Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination

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Abstract

Two highly contrasting variables summarizing the efficiency of transport of materials within the leaf are recognized as playing central roles in determining gas exchange and plant performance. This paper summarizes current approaches for the measurement of mesophyll conductance to CO2 ($g_m$) and leaf hydraulic conductance ($K_{leaf}$) and addresses the physiological integration of these parameters. First, the most common methods to determine $g_m$ and $K_{leaf}$ are summarized. Next, novel data compilation is analysed, which indicates that, across diverse species, $g_m$ is strongly linked with gas exchange parameters such as net CO2 assimilation ($A_{area}$) and stomatal conductance ($g_s$), and with $K_{leaf}$, independently of leaf vein length per leaf area. Based on their parallel responses to a number of environmental variables, this review proposes that $g_m$ is linked to the outside-xylem but not to the xylem component of $K_{leaf}$. Further, a mechanistic hypothesis is proposed to explain the interactions among all these and other physiological parameters. Finally, the possibility of estimating $g_m$ based on this hypothesis was tested using a regression analysis and a neurofuzzy logic approach. These approaches enabled the estimation of $g_m$ of given species from $K_{leaf}$ and leaf mass per area, providing a higher predictive power than from either parameter alone. The possibility of estimating $g_m$ from measured $K_{leaf}$ or vice-versa would result in a rapid increase in available data. Studies in which $g_m$, $K_{leaf}$, and leaf mass per area are simultaneously determined are needed in order to confirm and strengthen predictive and explanatory models for these parameters and importantly improve resolution of the integrated hydraulic-stomatal-photosynthetic system.

Key words: Coordination traits, leaf hydraulic conductance, leaf mass area, mesophyll conductance, photosynthesis, vein leaf area.

Introduction

The mesophyll conductance to CO2 assimilation ($g_m$; mol CO2 m$^{-2}$ s$^{-1}$) and the leaf hydraulic conductance ($K_{leaf}$; units mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) are two variables that importantly influence transport of materials within the leaf. These two variables have been increasingly recognized as playing central roles in determining gas exchange rates and plant performance. However, while both traits have independently been the focus of a large number of studies in the past two decades, very few have focused on their coordination. In this work, the aims were 3-fold: (i) to summarize current approaches and point to key resources for the measurement of $g_m$ and $K_{leaf}$; (ii) to present theory and a novel data compilation showing their physiological integration; and (iii) to indicate the importance of frontier research based on quantifying both variables.

Despite the fact that both $g_m$ and $K_{leaf}$ are named as ‘conductances’, $g_m$ and $K_{leaf}$ could not be more different in what they represent. These traits quantify the effectiveness of the transport of different substances, in partially distinct leaf tissues, by different physical processes, and are estimated with completely different measurement techniques. Thus,
$g_m$ represents the conductance of the diffusional pathway of gaseous CO₂ from the substomatal cavity through the mesophyll tissue into the cells and then into the chloroplasts where biochemical assimilation occurs (Flexas et al., 2012a,b). $K_{leaf}$ represents the conductance of the bulk flow pathway of liquid water from the petiole through the xylem throughout the leaf and across the bundle sheath surrounding the veins, through or around cells to the sites of evaporation (Tyree and Zimmermann, 2002; Sack and Holbrook, 2006). $g_m$ is measured using gas exchange systems in combination with other methods, whereas $K_{leaf}$ is typically measured using hydraulic apparatus. While $g_m$ is known to be composed of the conductances of a number of components of the diffusional pathway, including those of the intercellular air spaces ($g_{iac}$), cell walls ($g_{cw}$), and chloroplast outer membrane ($g_{cm}$), among others (Flexas et al., 2012b), in practice it has not yet been directly decomposed into its fractions due to current technical limitations. By contrast, $K_{leaf}$ has been considered in terms of its subcomponents, the conductance of the outside-xylem pathways ($K_{ox}$) (including apoplastic, symplastic, and cell-to-cell pathways for water movement), and the xylem pathways ($K_{x}$), i.e. the pathways inside the veins, both of which are strongly influenced by the vein length per leaf area, $VLA$, also known as ‘vein density’; reviewed in Sack and Scoffoni, 2013a). These parameters—$g_{xm}$, $K_{leaf}$, $K_{ox}$, $K_{x}$, and $VLA$—are those discussed in detail most in this paper, with other physiological and structural traits being briefly defined at their first appearance.

Despite their representing the transport of contrasting substances according to different physics, both $g_m$ and $K_{leaf}$ contribute strongly to the determination of maximum rates of photosynthesis and its dynamics under shifting environmental conditions, and they partly share a mechanistic basis. In reviewing the measurement techniques and analysing what can be derived from their integration, this work promotes a new approach towards clearer resolution of the complete hydraulic-stomatal-photosynthetic system that fuels plant growth. This review also highlights that the concept of ‘optimization’ of water use and carbon gain now needs to consider xylem and outside-xylem flow pathways, and within-leaf gas phase delivery to chloroplasts.

**Current approaches to the measurement of mesophyll conductance ($g_m$)**

There are currently three major approaches commonly used for the estimation of $g_m$, although other methods have been described (see details in Warren, 2006; Pons et al., 2009; Flexas et al., 2012a). These three current approaches are (i) the online carbon isotope discrimination method (i.e. the ‘Evans’ method, after Evans et al., 1986); (ii) the combined chlorophyll fluorescence and gas exchange method (i.e. the ‘Harley’ method, after Harley et al., 1992); and (iii) the curve-fitting method (i.e. the ‘Ether’ method, after Ethier and Livingston, 2004). All three methods have advantages and drawbacks.

