







ORIGINAL RESEARCH

Low baseline intraspecific variation in leaf pressure-volume traits: Biophysical basis and implications for spectroscopic sensing

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Funding information

Anthony and Jeanne Pritzker Family Foundation; National Science Foundation, Grant/Award Numbers: 1735325, 1951244, 2017949

Edited by J.M. Torres-Ruiz

Abstract

Intra-specific trait variation (ITV) plays a role in processes at a wide range of scales from organs to ecosystems across climate gradients. Yet, ITV remains rarely quantified for many ecophysiological traits typically assessed for species means, such as pressure volume (PV) curve parameters including osmotic potential at full turgor and modulus of elasticity, which are important in plant water relations. We defined a baseline “reference ITV” (ITV_{ref}) as the variation among fully exposed, mature sun leaves of replicate individuals of a given species grown in similar, well-watered conditions, representing the conservative sampling design commonly used for species-level ecophysiological traits. We hypothesized that PV parameters would show low ITV_{ref} relative to other leaf morphological traits, and that their intraspecific relationships would be similar to those previously established across species and proposed to arise from biophysical constraints. In a database of novel and published PV curves and additional leaf structural traits for 50 diverse species, we found low ITV_{ref} for PV parameters relative to other morphological traits, and strong intraspecific relationships among PV traits. Simulation modeling showed that conservative ITV_{ref} enables the use of species-mean PV parameters for scaling up from spectroscopic measurements of leaf water content to enable sensing of leaf water potential.

1 | INTRODUCTION

Functional traits are widely used to discern and explain patterns and processes across scales from organ and species-level physiology to ecosystem function (Albert et al., 2011; Bartlett et al., 2014; Medeiros et al., 2019; Violle et al., 2007). Plant-water relations traits are critical for determining species' responses across aridity gradients, including, for example, pressure-volume (PV) curve traits estimated from the relationship between water potential and relative water content: turgor loss (wilting) point ($\pi_{t_{ip}}$), osmotic potential at full turgor (π_o), modulus of elasticity (ϵ), relative water content at turgor loss point ($RWC_{t_{ip}}$) and capacitances at full turgor (C_{ft}) and at turgor loss ($C_{t_{ip}}$; see Table 1 for terms, symbols, and definitions; Bartlett et al., 2016; Bartlett, Scoffoni, & Sack, 2012; Brodribb et al., 2020; de Bello

et al., 2011; Rosas et al., 2019; Sack & Pasquet-Kok, 2011). Species mean values for these traits are becoming increasingly available (Kattge et al., 2020), yet there has been little consideration of intra-specific trait variation (ITV) in most PV parameters. PV parameters influence drought responses: $\pi_{t_{ip}}$ and $RWC_{t_{ip}}$ correspond closely on average to the threshold for stomatal closure during dehydration (Henry et al., 2019; Trueba et al., 2019) and that for incipient cell damage (John et al., 2018); the ϵ and π_o are underlying cellular traits quantifying wall stiffness and osmotic concentration; and C_{ft} and $C_{t_{ip}}$ are water storage parameters (Bartlett, Scoffoni, & Sack, 2012). Further, PV parameters can be applied to the prediction of leaf water status based on electromagnetic radiation, an increasingly popular approach (Cotrozzi et al., 2017; Rapaport et al., 2017; Sapes et al., 2022; reviewed in Browne et al., 2020 Table S1). Thus,

TABLE 1 Leaf water status, structural composition and pressure-volume curve parameter symbols, units, and definitions.

Variable	Symbol	Unit	Definitions
Leaf water status			
Leaf water potential	Ψ_{leaf}	MPa	Water status variable indicating the bulk leaf average chemical potential of water within leaf cells
Osmotic potential	Ψ_s	MPa	Component of leaf water potential relating to the concentration of cell solutes
Pressure potential	Ψ_p	MPa	Turgor pressure against the cell walls
Relative water content	RWC	$g \cdot g^{-1}$	Water status index, the ratio of the mass of water in the leaf to that in the saturated leaf
Water mass per area	WMA	$g \cdot m^{-2}$	Water status index, the ratio of the mass of water in the leaf to the leaf area, also known as the “equivalent water thickness”
Structural composition			
Saturated water content	SWC	$g \cdot g^{-1}$	Mass of water in fully saturated leaves relative to dry mass
Leaf thickness	LT	cm	Thickness of leaf lamina
Leaf mass per area	LMA	$g \cdot m^{-2}$	Ratio of mass of dry leaf lamina to leaf area
Saturated water mass per area	SWMA	$g \cdot m^{-2}$	Mass of water in fully saturated leaves relative to leaf area; equivalent to the product of leaf mass per area and saturated water content
Leaf dry matter content	LDMC	$mg \cdot g^{-1}$	Ratio of dry mass to leaf fresh mass at full saturation
Leaf density	ρ_{leaf}	$g \cdot cm^{-3}$	Leaf dry mass per saturated volume; equivalent to the quotient of LMA and leaf thickness
Pressure-volume curve parameters (symplastic basis denoted by an “s” in the subscript)			
Water potential at turgor loss point	π_{tlp}	MPa	Bulk chemical potential of water within leaf cells at “wilting point”, equivalent to the solute potential of cells at the point of turgor loss
Osmotic potential at full turgor	π_o	MPa	Bulk component of water potential relating to the solute concentration in cells (Ψ_s) at full turgor (i.e., at saturation)
Modulus of elasticity	$\epsilon; \epsilon_s$	MPa	Bulk stiffness of leaf cell walls as quantified by slope of the relationship between Ψ_p and relative water content above Ψ_{tlp}
Relative water content at turgor loss point	$RWC_{\text{tlp}}; RWC_{\text{tlp},s}$	$g \cdot g^{-1}$	Leaf hydration at which cells lose turgor
Capacitance at full turgor, relative	$C_{\text{ft}}; C_{\text{ft},s}$	MPa^{-1}	Relative water storage capacity at full turgor
Capacitance at Ψ_{tlp} , relative	$C_{\text{tlp}}; C_{\text{tlp},s}$	MPa^{-1}	Relative water storage capacity for leaves dehydrated beyond turgor loss point
Absolute capacitance at full turgor	$C_{\text{ft},\text{abs}}$	$mol \cdot m^{-2} \cdot MPa^{-1}$	Symplastic absolute water mass capacitance at full turgor normalized by leaf area
Apoplastic fraction	a_f	unitless	Extracellular fraction of water content. This is equivalent to $g^* \cdot g^{-1}$.
Terahertz spectroscopy			
Terahertz time-domain spectroscopy	THz-TDS	–	System of generating and detecting terahertz wavelength pulses interacting with a sample
Peak field ratio	PFR	$dBs \cdot dBs^{-1}$	Ratio of transmitted radiation through sample to radiation transmitted through an empty system

