* **38**, 4–6.

Scoffoni, C. and Sack, L. (2017) The causes and consequences of leaf hydraulic decline with dehydration. *J. Exp. Bot.* **68**, 4479–4496.

Scoffoni, C., Chatelet, D.S., Pasquet-kok, J., Rawls, M., Donoghue, M.J., Edwards, E.J. and Sack, L. (2016) Hydraulic basis for the evolution of photosynthetic productivity. *Nat. Plants* **2**, 16 072.

Scoffoni, C., Albuquerque, C., Brodersen, C., Townes, S.V., John, G.P., Bartlett, M.K., Buckley, T.N., McElrone, A.J. and Sack, L. (2017) Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant Physiol.* **173**, 1197–1210.

Scoffoni, C., Albuquerque, C., Cochard, H. *et al.* (2018) The causes of leaf hydraulic vulnerability and its influence on gas exchange in *Arabidopsis thaliana*. *Plant Physiol.* **178**, 1584–1601.

Skelton, R.P., Brodribb, T.J., McAdam, S.A.M. and Mitchell, P.J. (2017) Gas exchange recovery following natural drought is rapid unless limited by loss of leaf hydraulic conductance: evidence from an evergreen woodland. *New Phytol.* **215**, 1399–1412.

Song, X. and Barbour, M.M. (2016) Leaf water oxygen isotope measurement by direct equilibration. *New Phytol.* **211**, 1120–1128.

Sorrentino, G., Haworth, M., Wahbi, S., Mahmood, T., Zuomin, S. and Centritto, M. (2016) Abscisic acid induces rapid reductions in mesophyll conductance to carbon dioxide. *PLoS One*, **11**, e0148554.

Sperry, J.S., Meinzer, F.C. and McCulloh, K.A. (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant Cell Environ.* **31**, 632–645.

Steudle, E., Smith, J.A.C. and Lüttge, U. (1980) Water-relation parameters of individual mesophyll cells of the crassulacean acid metabolism plant *Kalanchoë daigremontiana*. *Plant Physiol.* **66**, 1155–1163.

Stratton, L., Goldstein, G. and Meinzer, F.C. (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. *Plant Cell Environ.* **23**, 99–106.

Theroux-Rancourt, G. and Gilbert, M.E. (2016) The light response of mesophyll conductance is controlled by structure across leaf profiles. *Plant Cell Environ.* **40**, 726–740.

Theroux-Rancourt, G., Ethier, G. and Pepin, S. (2014) Threshold response of mesophyll CO_2 conductance to leaf hydraulics in highly transpiring hybrid poplar clones exposed to soil drying. *J. Exp. Bot.* **65**, 741–753.

Trifilo, P., Raimondo, F., Savi, T., Lo Gullo, M.A. and Nardini, A. (2016) The contribution of vascular and extra-vascular water pathways to drought-induced decline of leaf hydraulic conductance. *J. Exp. Bot.* **67**, 5029–5039.

Tyree, M.T. and Ewers, F.W. (1991) The hydraulic architecture of trees and other woody-plants. *New Phytol.* **119**, 345–360.

Tyree, M.T. and Hammel, H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J. Exp. Bot.* **23**, 267–282.

Tyree, M.T. and Jarvis, P.G. (1982) Water in tissues and cells. In *Physiological Plant Ecology II* (Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H., eds). Berlin, Heidelberg: Springer, pp. 35–77.

Tyree, M.T. and Yang, S.D. (1990) Water-storage capacity of thuja, tsuga and acer stems measured by dehydration isotherms - the contribution of capillary water and cavitation. *Planta*, **182**, 420–426.

Tyree, M.T., Snyderman, D.A., Wilmot, T.R. and Machado, J.L. (1991) Water relations and hydraulic architecture of a tropical tree (*Schefflera-Morototoni*) - data, models, and a comparison with 2 temperate species (*Acer-Saccharum* and *Thuja-Occidentalis*). *Plant Physiol.* **96**, 1105–1113.

Venturas, M.D., Sperry, J.S. and Hacke, U.G. (2017) Plant xylem hydraulics: what we understand, current research, and future challenges. *J. Integr. Plant Biol.* **59**, 356–389.

Venturas, M.D., Sperry, J.S., Love, D.M., Frehner, E.H., Allred, M.G., Wang, Y. and Anderegg, W.R.L. (2018) A stomatal control model based on optimization of carbon gain versus hydraulic risk predicts aspen sapling responses to drought. *New Phytol.* **220**(3), 836–850.

Vergeynst, L.L., Dierick, M., Bogaerts, J.A., Cnudde, V. and Steppe, K. (2014) Cavitation: a blessing in disguise? New method to establish vulnerability curves and assess hydraulic capacitance of woody tissues. *Tree Physiol.* **35**, 400–409.

Vitali, M., Cochard, H., Gambino, G., Ponomarenko, A., Perrone, I. and Lovisolo, C. (2016) VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine. *Physiol. Plant.* **158**, 284–296.

Wang, X., Du, T., Huang, J., Peng, S. and Xiong, D. (2018) Leaf hydraulic vulnerability triggers the decline in stomatal and mesophyll conductance during drought in rice (*Oryza sativa*). *J. Exp. Bot.* **69**, 4033–4045.

Xiong, D., Flexas, J., Yu, T., Peng, S. and Huang, J. (2017) Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO_2 in *Oryza*. *New Phytol.* **213**, 572–583.

Xiong, D., Douthe, C. and Flexas, J. (2018) Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. *Plant Cell Environ.* **41**, 436–450.

Zeppel, M.J.B., Lewis, J.D., Phillips, N.G. and Tissue, D.T. (2014) Consequences of nocturnal water loss: a synthesis of regulating factors and implications for capacitance, embolism and use in models. *Tree Physiol.* **34**, 1047–1055.

Zhang, Y.J., Meinzer, F.C., Qi, J.H., Goldstein, G. and Cao, K.F. (2013) Midday stomatal conductance is more related to stem rather than leaf water status in subtropical deciduous and evergreen broadleaf trees. *Plant Cell Environ.* **36**, 149–158.

Zhang, Y.J., Rockwell, F.E., Graham, A.C., Alexander, T. and Holbrook, N.M. (2016) Reversible leaf xylem collapse: a potential "circuit breaker" against cavitation. *Plant Physiol.* **172**, 2261–2274.

Zhu, S.-D., Chen, Y.-J., Ye, Q., He, P.-C., Liu, H., Li, R.-H., Fu, P.-L., Jiang, G.-F. and Cao, K.-F. (2018) Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. *Tree Physiol.* **38**, 658–663.

Zsögön, A., Alves Negrini, A.C., Peres, L.E.P., Nguyen, H.T. and Ball, M.C. (2015) A mutation that eliminates bundle sheath extensions reduces leaf hydraulic conductance, stomatal conductance and assimilation rates in tomato (*Solanum lycopersicum*). *New Phytol.* **205**, 618–626.

Zwieniecki, M.A., Brodribb, T.J. and Holbrook, N.M. (2007) Hydraulic design of leaves: insights from rehydration kinetics. *Plant Cell Environ.* **30**, 910–921.