The Evans method is based on the idea that the discrimination of Rubisco against $^{13}$C depends on the CO₂ concentration at the carboxylation sites inside the chloroplasts ($C_i$). When using common infra-red gas analysers (IRGAs), the CO₂ concentration in intercellular air spaces ($C_{ic}$) is estimated; thus, one can estimate a ‘theoretical’ discrimination ($\Delta^{13}C$) assuming that $C_{ic}=C_i$. The difference between $\Delta^{13}C$ and the actual measured discrimination ($\Delta^{13}C_{mea}$) can be then used to estimate $C_i$, which enables estimation of $g_m$ as $g_m=A/\Delta^{13}C_{mea}$, where $A_N$ is the net CO₂ assimilation determined with the IRGA. This method requires the use of a mass spectrometer or a tuneable laser diode for the measurement of $\Delta^{13}C$, coupled to an IRGA for the measurement of $A_N$ and $C_i$, and a priori knowledge of carbon isotope discrimination throughout the CO₂ assimilation process: that is, (i) during CO₂ diffusion in air from outside the leaf through stomata into the intercellular air spaces; (ii) during Rubisco and PEPCase carboxylation (and the relative rate of the two reactions); and (iii) during respiration and photorespiration. All these values are considered as constants, which introduces uncertainty in the estimation, because potential differences across species and/or experimental conditions have not yet been fully quantified (Flexas et al., 2012a). Another drawback of this method is that it cannot be used directly under field conditions, although the method may be applied by collecting air from gas exchange chambers in the field into flasks and later performing an ‘off-line’ discrimination analysis in the laboratory, or by using the discrimination of recently synthesized sugars after their extraction in the laboratory (see Flexas et al., 2012a for details). Further, the precision of tuneable laser diodes and mass spectrometers (except dual-inlet configurations) is often too low for the reliable determination of the carbon isotope discrimination of a small area of leaf, and thus larger-than-typical gas exchange cuvettes are generally required (Pons et al., 2009; Flexas et al., 2012a). The main advantage of this method is that it is the only one based on combining information from two completely independent measurements (gas exchange and carbon isotopic discrimination) determined from the exact same photosynthetic tissue.

The Harley method is based on the assumption that all the energy and reducing power generated by the electron transport reactions in the chloroplast thylakoid membranes is used to fuel either photosynthetic carboxylation or oxygenation (i.e. photorespiration), while their use in other processes (e.g. photosynthetic nitrate reduction, Mehler reaction) is negligible. The $g_m$ can thus be estimated from measurements of the rate of electron transport (ETR) using a chlorophyll fluorimeter, and measurements of the $A_N$ and the rate of day respiration using an IRGA. The ETR is used to estimate the gross photosynthetic rate, and hence the net CO₂ assimilation can be used to estimate the fractions of thylakoid energy used in carboxylation and oxygenation, and then the $C_i$ can be estimated using a priori knowledge of the Rubisco specificity factor or the CO₂ photocompensation point (F*) and other factors that may be obtained using independent methods (further details can be found in Warren, 2006; Pons et al., 2009; and Flexas et al., 2012a). A main drawback of this method is that ETR is estimated from the fluorescence of the top cell layers of the leaf, while photosynthetic gas exchange is measured from the bulk leaf enclosed in the IRGA cuvette. Thus,
basing a $g_m$ estimate on these two mismatched measurements can introduce uncertainty, especially in thick leaves. Another drawback of this approach is the requirement of many additional measurements for the estimation of $g_m$, such as leaf absorbance (required for accurate estimation of ETR), day respiration, and $\Gamma^*$. The main advantage of this method is that it is easier to use under field conditions, while still combining two independent measurements for the estimation of $g_m$ (Pons et al., 2009; Flexas et al., 2012a).

Finally, the Ethier method is based on the idea that a finite $g_m$ increases the curvature of the graph plotted of the measured response of $A_i$ to $C_i$ (i.e. the $A-C_i$ curve). Using a specifically designed curve-fitting based on the Farquhar et al. (1980) model of photosynthesis, one can estimate an average $g_m$ in addition to the standard parameters yielded from $A-C_i$ curve analysis (i.e. the maximum velocities of carboxylation and electron transport; respectively $V_{\text{cmax}}$ and $J_{\text{max}}$). The obvious advantage of this method is that it only requires the use of an IRGA. However, a drawback of this method, as for the Evans method, is the need for a priori knowledge of $\Gamma^*$ and other Rubisco kinetics and their temperature responses, all of which are typically treated as constants based on the values reported for tobacco by Bernacchi et al. (2002), thus not accounting for potential differences across species and/or experimental conditions. The other major drawback of this method is its statistical extraction of values for at least three unknowns ($g_m$, $V_{\text{cmax}}$ and $J_{\text{max}}$) from a single curve-fitting exercise, which it is often statistically weak, unless one has exceptionally clean and smooth $A-C_i$ curves with a large number of points or $C_i$ intervals.

Because all three methods present a number of drawbacks and require many assumptions that are often not fully tested, several have recommended the use of at least two methods simultaneously to report differences in $g_m$ among species or treatments (Pons et al., 2009; Flexas et al., 2012a).

**Current approaches to the measurement of leaf hydraulic conductance ($K_{\text{leaf}}$)**

The leaf hydraulic conductance ($K_{\text{leaf}}$) is determined as the ratio of the water flow rate through the leaf to the water potential gradient driving force for water movement across the leaf. The $K_{\text{leaf}}$ summarizes the behaviour of a complex system: water moves through the petiole and through several orders of veins, exits into the bundle sheath and passes through (via symplastic or cell-to-cell pathways) or around (via apoplastic pathway) mesophyll cells before evaporating into the airspace and being transpired from the stomata (Sack and Tyree, 2005; Sack and Holbrook, 2006). The $K_{\text{leaf}}$ has been quantified for over 20 years, with a dramatic increase since the late 1990s, and a number of different approaches have been used based on different techniques. Currently, most researchers measure $K_{\text{leaf}}$ for excised leaves, typically using one of four methods: (i) the evaporative flux method (EFM); (ii) the rehydration kinetics methods (RKM); (iii) the high pressure flowmeter (HPFM); and (iv) the vacuum pump method (VPM). A minority of researchers use (v) *in vivo* methods for estimating $K_{\text{leaf}}$. All these methods have advantages and drawbacks in terms of logistics, field portability, biological realism, and precision.