Note: Empirically estimated parameters are presented with hats within the text.

measurements of water mass per unit leaf area (WMA) derived from spectroscopy can be scaled up to Ψ_{leaf} , based on inputs of species mean π_o , ϵ , π_{tlp} , RWC_{tlp} and saturated water mass per area (the product of leaf mass per area and saturated water content; Figure 1). The aim of this study is to consider the ITV and inter-relationships of PV parameters at the scale of most measurements in the literature, and its importance for upscaling for the spectroscopic estimation of leaf water potential.

Quantifying ITV is crucial to effectively predict many population, community, and ecosystem-scale processes (Kraft et al., 2014). Theory

holds that ITV especially benefits resource capture under patchy spatial and temporal availability (Bolnick et al., 2011; Funk, 2008; Nicotra et al., 2010). Yet, ITV can be a “fuzzy” concept: its definition and functional importance depend on scale and environmental conditions (synthesized in Table 2; Albert et al., 2010; Dawson et al., 2021; Reich et al., 2003). Thus, ITV can be assessed within or among co-existing individuals of a species or among populations and may reflect genetic variation and phenotypic plasticity, arising from ontogeny and environmental differences, including climate, soil and/or microclimate. Indeed, ITV can arise even among adjacent leaves on a given plant

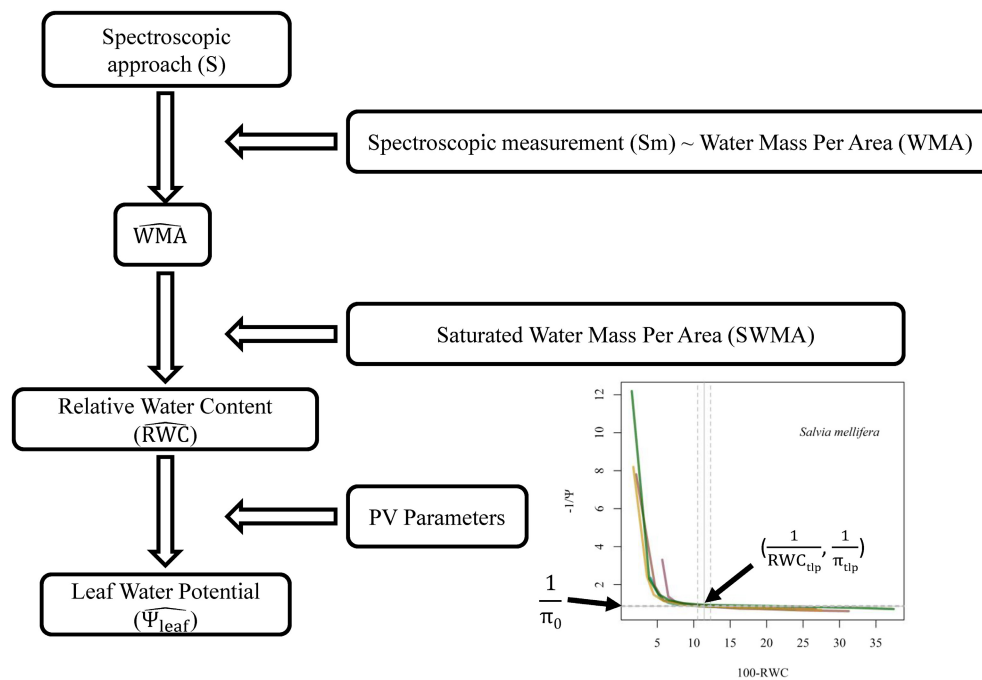


FIGURE 1 Diagram of inputs used to predict of leaf water status variables from spectroscopy-based measurements and leaf traits from spectroscopy approaches (S) (extended from Browne et al. (2020)). Energy transmitted from leaf tissue is indicative of the tissue “equivalent water thickness” or water mass per area (WMA). A function relating WMA to the spectroscopic measurement (S_m) across leaves varying in hydration state enables prediction of WMA. Relative water content (RWC) is then estimated by including the saturated water mass per area (i.e., the product of leaf mass per area and saturated water content). Last, leaf water potential is determined using species-mean pressure-volume (PV) curve parameters.

TABLE 2 Intraspecific variation can arise from multiple sources and across multiple temporal, spatial and conceptual scales, each reflecting multiple underlying processes. Traits vary among individuals of the same species due to three sources, that is, genomic variation, developmental and ontogenetic variation, and environmental variation, and can vary within an individual, across individuals of a community, and across populations, with examples provided for each; as noted, typically, the sources and scales cannot be fully disentangled.

Source	Scales		
	Within a canopy	Across individuals of a given ecotype	Across populations/ecotypes
Genomic variation	Among leaves of different heights in a tree, separated by long mitotic history (e.g., within long-lived trees such as oaks (Plomion et al., 2018, Scholes & Paige, 2015))	Among individuals of annual plant species from a given community (Fridley & Grime, 2010)	Across ecotypes of <i>Arabidopsis thaliana</i> grown in a common garden (Sartori et al., 2019)
Developmental and ontogenetic variation	Among sun leaves on a given tree branch (e.g., variation due to hydraulic sectoriality (Orians et al., 2005))	Between trees of different ages in a community (Nicotra et al., 2010); also includes genomic variation	Between trees of different ages of different communities; also includes genomic variation, and possibly environmental variation
Environmental variation	Between sun and shade leaves (Niinemets et al., 2004, Sack et al., 2006)	Among individuals of a species in different microsites within a community (Williams et al., 2020); also includes genomic variation and possibly ontogenetic variation	Among individuals of different populations; also includes genomic variation and possibly ontogenetic variation

Note: The cell in **bold-face** represents the types of variation involved in the baseline, “reference” intraspecific trait variation (ITV_{ref}) considered in this study, that is, variation across individuals in “typical” sun leaves.