The EFM is based on driving transpiration ($E$) for a leaf with petiole attached to a potometer, and when flow (and leaf water potential, $\Psi_{\text{leaf}}$) reaches a steady state, subsequently determining the water potential driving force across the leaf ($\Delta \Psi_{\text{leaf}}$), with $K_{\text{leaf}}=E/\Delta \Psi_{\text{leaf}}$. Resources for describing EFM measurement include Sack et al. (2002), Brodribb et al. (2007), Scoffoni et al. (2008, 2012), Guyot et al. (2012), and a recent movie (Sack and Scoffoni, 2013b). A drawback of the EFM is its relying on estimating the water potential driving force for water movement from equilibrated, non-transpiring leaf tissue, using the pressure chamber (or another technique, for example psychrometry) which introduces some uncertainty, as the true driving force during transpiration may be stronger, by an amount that depends on where in the leaf the water is evaporating and the precise transpiration pathways, which are largely unknown (reviewed by Sack et al., 2002; Sack and Tyree, 2005; Sack and Holbrook, 2006; Guyot et al., 2012; Scoffoni et al., 2012). The main advantages of the EFM are its simplicity, practicality, and realism, as the transpiration of the excised leaf is just as *in vivo*, driven by the vapour pressure deficit (VPD) of the ambient air. Additional advantages are the ability to manipulate light, and potentially temperature and humidity around the leaf, and also to estimate stomatal conductance ($g_s$) during the measurement (Guyot et al., 2012; Sack and Scoffoni, 2013b). A measurement can be made in 30–60 min.

The RKM is based on estimating the $K_{\text{leaf}}$ during the uptake of water into a partially dehydrated leaf. In an early version of this method, $K_{\text{leaf}}$ was estimated from (i) the initial $\Psi_{\text{leaf}}$ determined from equilibrated adjacent leaves, (ii) the final $\Psi_{\text{leaf}}$ after rehydration, (iii) the rehydration time, and (iv) the water uptake during rehydration, which was in turn estimated from the water potential measurements, and the leaf water storage capacitance estimated from pressure volume curves; resources for that measurement include Brodribb and Holbrook (2003) and Brodribb et al. (2005). A more recent, refined version of the RKM, the ‘Dynamic-RKM’, involves directly measuring the water uptake during rehydration, rather than estimating this based on the capacitance. Resources for this measurement include Brodribb and Cochard (2009), Blackman and Brodribb (2011), and Brodribb et al. (2011). A drawback of either version of the RKM is its relying on estimating the water potential driving force for rehydration from equilibrated leaf tissue which introduces some uncertainty, as the true driving force during rehydration may be stronger. Further drawbacks include uncertainty in some of the inputs into the calculation, such as noisiness in the data arising from determining initial and final $\Psi_{\text{leaf}}$ from different, albeit approximately equilibrated leaves and the precise rehydration time (since the leaf mesophyll may continue to rehydrate even after the petiole is removed from water). Additional drawbacks of this approach include the uncertainty of the flow pathways during rehydration and the degree that these pathways mimic those of transpiration. The advantage of this measurement is its rapidity, as measurements can be made within minutes.
The HPFM typically involves forcing water through a system of tubing including a high-resistance segment of tubing placed between two pressure transducers, through the petiole, into a leaf until it leaks from the stomata and a steady state flow is established. The \( K_{\text{leaf}} \) is measured using the known pressure applied and the drop in pressure in the high-resistance tubing relative to that across the leaf (resources include Yang and Tyree, 1994; Sack et al., 2002; Tyree et al., 2005). Drawbacks include the inability to measure partially dehydrated leaves, which is often desirable to determine the dynamics of \( K_{\text{leaf}} \) with dehydration. Further, this measurement approach involves artificial conditions arising in the leaf, including the flooding of mesophyll and forcing of water through the airspaces out of the stomata. The question has been raised of whether stomatal closure might influence the measurement (Sack et al., 2002; Rockwell et al., 2011) but detailed tests indicated that this was a negligible factor (Sack et al., 2002; Tyree et al., 2005; Cochard et al., 2007; Nardini et al., 2010). Rockwell et al. (2011) also hypothesized that the flooding of the mesophyll would possibly reduce the \( K_{\text{leaf}} \) but data have not been conclusive. A major advantage of this method is the ease of controlling light and/or temperature, as the leaf can be maintained submerged in a temperature-controlled water bath. A measurement can be made in 30–60 min. Several modified versions of this approach have been developed using the pressure chamber to drive flow, either by forcing water out of the leaf lamina cells through the petiole protruding from the chamber (Franks, 2006), or by submerging a shoot underwater in the pressure chamber and forcing water through the stomata into the mesophyll, and thereby into the xylem and eventually out of the cut shoot protruding from the chamber (Postaire et al., 2010; Prado et al., 2013).

The VPM is based on drawing water out of the leaf using subatmospheric pressures and determining \( K_{\text{leaf}} \) as the slope of flow rate against pressure (Martre et al., 2001; Nardini et al., 2001; Sack et al., 2002). Drawbacks of this method are lack of realism in subatmospheric pressure as a driving force for water flow within the leaf airspaces, a lack of field portability and lengthy measurement times (up to 2 h per leaf). Further, some leaves show non-linearities of flow against pressure, implying changes in flow pathways within the leaf, or damaged cells (e.g. Martre et al., 2001). Notably, while the HPFM and VPM are least realistic and have drawbacks, a major advantage of these methods is the ability to calculate \( K_{\text{leaf}} \) directly, rather than as a ratio of two separate measurements as occurs for EFM and dynamic RKM (i.e. from \( E \) and \( \Delta \Psi \)). Consequently, the data tend to be less noisy and fewer replicates are often necessary (e.g. 5–6 for HPFM or VPM; whereas often >10 are required for EFM and RKM). Thus, if few leaves are available for measurement, e.g. due to species rarity or complex experimental treatments, then these methods can be especially attractive.

\textit{In vivo} measurements of \( K_{\text{leaf}} \) have been made (i.e. leaf transpiration (\( E \)) determined while still attached to the plant). The \( E \) can be estimated from sapflow methods or gas exchange measurements, and the water potential driving force estimated as the difference between bagged and unbagged leaf water potential (reviewed in Sack and Tyree, 2005). \textit{In vivo} measurements can be subject to instability, without clear steady transpiration, and thus are frequently noisy. Further, these methods do not allow the control of environmental conditions as for methods applied to excised leaves. A major challenge for ongoing methods development is an \textit{in vivo} method that is accurate and reliable, which would enable the characterization of \( K_{\text{leaf}} \) dynamics in real time and \textit{in vivo}, rather than on excised leaves (cf. Scoffoni et al., 2012).

### The integration of mesophyll conductance and leaf hydraulic conductance

**Meta-analysis of the coordination of \( g_m \) and \( K_{\text{leaf}} \)**

The \( g_m \) and \( K_{\text{leaf}} \) might be expected to be coordinated, due to their both being critically involved in leaf function and determinants of photosynthetic rate, and further they may depend in part on some shared structural and/or physiological traits, leading to a partially integrated mechanistic basis. Thus, Griffiths and Helliker (2013) suggested that water transport efficiency, as determined by hydraulic supply and regulation by stomata, might influence \( g_m \), but the question has not been investigated directly with data. Using a newly compiled dataset for diverse species (Supplementary Table S1, available at JXB online) for \( g_m \), \( K_{\text{leaf}} \), stomatal conductance (\( g_s \)), and light-saturated photosynthetic rate, the current work found novel trends for a coordination among these traits on both a leaf area- and a leaf mass-normalized basis (Fig. 2A–E). In this dataset was found the coordination of \( g_m \), \( g_s \), and photosynthetic rate per leaf area (\( A_{\text{area}} \)) previously reported in a number of recent papers (Fig. 1A and B; see Flexas et al., 2002, 2004, 2012b, 2013). There was also a strong correlation of \( A_{\text{area}} \) with \( K_{\text{leaf}} \) (Fig. 2A and C), as has been previously reported across species (Brodribb and Holbrook, 2006; Franks, 2006; Walls, 2011). Only a non-significant empirical trend was found between \( K_{\text{leaf}} \) and \( g_s \) in these data, although such a relationship has been reported previously (Franks, 2006). The current work also discovered a correlation of \( g_m \) with \( K_{\text{leaf}} \) with across diverse species. A previous heuristic indication that a relationship of \( g_m \) with \( K_{\text{leaf}} \) might exist was provided by Flexas et al. (2012b), who had found a correlation across diverse species of \( g_m \) with hydraulic conductances of various organs, for related species within the same genus or family, when excluding a number of outliers. This new analysis shows that this trend holds strongly across diverse dicot species. These trends were all strengthened by placing traits on a leaf-mass basis (Fig. 2D–F; see following section for additional discussion of area- and mass-based normalization).

Implications of the coordination of \( K_{\text{leaf}} \) with \( g_m \) exist at multiple scales. The correlation of \( K_{\text{leaf}} \) with \( g_m \) but not significantly with \( g_s \), in this work’s dataset implies a shared mechanistic basis for trait coordination (as will be discussed). The typical explanation for the correlation of \( K_{\text{leaf}} \) with \( g_s \) and \( A_{\text{area}} \) across species is based on optimal hydraulic design, such that plants should have a \( K_{\text{leaf}} \) that matches their \( g_s \) to match hydraulic supply with demand, when operating within a narrow range of soil water potential, leaf water potential,
and VPD (to be explained further). However, across species adapted to varying environments, such correlation of $K_{\text{leaf}}$ with $g_s$ would be expected to be weak. The correlation of $K_{\text{leaf}}$ with $g_m$ across species implies an additional co-selection of high $g_m$ to enhance greater CO₂ assimilation to optimize performance for species with high $K_{\text{leaf}}$ and $g_s$ to enable higher rates of gas exchange. This finding suggests that, while historically it has been thought that water use and assimilation are coordinated within the plant due simply to their co-regulation by stomata, that the concept of ‘optimization’ of water use and carbon gain now needs to consider xylem and outside-xylem flow pathways, and within-leaf gas phase delivery to chloroplasts. Indeed, this correlation of $K_{\text{leaf}}$ and $g_m$ indicates the possibility of partial overlap in their mechanistic basis (i.e. due to their both depending on mesophyll structure and physiology (Sack and Scoffoni, 2013a; to be discussed further). Thus, in these data, it appeared that $K_{\text{ox}}$ was a major influence on $K_{\text{leaf}}$ and independent of $VLA$, and that $K_{\text{leaf}}$ correlated with $g_m$ due to their influence by a common factor, and that this contributed in part to the correlation of $K_{\text{leaf}}$ with $A_{\text{area}}$, a point to be further discussed.

**Coordination on a leaf-area versus leaf-mass basis**

Traditionally, gas exchange variables, $g_m$ included, have been normalized by dividing by leaf area but also often expressed on a per leaf-mass basis by dividing the area-normalized rates by leaf mass per area ($LMA$). By contrast, hydraulic supply to leaves has been traditionally only expressed on a leaf-area basis; however, recent studies have begun also to normalize $K_{\text{leaf}}$ on a leaf-mass basis ($K_{\text{leaf, mass}}$; Niinemets and Sack, 2006; Nardini et al., 2012; Simonin et al., 2012). Recent work has demonstrated that some trait correlations, and in particular those among ‘leaf economics’ variables expressed on a mass basis, such as photosynthetic rate per leaf mass ($A_{\text{mass}}$), nitrogen concentration per leaf mass ($N_{\text{mass}}$), and $LMA$, arise in part automatically, or by statistical necessity, given their basis of expression per unit leaf area or per unit leaf dry mass (Lloyd et al., 2013; Osnas et al., 2013). Thus, correlations among mass-based traits and $LMA$ may result when mass-based traits are determined by dividing area-based traits by $LMA$ (e.g. $K_{\text{leaf, mass}} = K_{\text{leaf}} / LMA$). Similarly, correlations among area-based traits and $LMA$ may result when area-based traits are determined by multiplying mass-based traits by $LMA$ (e.g. $N_{\text{area}} = N_{\text{mass}} \times LMA$) (Lloyd et al., 2013; Osnas et al., 2013; Sack et al., 2013). This review follows Sack et al. (2013) in referring to such correlations as ‘innate correlations’. These linkages may arise due to the calculation of variables, but still represent physical phenomena important to plant function. Leaves built...
of cells with thicker cell walls will tend to have higher LMA and to have ‘diluted’ physiological rates and conductances that are expressed on a mass basis, and thus lower $A_{mass}$ and $K_{leaf,mass}$. The resulting negative correlations of $K_{leaf,mass}$ and $A_{mass}$ with LMA relevant to leaf economic performance: leaves with high LMA will yield less return in hydraulic conductance and photosynthetic rate over short timescales but have benefits over long leaf lifetimes (Westoby et al., 2013). Thus, even if they arise in part innately, relationships among mass-based traits are considered by most to represent mechanistic trait linkages with ecological significance, especially given that mass-based traits scale up most directly to influencing whole-plant relative growth rate (to be discussed). Thus, the correlation of $K_{leaf}$ with $g_m$ on a mass basis (Fig. 2D–F) indicates a coordination that would scale up to influencing whole plant carbon budgets.