shoot, reflecting developmental plasticity arising from gene expression variation and subtle differences in microclimate (Table 2). In many contexts, ITV can account for important trait variation even relative to interspecific differences, most notably among sun and shade leaves

(Albert et al., 2011; Baird et al., 2017; Delgrange et al., 2004; Givnish, 1988; Siefert et al., 2015; Valladares et al., 2000). One reason that many leaf-level ecophysiological traits have not been assessed for ITV is that the typical determination of species mean trait values

involves selecting “typical” sunlit, fully exposed and mature leaves sampled from replicate plants of given species, often growing in close proximity. This approach is justified to achieve accuracy and precision in the determination of species trait means for exposed leaves with the highest level of photosynthetic activity (e.g., Albert et al., 2011; González-Villagra et al., 2022; Jones & Turner, 1980; Novoplansky et al., 1989; Watson & Casper, 1984). Here, we defined a “reference” ITV (ITV_{ref}) at this scale of typical measurement, that is, representing the variation among sun leaves of replicate plants in similar growing conditions. The ITV_{ref} is expected to be low relative to other scales of ITV, such as comparison of sun versus shade leaves, or of populations across soil or climatic gradients (Rosas et al., 2019). Our intention in clarifying the ITV_{ref} of PV parameters is two-fold. First, the understanding of the relative variation of different traits provides key insights into mechanisms of adaptation, that is, indicating the constraints on traits and their correlations within and across environments. Second, the variation among sun leaves of given species in PV parameters has urgent implications for spectroscopic sensing of water potential that relies on species mean PV parameters.

We hypothesized conservative ITV_{ref} in PV parameters relative to other leaf traits. Notably, plants typically photosynthesize and grow within a narrow range of cell hydration, and PV parameters either directly represent thresholds below which functions decline and damage may occur (π_{tip} and RWC_{tip}), or contribute to the determination of these thresholds (π_o , ϵ , C_{ft} , C_{tip}). Thus, PV curves for sun leaves of given species should be conservative to preserve hydration during short-term but often extreme changes in canopy microclimates. By contrast, other leaf traits related to light capture, such as leaf area, leaf mass per area, leaf thickness, leaf dry matter content, and leaf density, may be more variable as light conditions may vary greatly within a canopy to optimize irradiance capture, nitrogen allocation, and/or carbon gain relative to water loss. Indeed, our hypothesis for the conservative ITV of PV traits for sun leaves is analogous to theory at the whole plant scale considering ITV across spatial resource gradients. Thus, optimization hypotheses predict that organ level traits may show lower ITV than whole-plant traits, such as growth rate and water use efficiency, which relate to stronger environmental variation (Marks, 2007; Siefert et al., 2015; Zhou et al., 2022; but see Herrera, 2017). One study of species populations across an aridity gradient reported a conservative ITV for π_{tip} (Rosas et al., 2019), that is, coefficient of variation of 15% relative to leaf mass per area, which had a value of 46% across that aridity gradient.

We also examined the trait–trait relationships among PV parameters and other leaf structural traits. Notably, trait–trait relationships observed across species may not hold within given species and vice versa (Messier et al., 2018; Rosas et al., 2019). Yet, theory developed across species proposed that PV parameters are intrinsically related, with “higher-level” traits π_{tip} , RWC_{tip} , and C_{ft} arising as biophysical functions of underlying traits: ϵ , π_o , and apoplastic fraction (a) (Bartlett, Scoffoni, & Sack, 2012). Thus, we hypothesized that relationships for replicate sun leaves of given species would follow the biophysical relationships established across species. Indeed, these strong

relationships would provide an explanation for why certain PV parameters would be conservative; if π_{tip} needs to be conservative to maintain hydration above a given threshold for leaf function, and π_{tip} is biophysically determined by π_o and ϵ , then these parameters too would need to be conservative. Further, we tested whether, as expected from the inter-relationship of PV parameters, the variation in the PV parameters would be correlated across species, such that species with high ITV_{ref} in one trait would show high ITV_{ref} in other traits.

Finally, we tested whether ITV_{ref} in PV traits is typically sufficiently conservative such that species mean PV parameters can enable accuracy and precision in the spectroscopic estimation of Ψ_{leaf} from leaf water content, using as an example terahertz (THz) radiation (Figure 1; Table 1). Such sensing is an urgent priority for climate-forward irrigation system design and, potentially, for in-situ gas exchange systems that would estimate water potential simultaneously (Jepsen et al., 2011; Li et al., 2018; Lu & Zhuang, 2010).

Thus, overall, we hypothesized that ITV_{ref} of PV parameters would be lower than for other morphological traits, that PV parameters (and their variation) would show strong inter-relationships within species, and that the low ITV_{ref} for PV parameters would enable robust predictions of leaf water potential from spectroscopic data using species-mean parameters. To test these hypotheses, we compiled a database for ITV_{ref} of PV parameters for 50 species and additional leaf traits for 39 species based on new data and published studies (Bartlett, Scoffoni, Ardy, et al., 2012; John et al., 2018; Maréchaux et al., 2015; Pivovarov et al., 2014; Scoffoni et al., 2011; Scoffoni et al., 2014).

2 | MATERIALS AND METHODS

2.1 | Data collection and compilation, and pressure volume curve construction

We constructed PV curves for 12 native California woody shrub species within six genera, selected for variation in native habitat (Table S1). Juveniles of each species were acquired in 1 L pots in April 2019 (Rancho Santa Ana Botanic Garden, Claremont, CA) and grown for 11 months to heights of 1–2.5 m in the Plant Growth Center at the University of California, Los Angeles (minimum, mean and maximum values for sunny days during a representative 2 week period between 1000 and 1400 h for temperature, 20.4°C, 22.0°C, 23.8°C; for relative humidity 30.1%, 35.6%, 43.1%; and for irradiance 64.2, 283, 1090 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Leaf pressure volume curves were constructed in March–May 2020 using the bench drying method (Sack & Pasquet-Kok, 2011). Shoots with fully developed sun leaves from six individuals were harvested in the afternoon of the day prior to measurements and transported to the lab in dark plastic bags with wet paper towels. From each shoot, two nodes were recut under deionized water, and shoots were rehydrated overnight under plastic. Leaves were repeatedly weighted with an analytical balance (0.01 mg; MS205DU Mettler Toledo) and Ψ_{leaf} was determined using a pressure

chamber (0.001 MPa resolution, Plant Moisture Stress Model 1000; PMS Instruments Co). When “plateau effects” were detected during early dehydration, these measurements were excluded before estimation of pressure-volume parameters (Kubiske & Abrams, 1990). We constructed PV curves for one leaf from each of five to six individuals, except for *Clematis lasiantha*, for which we measured two leaves of each of three individuals due to limited availability.