**Common dynamic responses of $g_m$ and $K_{leaf}$**

The previous section showed the coordination of steady-state, maximum values of $g_m$ and $K_{leaf}$ (i.e. under non-stress conditions). However, both parameters are dynamic at a
range of time scales from minutes to hours to days to seasons to years (Sack and Holbrook, 2006; Flexas et al., 2007, 2008). The short-term dynamics (seconds to hours) of both variables have attracted increasing attention because of their likely ability to influence instantaneous and daily means for water use and photosynthetic carbon gain.

Indeed, $g_m$ and $K_{leaf}$ show responses which are similar in direction and extent to a number of environmental variables (Table 1). In response to increased leaf temperature, $g_m$ increases to an optimum, depending on the species and acclimation conditions, and then decreases thereafter (Bernacchi et al., 2002; Warren and Dreyer, 2006; Yamori et al., 2006; Warren, 2008a), although the latter decrease has been debated (Evans and von Caemmerer, 2013). The $g_m$ also shows a longer-term acclimation, i.e. for plants growing and producing leaves under different temperature regimes (Yamori et al., 2006; Bunce, 2008; Flexas et al., 2008; Warren, 2008a). The $K_{leaf}$ also increases in response to temperature, both due to the increase in viscosity of water and to the greater permeability of membranes in the outside-xylem pathways (Sack et al., 2004). Few studies have analysed the short-term dynamics of $g_m$ in response to irradiance, yet most of them except Tazoe et al. (2009) have found a positive correlation (Gorton et al., 2003; Flexas et al., 2007; Doutte et al., 2011), and $g_m$ seems to be modulated by light quality as well (Loreto et al., 2009); further, shade leaves have lower $g_m$ than sun leaves (Hanba et al., 2002; Piel et al., 2002; Warren et al., 2007; Monti et al., 2009). These responses are similar in direction to those of $K_{leaf}$, which in a number of species increases in the short-term with irradiance, and depends on light quality, and increases over the long term during acclimation to sun versus shade (e.g. Sack et al., 2002, 2003; Tyree et al., 2005; Cochard et al., 2007; Scoffoni et al., 2008; Brodribb and Jordan, 2011; Sellin et al., 2011).

Of all environmental conditions, the influence of water stress (from short-term leaf dehydration to long-term drought or exposure to salinity) is by far the one for which most reports on the dynamics of $g_m$ and $K_{leaf}$ have been published. Regardless of the velocity of water stress imposition, $g_m$ strongly decreases in response to water stress in a wide range of species (e.g. Bongi and Loreto, 1989; Rouspard et al., 1996; Flexas et al., 2002, 2006a, 2009; Diaz-Espejo et al., 2007; Galmés et al., 2007; Perez-Martin et al., 2009; Misson et al., 2010; Galle et al., 2011). Similarly, the strong decline of $K_{leaf}$ with dehydration has been studied in over 100 species (e.g. Nardini et al., 2001; Brodribb and Holbrook, 2003; Scoffoni et al., 2012). The bulk of studies show a strong decline of $g_m$ with leaf dehydration; only a few studies have reported minor effects on $g_m$ (e.g. Warren et al., 2011). Similarly, the $K_{leaf}$ declines with water stress in all species, but the steepness and shape of this decline varies across species, with more drought-sensitive species experiencing stronger declines at mild dehydration (Scoffoni et al., 2012).

However, based on the data available, the responses of $g_m$ to CO$_2$ and VPD do not parallel those of $K_{leaf}$. A large number of studies using different species and methods to estimate $g_m$ have observed that low CO$_2$ increases $g_m$ (Centritto et al., 2003), and rapidly increasing CO$_2$ results in strong decreases of $g_m$ (Düring, 2003; Flexas et al., 2007; Hassiotou et al.,

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**Fig. 3.** Independence of (A) mesophyll conductance, (B) leaf hydraulic conductance, (C) stomatal conductance, and (D) maximum assimilation rate per area ($A_{max}$) from leaf vein length per area across diverse species in a compiled database. Gymnosperms (grey circles; $n=3$ in A, C, and D), monocots (open circles; $n=3$ in A, C, and D), and dicots (filled circles; $n=31, 14, 30,$ and $31$ in A, B, C, and D, respectively) are represented. Correlation coefficient and significance were obtained from standard major axes fitted through the dicot dataset only. NS, $P>0.05$. 

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Table 1. Coordinated dynamics of leaf hydraulic and mesophyll conductance with short-term changes water status, irradiance, vapour pressure deficit (VPD), temperature, and CO₂

<table>
<thead>
<tr>
<th>Resulting response of $g_m$</th>
<th>Resulting response of $K_{leaf}$</th>
<th>Example references</th>
<th>Example references</th>
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<tbody>
<tr>
<td>Declining water status</td>
<td>Decrease, 0–100%</td>
<td>Decrease, up to 100%</td>
<td>Nardini et al. (2001); Brodribb and Holbrook (2003); Blackman et al. (2010); Scoffoni et al. (2012)</td>
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<tr>
<td>Increasing light</td>
<td>Increase, 0–250%</td>
<td>Increase, 0–367%</td>
<td>Sack et al. (2002); Tyree et al. (2005); Coehard et al. (2007); Scoffoni et al. (2008)</td>
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<td>Increasing VPD</td>
<td>Decrease, 0–60%</td>
<td>Increase, up to 401%</td>
<td>Levin et al. (2007)</td>
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<tr>
<td>Increasing temperature</td>
<td>Increase, 0–400%</td>
<td>Increase, up to 175%</td>
<td>Sack et al. (2004)</td>
</tr>
<tr>
<td>Increasing CO₂</td>
<td>Decrease, 0–90%</td>
<td>=</td>
<td>Bunce (2006)</td>
</tr>
</tbody>
</table>

$^a$The $g_m$ scaled with temperature with a slope of 5% per degree Celsius (Evans and von Caemmerer, 2013).