Additionally, we compiled a dataset of PV curves from six previously published studies (Bartlett, Scoffoni, Ardy, et al., 2012; John et al., 2018; Maréchaux et al., 2015; Pivovarov et al., 2014; Scoffoni et al., 2011; Scoffoni et al., 2014), for a total of 50 unique species diverse in phylogeny, habitat type, and drought tolerance parameters (Table S2). Data were for adult plants growing in urban or wild forests. For five species (*Cercocarpus betuloides*, *Comarostaphylis diversifolia*, *Encelia farinosa*, *Platanus racemosa*, and *Quercus agrifolia*), PV curve data were collected from two studies and were included as independent records. In each study, mature sun leaves were sampled from 3 to 6 replicate individuals, grown in similar conditions and in close proximity. For uniformity, the original data were acquired and re-analyzed by constructing PV curves and extracting parameters (Sack & Pasquet-Kok, 2011). For the PV parameters that can be expressed in relation to either the total or the symplastic water content (i.e., RWC, ϵ , C_{ft} and C_{tip} ; Koide et al., 2000; Tyree & Hammel, 1972), we analyzed both versions. Symplastic parameters rely on the accurate determination of the apoplastic fraction, which is not always possible (e.g., Lenz et al., 2006; Sack et al., 2003). We determined the apoplastic fraction as the x-intercept of the linear relationship between the negative inverse of Ψ_{leaf} and 100-RWC; that is, the RWC at which declining leaf water potential (and solute potential) tends to negative infinity (Bartlett, Scoffoni, & Sack, 2012). We calculated the symplastic RWC (RWC_s), symplastic modulus of elasticity (ϵ_s), and symplastic capacitance at full turgor ($C_{ft,s}$) and turgor loss point ($C_{tip,s}$) as:

$$RWC_s = \frac{RWC - \frac{a_f}{100}}{1 - \frac{a_f}{100}} \quad (1)$$

$$\epsilon_s = \frac{\Delta\Psi_p}{\Delta RWC_s} \quad (2)$$

$$C_{ft,s} = \frac{\Delta RWC_s}{\Delta\Psi_{leaf}} = \frac{SD(RWC_s)}{SD(\Psi_{leaf})}, \Psi_{leaf} > \pi_{tip} \quad (3)$$

$$C_{tip,s} = \frac{\Delta RWC_s}{\Delta\Psi_{leaf}} = \frac{SD(RWC_s)}{SD(\Psi_{leaf})}, \Psi_{leaf} < \pi_{tip} \quad (4)$$

Notably, for capacitances, which are defined as the slope of RWC versus Ψ_{leaf} , we used the quotient of standard deviations, which represents the slope of the standard major axis, which has the advantage of symmetry in y and x (Warton et al., 2011). Absolute leaf area specific capacitance at full turgor ($C_{ft,abs}$) was determined as:

$$C_{ft,abs} = C_{ft} \cdot \frac{SWMA}{18} \quad (5)$$

where SWMA is the saturated water mass per leaf area (=leaf mass per area \times saturated water content per leaf mass; $g\ m^{-2}$) and $18\ g\ mol^{-1}$ is the molar mass of water.

We included data for species for which there were ≥ 5 individual leaf values after statistical outliers were removed to improve the estimation of PV parameters. We tested for outliers in each species' PV parameter dataset, when traits differed by more than two-fold in their values across the sun leaves, by applying the Dixon Q outlier test (*dixon.test* function in R 4.2.1; *outliers* package version 0.14; Komsta, 2011; R Core Team, 2022; Table S3). The typical cause of outlier PV parameter values was a paucity of points (1) above the turgor loss point, leading to uncertainty in the estimation of saturated water content and, thereby, of leaf relative water content, or (2) below turgor loss point, leading to uncertainty in the estimation of water potential and relative water content at turgor loss point. When an outlier was found in a parameter, the raw pressure volume curve values were checked for any errors, and if errors could not be corrected, given leaves were removed; 1/6 leaves were removed for *Betula occidentalis*, *Camellia sasanqua*, *Fraxinus dipetala*, *Helianthus annuus* and *Qualea rosea* and data for 24 species records were removed due to no longer being represented by ≥ 5 individual leaf values. The parameters for which outliers were most frequent were bulk modulus of elasticity (9/383 leaves), capacitance at full turgor (8/383 leaves), and their symplastic counterparts (symplastic modulus and symplastic capacitance at full turgor; 6/383 each). Unrealistic negative apoplastic fraction estimates were also removed from the data set; 23/53 species had ≥ 5 non-negative a_f values and were analyzed for a_f and symplastic PV parameters. We also tested the effect of excluding outliers (see subsequent section).

For 39 species that were measured in this study and in the published literature, data for means and standard deviations for leaf mass per area (LMA), leaf area (LA), leaf dry matter content (LDMC), and leaf thickness (LT) were compiled for sun leaves from the same individuals as those sampled for the pressure volume curve parameters (Table S4). We estimated leaf density (ρ_{leaf}) from mean LMA and LT as:

$$\rho_{leaf} = \frac{LMA}{LT} \quad (6)$$

Standard deviation for ρ_{leaf} was calculated based on those of LMA and LT by propagation of error (Beers, 1957). We did not remove outliers from morphological traits because raw individual data were not available from some studies, and thus coefficients of variation were determined from mean, standard errors, and sample number.

2.2 | Statistics: Within-species trait variability

As a measure of ITV_{ref} for PV parameters, we used the coefficient of variation (CV; Albert et al., 2010):

$$ITV_{ref} = \frac{SD(\text{trait}_{\text{species}})}{\text{mean}(\text{trait}_{\text{species}})} \cdot 100 \quad (7)$$

Where $\text{trait}_{\text{species}}$ represents the measured values for the sun leaves of a given trait for a given species. To compare differences in ITV_{ref} between trait types (i.e., morphological and pressure volume parameters), we performed a nested ANOVA with species' ITV_{ref} values for traits nested within trait type using `aov` from the `stats` package in R (R Core Team, 2022). We also conducted this test while including the outlier leaves in the calculation of ITV_{ref} for PV parameters π_o , $\pi_{t|p}$, ϵ , $RWC_{t|p}$, C_{ft} , and $C_{t|p}$.

2.3 | Statistics: Testing theory for the basis of intraspecific relationships among PV parameters and additional morphological traits

We tested the relationships among PV traits across sun leaves of given species (using the `cor.test` function for R software; version 4.2.1; R Core Team, 2022). We used absolute values for negative PV parameters (i.e., π_o and $\pi_{t|p}$). We considered relationships significant if $p < 0.05$ for both Spearman and Pearson correlation tests, with Pearson tests conducted on untransformed or log-transformed data, that is, respectively testing linear and nonlinear (power law) relationships; we report in the text the strongest correlation coefficient (Table S5) and present relationships among PV parameters with correlation coefficient fitted with standard major axes using the `smatr` R package (Warton et al., 2011).

To determine whether the theory for trait relationships previously established across leaves of diverse species would apply within species, we tested whether $\pi_{t|p}$ and $RWC_{t|p}$ and leaf capacitance at full turgor (C_{ft}) are biophysical functions of π_o and ϵ . According to Bartlett, Scoffoni, and Sack (2012),

$$\widehat{\pi_{t|p}} = \frac{\pi_o \epsilon_s}{\pi_o + \epsilon_s} \quad (8)$$

$$RWC_{t|p,s} = \frac{\pi_o + \epsilon_s}{\epsilon_s} \quad (9)$$

$$\widehat{C_{ft}} = \frac{(100 - RWC_{t|p})}{0 - \Psi_{t|p}} \quad (10)$$

Combined with Equations 8 and 9 and simplified:

$$\widehat{C_{ft}} = \frac{(100 - a_f)(\pi_o + \epsilon)}{\epsilon^2} \quad (11)$$

where π_o is the osmotic potential at full turgor, ϵ is the symplastic modulus of elasticity, and a_f is the apoplastic fraction.

We tested the application of these relationships intraspecifically for $\pi_{t|p}$, $RWC_{t|p}$, and C_{ft} using ordinary least squares (OLS) regressions of observed values versus values predicted based on Equations (8–10), respectively (Table S6).

2.4 | Statistics: Testing for correlations across species in the ITV_{ref} of the PV parameters

To test whether species with high ITV_{ref} in a given trait show high ITV_{ref} in other traits, we analyzed correlations of ITV_{ref} among PV parameters across all species using `cor.test` function for R software (version 4.2.1; R Core Team, 2022; Table S7).

2.5 | Testing the impact of intraspecific variation in PV parameters on remotely sensed leaf water potential

We considered the influence of intraspecific variation in PV parameters on the accuracy and precision of scaling up from spectroscopic determination of leaf water content to the estimation of Ψ_{leaf} (Figure 1). We conducted a sensitivity analysis of the impact of error in each variable relative to other sources of error on the estimation of Ψ_{leaf} for the illustrative case of scaling up from THz time-domain spectroscopy based on previously published data (Browne et al., 2020). THz radiation falls within the 100 GHz–10 THz frequency range or 10–1000 μm wavelength range (Mittleman et al., 1996), and its transmission through a sample is very sensitive to liquid water (Hecht, 2002; Rønne et al., 1999; Thrane et al., 1995), such that the peak field ratio (PFR), the ratio of transmitted radiation through a sample to total propagated radiation, can predict leaf water mass per area (WMA) (Baldacci et al., 2017; Gente et al., 2018), which can be upscaled to Ψ_{leaf} using saturated leaf water content per leaf area (SWMA, itself a function of SWC and LMA) and PV parameters (Figure 1; Browne et al., 2020).

First, to determine how variation in input parameters would limit the prediction of leaf water potential, we tested the intrinsic sensitivity of Ψ_{leaf} to increases in pressure volume curve parameters (both $\pi_{t|p}$ and ϵ) and saturated water mass per area. We estimated a base model using species mean parameters and sampled values of PV parameters and SWMA. Then, we increased each of the input parameters by 5%–100%.

We estimated the root mean square error (RMSE) as:

$$RMSE = \sqrt{(\text{observed} - \text{fitted})^2} \quad (12)$$

Where the observed values are the estimated Ψ_{leaf} from the base model and the fitted values are estimated Ψ_{leaf} when increasing input parameters in the intrinsic sensitivity analysis.

Next, we used previously published data for the estimation of Ψ_{leaf} based on \ln (PFR) for two species, *Hedera canariensis* and *Platanus racemosa* (Browne et al., 2020). We chose five \ln (PFR) values representing a range of leaf hydration, i.e., 95%, 80%, 75%, 70%, and 65% of the saturated water mass per area (−1.69, −1.27, −1.13, −0.99, −0.85 dBs dBs^{−1} for *H. canariensis* and −1.14, −0.95, −0.89, −0.83 and −0.76 dBs dBs^{−1} for *P. racemosa*). We conducted three types of simulations to estimate the influence of error in specific input variables on the precision of Ψ_{leaf} estimates, that is, by sampling with replacement from a normal distribution with the mean and standard

TABLE 3 Simulations testing the influence of error in variables on the prediction of leaf water potential scaling up from the log transformed peak field ratio, \ln (PFR), which relates to water mass per area (WMA; Figure 1). For each bootstrapping simulation we used either the mean value of each parameter or values sampled from a constructed distribution with the same mean and standard deviation from published data (Browne et al., 2020), or using our own database of traits.