2009; Vrábl et al., 2009; Yin et al., 2009; Bunce, 2010; Douthe et al., 2011). Only one study in wheat showed no response of $g_m$ to increased CO₂ in the short term (Tazoe et al., 2009). In the long term, Singsaas et al. (2003) reported decreased $g_m$ with higher CO₂ only in some species and not in many others. Velikova et al. (2009) showed in Platane orientalis that new leaves emerged when plants were already growing at high CO₂ showed much larger decreases of $g_m$ than pre-existing leaves. Salazar-Parra et al. (2012) also showed decreased $g_m$ with long-term high CO₂ in grapevines. By contrast, recent studies have found no effect of high CO₂ on $K_{leaf}$ in soybean (Bunce, 2006; Locke et al., 2013). More studies will be needed to confirm such a lack of response in $K_{leaf}$ to CO₂.

The response of $g_m$ to VPD is less certain. Under a sudden increase in VPD, Bongi and Loreto (1989) observed a decreased $g_m$ in Olea europaea, whereas Warren (2008b) did not observe any response in several species, including Eucalyptus regnans, Phasolus vulgaris, and Solanum hycoperscium. Perez-Martin et al. (2009) observed longer-term acclimation of $g_m$ to VPD in Olea europaea and Vitis vinifera. One study has shown an increase in $K_{leaf}$ in response to VPD (Levin et al., 2007).

Thus, the similar responses of $g_m$ and $K_{leaf}$ to several environmental factors—notably, temperature, light, and leaf water status—provide another line of evidence for their mechanistic coordination and integrated influence on leaf function under dynamic conditions.

Mechanistic basis for the coordination: the outside-xylem tissues

Previous work on the coordination of $K_{leaf}$ with photosynthetic rate per leaf area ($A_{area}$) has emphasized the influence of $K_{leaf}$ on stomatal conductance ($g_s$), mediated by leaf water potential (Sack and Holbrook, 2006; Holloway-Phillips and Brodribb, 2011). However, this work’s novel finding of a coordination of $g_m$ and $K_{leaf}$ in the compiled data, in which $g_m$ was uncorrelated with $K_{leaf}$, implies that the coordination of $A_{area}$ with $K_{leaf}$ is due to more than simply the scaling of hydraulic and stomatal conductances. It is posited that properties of the outside xylem pathways which influence $K_{leaf}$ and $g_m$ in common underlie this coordination (see network diagram in Fig. 4). Based on a previously developed conceptual model for the coordination of leaf traits (Sack and Scoffoni, 2013a; Sack et al., 2013) synthesized from previous studies of a wide range of species sets, the common basis of $K_{leaf}$ and $g_m$ can be clarified. In brief, $K_{leaf}$ and its components, the vein xylem hydraulic conductance ($K_v$) and the outside-xylem hydraulic conductance ($K_{ox}$), are influenced by a number of leaf vein traits (Coehard et al., 2004; Sack et al., 2004, 2005; McKown et al., 2010; Sack and Scoffoni, 2013b):

$$K_{leaf} = (K_v^{-1} + K_{ox}^{-1})^{-1}$$

(1)

The $K_v$ is influenced by the vein cross-sectional conductivity of each vein order, which is in turn influenced by the numbers and dimensions of the xylem cells. Further, $K_v$ and $K_{ox}$ depend on the VLA and the number and size of free-ending veins (FEVs), and the sizes, numbers, and permeability of the bundle sheath and bundle sheath extensions (BS and BSEs) along with the mesophyll water flow pathways (MPs). Additional vein traits influence the sensitivity of $K_v$ to xylem embolism: the major vein length per area (major VLA) and the topology of the vein system. These aspects of the vein
system influence the abundance of redundant high conductivity pathways around embolized xylem conduits.

Because the leaf is an important hydraulic limitation in the plant, and $K_{\text{leaf}}$ scales with the whole plant hydraulic conductance ($K_{\text{plant}}$) (Sack et al., 2003; Sack and Holbrook, 2006), vein traits scale up to influencing $K_{\text{plant}}$ by the Ohm’s law analogy, leaf water potential ($\Psi_{\text{leaf}}$) at a given transpiration rate ($E$):

$$\Delta \Psi_{\text{leaf-to-soil}} = E / K_{\text{plant}}$$

$$\Psi_{\text{leaf}} = E / K_{\text{plant}} + \Psi_{\text{soil}}$$

where $\Delta \Psi_{\text{leaf-to-soil}}$ is the water potential gradient between leaf and soil, and $\Psi_{\text{soil}}$ is the soil water potential. Coordination with stomatal conductance arises because

$$E = g \times \text{VPD}$$

where VPD is the vapour pressure deficit, and $g$ is leaf conductance to water vapour, primarily driven by the stomatal conductance ($g_s$) under wind of sufficient speed that the boundary layer conductance ($g_b$) is not limiting.

$$\Psi_{\text{leaf}} = (g_s \times \text{VPD}) / K_{\text{plant}} + \Psi_{\text{soil}}$$
Given these relationships, in many cases across species, \( g_s \) must be coordinated with \( K_{\text{plant}} \) (i.e. matching hydraulic demand with supply; Tyree and Zimmermann, 2002; Sack et al., 2005; Sack and Holbrook, 2006). That would be the case when \( \Psi_{\text{leaf}} \) varies only within narrow limits at a given range of VPD and \( \Psi_{\text{soil}} \). However, for plants under substantially different soil moisture or VPD regimes, the coordination of \( g_s \) and \( K_{\text{plant}} \) may shift (a point to be further discussed). For a given species, the \( g_s \) is a function of the maximum stomatal conductance \( g_{\text{max}} \) and the degree that stomatal closure declines as the leaf dehydrates to a given \( \Psi_{\text{leaf}} \). The \( g_{\text{max}} \) is a function of stomatal dimensions and numbers (quantified as stomatal size, density, index, and pore area index; Sack et al., 2003; Franks and Farquhar, 2007).

Ultimately, the photosynthetic rate per leaf area \( (A_{\text{area}}) \) is importantly controlled by this hydraulic-stomatal coordination. The \( A_{\text{area}} \) is determined by the chloroplastic CO₂ concentration \( (C_p) \) and biochemical parameters including the electron transport rate \( (J_{\text{max}}) \) and the maximum rate of carboxylation \( (V_{\text{cmax}}) \), while \( C_p \) is depends on the ambient CO₂ concentration \( (C_a) \), \( g_s \), and mesophyll conductance \( (g_{\text{m}}; \text{Farquhar et al., 2001; Flexas et al., 2012b}) \). The \( J_{\text{max}} \) and \( V_{\text{cmax}} \) depend on the activity of light and carbon reactions per leaf area, and would relate to leaf nitrogen concentration per area \( (N_{\text{area}}) \), as would the respiration rate \( (R_{\text{area}}) \) (Evans et al., 2000).