Simulation	\ln PFR \sim WMA	PV parameters	SWMA
A	Species mean	Sampled distribution	Species mean
B	Species mean	Species mean	Sampled distribution
C	Species mean	Sampled distribution	Sampled distribution

deviation of the sample data while keeping other variables constant at their mean value (Table 3). In simulation A, we added error to the PV parameters, that is, the modulus of elasticity and osmotic potential at full turgor. In simulation B, we added error to the saturated water mass per area (SWMA) based on its mean and standard deviation (Browne et al., 2020; Table S2). In simulation C, we added error to both the PV parameters and SWMA. For each set of simulations (A–C), we estimated 1000 relationships of PFR to Ψ_{leaf} (Figure 1). In detail, for simulating each relationship of \ln PFR to WMA, for each of the five PFR values, we first made predictions of WMA ($\widehat{\text{WMA}}$) using species-specific WMA \sim \ln PFR relationships:

$$\widehat{\text{WMA}} = a \cdot \ln \text{PFR} + b \quad (13)$$

Where a and b are a species-specific slope and intercept, respectively, determined empirically by Browne et al. (2020). Next, we predicted the relative water content ($\widehat{\text{RWC}}$) as:

$$\widehat{\text{RWC}} = \frac{\widehat{\text{WMA}}}{\text{SWMA}}, \quad (14)$$

where saturated water mass per area is:

$$\text{SWMA} = \text{LMA} \cdot \text{SWC} \quad (15)$$

To scale up from $\widehat{\text{RWC}}$ to Ψ_{leaf} , we estimated π_{tlp} and RWC_{tlp} (Equations 8 and 9, respectively). For simulations (A) and (C), we randomly sampled species-specific values of π_o and ε with replacement from normal distributions based on the mean and standard deviations of measured leaves of each species. Using calculated values of π_{tlp} and RWC_{tlp} , based on Equations (8) and (9), we then determined leaf osmotic potential (Ψ_s), leaf pressure potential (Ψ_p) and leaf water potential (Browne et al., 2020; Sack et al., 2018):

$$\widehat{\Psi}_s = \frac{\pi_o \cdot \pi_{\text{tlp}} (1 - \text{RWC}_{\text{tlp}})}{\pi_o (1 - \widehat{\text{RWC}}) + \pi_{\text{tlp}} (\widehat{\text{RWC}} - \text{RWC}_{\text{tlp}})} \quad (16)$$

and,

$$\widehat{\Psi}_p = \begin{cases} \pi_o \cdot \left(\frac{\widehat{\text{RWC}} - \text{RWC}_{\text{tlp}}}{1 - \text{RWC}_{\text{tlp}}} \right), & \text{if } \widehat{\text{RWC}} > \text{RWC}_{\text{tlp}} \\ 0, & \text{if } \widehat{\text{RWC}} < \text{RWC}_{\text{tlp}} \end{cases} \quad (17)$$

and,

$$\widehat{\Psi}_{\text{leaf}} = \widehat{\Psi}_s + \widehat{\Psi}_p \quad (18)$$

For simulation types B and C, we randomly sampled species-specific values of SWMA with replacement from normal distributions based on the mean and standard deviations of measured leaves of each species. When samples of SWMA were less than WMA, which would be impossible in reality, we resampled from the same distribution. We compared RMSE for the estimates of Ψ_{leaf} for each of these simulation types to determine the role of error in each input variable, in particular the role of uncertainty PV parameters relative to that of other input variables.

3 | RESULTS

3.1 | Assessing intraspecific variation in PV parameters

The ITV_{ref} , that is, the variation among sun leaves of different individuals of given species, quantified as a coefficient of variation, varied across measured PV parameters from $6.87 \pm 0.84\%$ to $34.1 \pm 2.1\%$ (mean \pm SE) for RWC_{tlp} and C_{ft} , respectively, and was on average $22.0 \pm 0.7\%$. The ITV_{ref} for PV parameters was on average significantly lower than those for other leaf structural traits, which varied from 12.3 ± 1.4 to $112 \pm 7.34\%$ for LT and LDMC, respectively, and on average $42.9 \pm 3.3\%$ (Nested ANOVA; $p < 0.001$; Figure 2; Table S8). This analysis was robust to the inclusion of outlier leaves in the calculation of ITV_{ref} for PV parameters π_o , π_{tlp} , ε , RWC_{tlp} , C_{ft} , and C_{tlp} , which increased the across-species mean ITV_{ref} values for these traits by 2.6%–5.4% (Nested ANOVA; $p < 0.001$; Table S8).

3.2 | Intraspecific correlations among PV parameters

Across leaves of given species, PV parameters were strongly correlated (Table S5), with relationships consistent with those previously reported across species, and the mechanistic linkages in Equations (8–11). Thus, relationships of π_{tlp} with π_o were found for 41/53 species (77% of species; mean $r = 0.81$), C_{ft} with ε for 50/53 species (94%; mean $r = -0.93$), RWC_{tlp} with ε for 24/53 species (45%; mean $r = 0.67$), RWC_{tlp} with C_{ft} for 32/53 species (60%; mean $r = -0.71$), and C_{tlp} with a_f for 14/23 species (57%; mean $r = -0.73$).

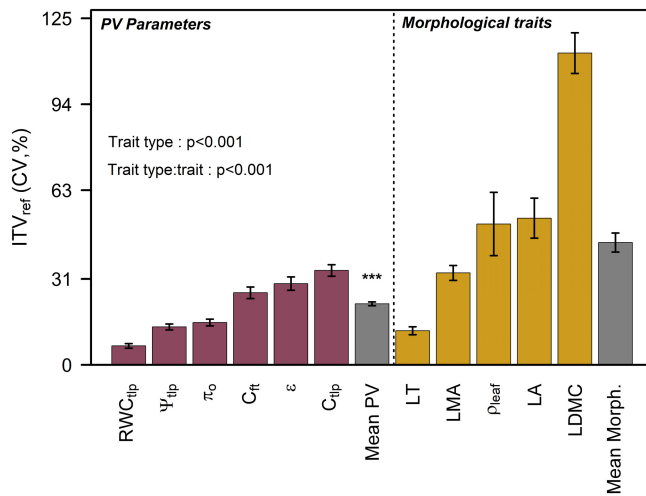


FIGURE 2 Mean intraspecific variation among sun leaves of plants growing in similar conditions (ITV_{ref}) for pressure volume curve parameters of 50 species and other morphological and compositional leaf traits for 39 species, separated by the dotted line and in each category ordered by increasing median coefficient of variation ($n = 12$ –50 species). Error bars indicate the standard error of the mean and asterisks denote the significant difference between mean of pressure volume curve and morphological and compositional leaf traits (***) $p < 0.001$; Nested ANOVA).

3.3 | Testing theory for the relationships among PV parameters within species

Our findings were consistent with theory established among species for biophysical relationships among PV parameters across sun leaves of given species, that is, the mechanistic determination of π_{tlp} , RWC_{tlp} , and C_{ft} based on ϵ , π_o , and a_f (Equations 8–11). Thus, we found strong relationships within species between the observed PV parameter values and those estimated based on theoretical formulae previously shown to hold across species (Bartlett, Scoffoni, & Sack, 2012; $r = 0.76$ –0.999; Figure 3; Table S6).

3.4 | Correlations of ITV_{ref} of PV parameters across species

We found strong across-species correlations among the ITV_{ref} values for different traits. Species with high intraspecific variation in one PV parameter also tended to have high variation in other PV parameters. The correlations of ITV_{ref} among variables that were biophysically related (Equations 8–11) were particularly strong, that is, for π_{tlp} and π_o ($r = 0.87$), RWC_{tlp} and ϵ ($r = 0.79$), C_{ft} and ϵ ($r = 0.85$; Figure 4), RWC_{tlp} (bulk) and apoplastic fraction (RWC_{tlp} $r = 0.56$), a_f and bulk capacitance at full turgor and at turgor loss point (C_{ft} $r = 0.38$; C_{tlp} $r = 0.51$) and symplastic capacitance at full turgor and π_o ($r = 0.56$; Table S7). Further, the ITV_{ref} for saturated water content correlated with those for ϵ and capacitance at full turgor ($r = 0.44$ and 0.48, respectively; Table S7).

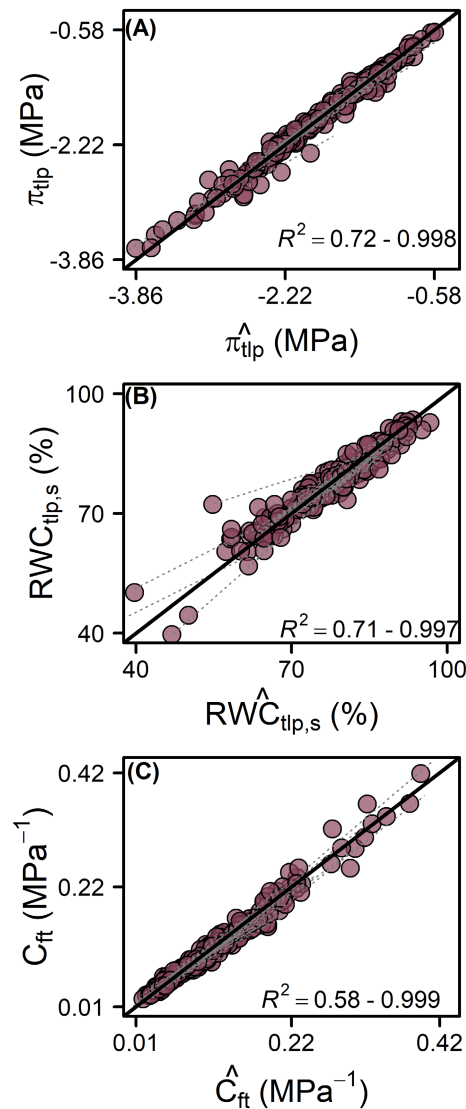


FIGURE 3 Tests of observed versus predicted values of leaf water potential at turgor loss point (π_{tlp} and $\widehat{\pi}_{tlp}$, respectively), relative water content at turgor loss point (RWC_{tlp} and \widehat{RWC}_{tlp} , respectively) and capacitance at full turgor (C_{ft} and \widehat{C}_{ft} , respectively) for 5–6 leaves of 40–44 species. Each point represents a leaf, and lines are plotted for all significant within-species regressions (gray dashed lines), with 1:1 line (solid black line). Values were predicted using Equations (8), (9) and (10) for $\widehat{\pi}_{tlp}$, \widehat{RWC}_{tlp} , and \widehat{C}_{ft} , respectively.

3.5 | Testing the influence of intraspecific variation in PV parameters in in-situ remote sensing of leaf water status

We tested whether ITV_{ref} for PV parameters is sufficiently conservative to enable precise predictions of leaf water potential from in-situ spectroscopic measurements of leaf water mass per area (WMA, a.k.a., “equivalent water thickness”), exemplified by the use of peak field ratio (PFR) based on THz transmission. Thus, we tested simulations differing in the variation (noise) in predicted Ψ_{leaf} values given the error added to different inputs. In our test of the intrinsic sensitivity