The \( K_{\text{c}} \) and \( K_{\text{m}} \) are dynamic, like \( K_{\text{soil}} \), and the hydraulic conductances elsewhere in the plant. All are sensitive to declining water potentials \( (\Psi) \) throughout the plant system, resulting from embolism in roots, stems, or leaves, tissue collapse, and/or biochemical changes. Further, declining water potential in or around the guard cells drive \( g_s \) decline (Hubbard et al., 2001; Guyot et al., 2012; Scoffoni et al., 2012). The susceptibilities of different tissues will thus scale up to impacting on \( K_{\text{soil}} \) and gas exchange.

Other leaf traits also influence higher-level function. The \( \text{LMA} \) (which itself is equal to the product of leaf thickness and density; \( \text{LT} \) and \( \text{LD} \) respectively), is typically negatively correlated with mass-based nitrogen concentration and rates of respiration and photosynthesis \( (N_{\text{mass}}, R_{\text{mass}} \text{ and } A_{\text{mass}} \text{ respectively}) \) (see previous discussion). A higher \( \text{LMA} \) typically corresponds with a greater leaf lifespan (LL; Wright et al., 2004).

Vein traits can thus influence whole-plant maximum relative growth rate \( (R_{\text{GRmax}}) \) (Evans, 1972; Poorter et al., 2009). The \( A_{\text{area}} \) is the major driver of the dry mass accumulation per leaf area (unit leaf rate, \( \text{ULR} \)). The \( R_{\text{GR}} \) is determined as the product of \( \text{ULR} \) and the leaf area ratio \( (LAR = \text{leaf area/plant mass}) \); the \( LAR \) itself is equal to the leaf mass fraction \( (L_{\text{MF}} = \text{leaf mass/plant mass}) \) divided by \( \text{LMA} \) plant maximum relative growth rate \( (R_{\text{GRmax}}) \) (Evans, 1972; Poorter et al., 2009).

The framework we have described for the potential relationships among vein traits, hydraulic traits and gas exchange traits is expected to arise clearly for some but not for all species sets. As explained above, the correlations among traits that would emerge due to equation 4 will do so only when species vary narrowly in their operating \( \Psi_{\text{leaf}}, \Psi_{\text{soil}}, \) and VPD. Thus, the particular coordination among traits in this framework will differ according to the habitat or environment of the species compared (Sack et al., 2005, 2013; Feild et al., 2011). Additionally, when traits are determined by two or more traits, the importance of those traits will depend on the species set. For example, because \( A_{\text{area}} \) depends on \( g_s \) and \( g_{\text{m}} \) and both are potentially coordinated with \( K_{\text{soil}} \), but differently depending on the species and its habitat, the coordination of \( A_{\text{area}} \) with \( g_{\text{m}} \) and with venation traits will differ among species sets, as expected according to the framework relating vein traits to gas exchange and whole-plant growth. Recent work has also indicated that higher \( \text{VLA} \) can also enable an increased phloem transport capacity that can also drive higher \( A_{\text{area}} \) (Fu et al., 2011; Nikinmaa et al., 2012; Sack and Scoffoni, 2013b). The \( A_{\text{area}} \) may also be influenced by nutrient delivery rates, which also may depend on vein traits (Shabala et al., 2002; Kerton et al., 2009; Gilliham et al., 2011), even in mature leaves which need to replace degraded proteins and pigments (Girardin et al., 1985; Niinemets et al., 2004).

Based on the coordination observed between \( g_{\text{m}}, K_{\text{leaf}} \), and \( A_{\text{area}} \) across diverse species and their partial similarity in responses to environmental conditions, this work hypothesizes that mesophyll structural and physiological traits that affect \( g_{\text{m}} \) and photosynthetic processes also have an influence on leaf hydraulic capacity. In principle, both \( K_{\text{m}} \) and \( g_{\text{m}} \) may depend on aquaporin activity (Ferrio et al., 2012). The mechanisms for coordinated dynamics of \( g_{\text{m}} \) and \( K_{\text{leaf}} \) remain to be clarified (Flexas et al., 2012b; Griffiths and Helliker, 2013). Further, the partially distinct dynamics of these traits (e.g. CO₂ response of \( g_{\text{m}} \) but not of \( K_{\text{leaf}} \)) are consistent with the partially independent pathways for the movement of water and CO₂ inside leaves. For instance, it is well known that water moves in leaves through xylem conduits within the veins and, upon exiting the bundle sheath, water moves through the mesophyll via apoplastic, symplastic, and/or cell-to-cell pathways (Sack and Holbrook, 2006). The \( g_{\text{m}} \) might be related only to the non-xylem component of \( K_{\text{leaf}} \). The question of whether CO₂ importantly moves through apoplastic, symplastic, or transmembrane cell-to-cell pathways has never been addressed, but it is probable that, in general, for \( C_3 \) plants, CO₂ diffuses simply from intercellular air spaces directly into each photosynthesizing mesophyll cell which acts as a final sink. If so, the ‘symplastic’ pathway for CO₂ does not exist, and then the ‘apoplastic’ and ‘transmembrane’ pathways are components of the diffusion into single cells. Thus, a CO₂ molecule from the intracellular air spaces has to first pass the apoplast/cell wall then across the cell membrane, the cytosol, and the chloroplast outer membrane to reach the site of carboxylation in the chloroplast matrix. These components are all potentially quantitatively important. In fact, cell-wall thickness has been found to represent a strong limitation on \( g_{\text{m}} \) (Peguero-Pina et al., 2012; Scafarò et al., 2011; Tosens et al., 2012; Tomás et al., 2013). Further, membrane aquaporins—which are known to facilitate transmembrane water but also CO₂ transport (Tyerman et al., 2002; Kaldenhoff et al., 2008)—also induce modifications of \( g_{\text{m}} \) (Hanba et al., 2004; Flexas et al., 2006b; Uehlein et al., 2008; Heckwolf et al., 2011).

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Thus, $g_m$ and $K_{ox}$ are both likely to be influenced in common by cell-wall and membrane properties that would affect the apoplastic and transmembrane pathways, respectively. Such overlap in mechanistic basis would explain the correlation between $g_m$ and $K_{leaf}$ (Fig. 1) and their parallel dynamics in response to changes in irradiance and water supply which might affect a wide range of leaf cells and/or aquaporins. However, different mechanisms operating at a range of scales might affect a wide range of leaf cells and/or aquaporins. However, different mechanisms operating at a range of scales might affect a wide range of leaf cells and/or aquaporins. Thus, studies in which $g_m$ and $K_{ox}$ are inter-related (Fig. 4) suggests that simple two parameter relationships as those shown in Fig. 1 may not capture the true co-determination among these two parameters and others. Biological processes are typically highly complex, dynamic and non-linear. Thus, artificial intelligence technology can be a useful mathematical approach to decipher and identify complex non-linear interactions between parameters controlling a process (Prasad and Dutta Gupta, 2008; Gago et al., 2010). Here, to develop predictive equations, the current employed neurofuzzy logic technology, a hybrid technology based on artificial neural networks (ANNs) combined with fuzzy logic, to retrieve and integrate knowledge hidden within the compiled dataset for the 17 species with known values for $K_{leaf}$, LMA, and $g_m$. This technology was described previously as a useful data mining tool to promote the understanding of complex processes in plant science (Gago et al., 2011). The current work used FormRules version 3.31 (2008; Intelligensys, UK), a commercial neurofuzzy logic software, implementing the ASMOD (Adaptive Spline MODeling of data) algorithm (Kavli and Weyer, 1994). This methodology generates models that are sums or products of the variables into smaller submodels that depend only on a subset of inputs using a global partitioning technique (Gago et al., 2011). A neurofuzzy logic submodel was successfully developed for the parameter (output) $g_m$ as a function of two variables (inputs): LMA and $K_{leaf}$. The inputs had independent effects on $g_m$. The work found a strong correlation between the experimentally measured and the predicted $g_m$ based on neurofuzzy logic analysis ($R^2=0.64$, $P<0.05$; Fig. 5A). The $g_m$ predicted by the model as a function of both LMA and $K_{leaf}$ are presented in a 3-D plot (Fig. 5B). Notably, increased $K_{leaf}$ was associated with slight increases in $g_m$, and the highest $g_m$ values were achieved at lower LMA values; the highest values of $g_m$ were observed when $K_{leaf}$ was high ($>14.5$ mmol m$^{-2}$ s$^{-1}$ MPa) and LMA was low ($<115$ g m$^{-2}$). The observed relationship suggests that it may be possible to estimate $g_m$ from $K_{leaf}$ and other simple parameters such as LMA or $A_{area}$ or, conversely, to estimate $K_{leaf}$ from a complete gas exchange analysis. It is important to bear in mind that the strength of ANNs arises from an ability to model complex non-linear relationships between inputs and outputs; however, the model yielded is a ‘black-box’, and interpretation is not possible, depending on other technologies such as fuzzy logic, genetic algorithms, or GEP (Genetic Expression Programming) (Gallego et al., 2011). In this sense, an exciting and promising research frontier is the use of ANNs coupled to GEP technology to develop predictive empirical equations relating hydraulics and mesophyll conductance; unfortunately, due to the current paucity of data for both parameters a validated empirical equation could not be generated with this technology. The neurofuzzy logic training parameters and software information can be seen in Supplementary Table S2. Also, in an attempt to retrieve an empirical equation, a multivariate regression analysis was performed on the same dataset (using R statistics software). A polynomial equation, with $g_m$ as the

Can empirical relationships be used to estimate $g_m$ from $K_{leaf}$ or vice-versa?

The strong coordination between $g_m$ and $K_{leaf}$ suggests that it may be possible to use knowledge of one of these parameters to at least roughly estimate the other. Measurement of $K_{leaf}$ and $g_m$ is rewarding but complex and time-consuming. Indeed values for both parameters were found for only 23 species in the compiled database (Supplementary Table S1). However, having data for these parameters for a wide range of species is crucial for whole-plant and ecosystem models. For instance, the time-response courses of $CO_2$ assimilation and its sensitivity to environmental change scenarios can be better modelled if dynamics of $g_m$ and $K_{leaf}$ are available, but the lack of information for many species precludes their use (Niinemets et al., 2009, 2011). The possibility of retrieving $g_m$ values from measured $K_{leaf}$ or vice versa would result in a fast expansion of the number of species for which these leaf traits are available.
dependent variable and \( K_{\text{leaf}} \), \( LMA \), and \( VLA \) as independent variables was fitted by stepwise multiple regressions. Similar results were obtained as with the neurofuzzy logic technology, although with a weaker predictive power (\( R^2 = 0.43, P < 0.05 \)). The following equation was obtained:

\[
g_m = 0.293 - 0.001LMA + 0.009K_{\text{leaf}} - 0.010VLA
\]

Studies in which \( g_m \), \( K_{\text{leaf}} \), and \( LMA \) are simultaneously determined are important to confirm and to obtain a truly predictive model or equation for these parameters (Fig. 5).

**Outstanding research possibilities emerging from the combined determination of \( g_m \) and \( K_{\text{leaf}} \)**

A new understanding of leaf physiology includes required knowledge of all transport processes, for water and \( CO_2 \), and the structures involved, i.e. from veins to mesophyll cells. The new generation of whole-plant physiologists will have combined skills required to measure gas exchange and hydraulic function, and the ability to interpret and utilize data of both types. Knowledge of the combined responses will enable a new ability to model the entire system, in turn allowing better predictions of gas exchange and its dynamics, and thus the influence of environmental factors on photosynthetic rate and plant growth. A detailed elucidation of the common basis of \( g_m \) and \( K_{\text{leaf}} \) (e.g. the shared aspect of the transport pathways and the role of aquaporins) can provide a clearer ability to scale up from molecules to whole leaf function, and potentially, via crop breeding, to influence both aspects of leaf function in parallel, with a synergistic influence on the integrated hydraulic-photosynthetic system.

**Supplementary material**

Supplementary data are available at *JXB* online.
- **Supplementary Table S1.** Multi-species dataset compilation.
- **Supplementary Table S2.** Training parameters setting for FormRules version 3.31.

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**References**