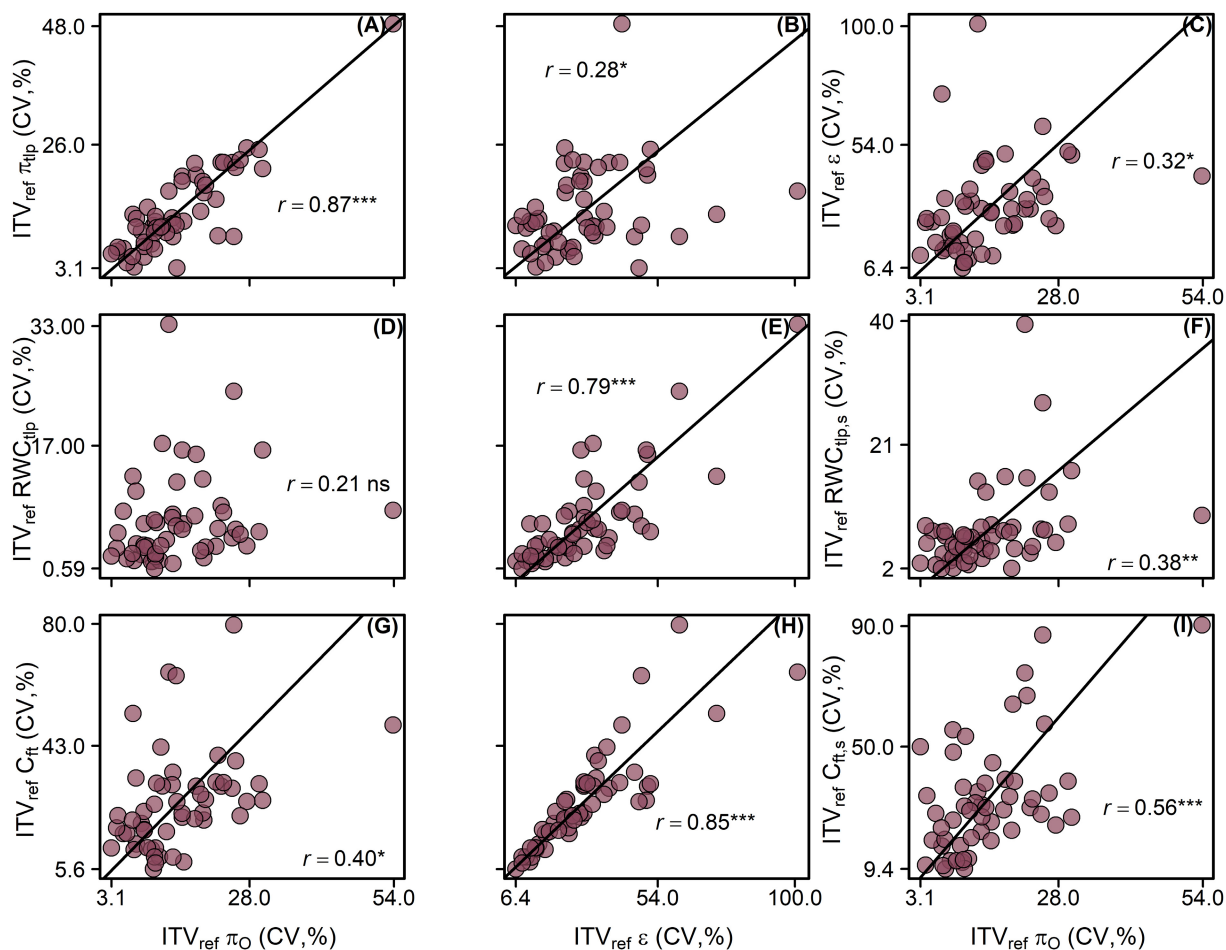


FIGURE 4 Correlations across species of the coefficient of variation (CV) across individual sun leaves of plants grown in similar, well-watered conditions (a measure of ITV_{ref}) for total (C_{ft}) and symplastic ($C_{ft,s}$) capacitance, total and symplastic relative water content at turgor loss point (RWC_{tip} and $RWC_{tip,s}$, respectively), and leaf water potential at turgor loss point (π_{tip}) with their mechanistic drivers according to biophysical theory, the leaf osmotic potential at full turgor (π_o) and modulus of elasticity (ϵ) for 53 species records. Statistical significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). There is a strong relationship among variation in “higher level” parameters and their constituents. Black solid lines were drawn with standard major axis.

of the increase in variation in predicted Ψ_{leaf} in relation to increasing each input variable by a given percentage of the mean, all else being equal, we found similar sensitivity to PV parameters and SWMA (Figure 5). The error in predicted Ψ_{leaf} increased gradually by ~ 0.05 MPa (RMSE) when 30% error was added to either the PV parameters or to SWMA, and then much more steeply thereafter, especially when error was added to both PV and SWMA (Figure 5). We also estimated the noise that would reduce predictive power using the RMSE for simulations based on sampling from the observed distributions of input parameters at each hydration level (i.e., at each input peak field ratio). For both *Hedera canariensis* and *Platanus racemosa*, there was similar noise in the prediction of Ψ_{leaf} due to error added to PV parameters and SWMA (Figure 6); however, when adding both together we saw compounding error for *H. canariensis* and compensation for *P. racemosa* (mean \pm SE for RMSE for simulations A, B, and C; 0.29 ± 0.01 , 0.24 ± 0.01 , and 0.35 ± 0.01 MPa for *H. canariensis*; 0.51 ± 0.03 MPa, 0.34 ± 0.02 MPa, and 0.34 ± 0.02 MPa for *P. racemosa*; $p < 0.001$ and

$p = 0.003$ for differences between species and among simulations, respectively; two-way ANOVA). Furthermore, across hydration states, the error in Ψ_{leaf} for *H. canariensis* declined slightly while RMSE increased with dehydration for *P. racemosa*. Overall, these results highlight the feasibility of predicting Ψ_{leaf} from PFR for sun leaves using species mean values for PV parameters and SWMA, with a RMSE resolution typically at < 0.5 MPa.

4 | DISCUSSION

Our analyses showed that PV parameters had low ITV_{ref} relative to other leaf morphological traits consistent with their importance as variables with strong influence on thresholds for dehydration responses. Further, the relationships among PV parameters and their variation supported biophysical hypotheses that were previously formulated across species. Our simulation modeling demonstrated that conservative ITV_{ref} for PV parameters validates the use of species-

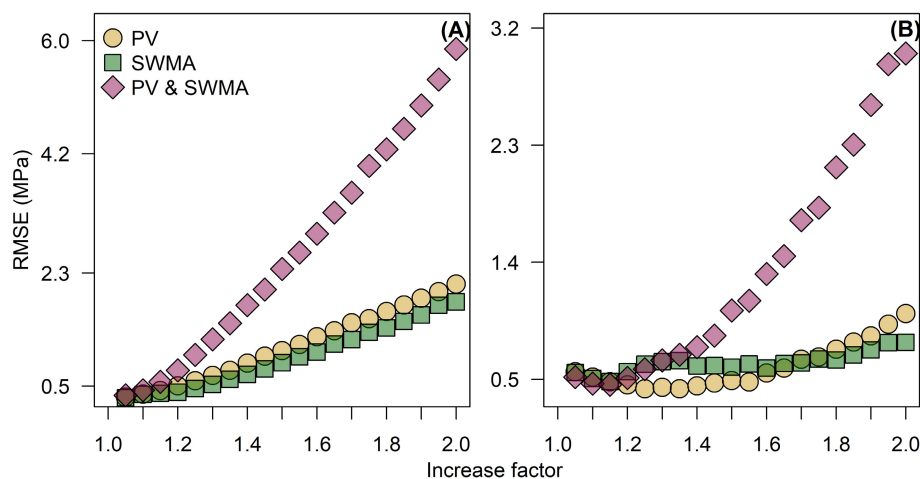


FIGURE 5 Test of model sensitivity to increasing parameters values for *Hedera canariensis* (A) and *Platanus racemosa* (B). We estimated the error in predicted leaf water potential (Ψ_{leaf}) using the root mean square error (RMSE; MPa) by increasing the sampled parameters, that is, for pressure volume curve (PV) parameters (π_{tip} and ϵ); for saturated water mass per area (SWMA); and for PV parameters and SWMA together, in our model (Figure 1). We increased sampled parameters by 5%–100%, represented here as multiplying parameters by 1.05–2.0.

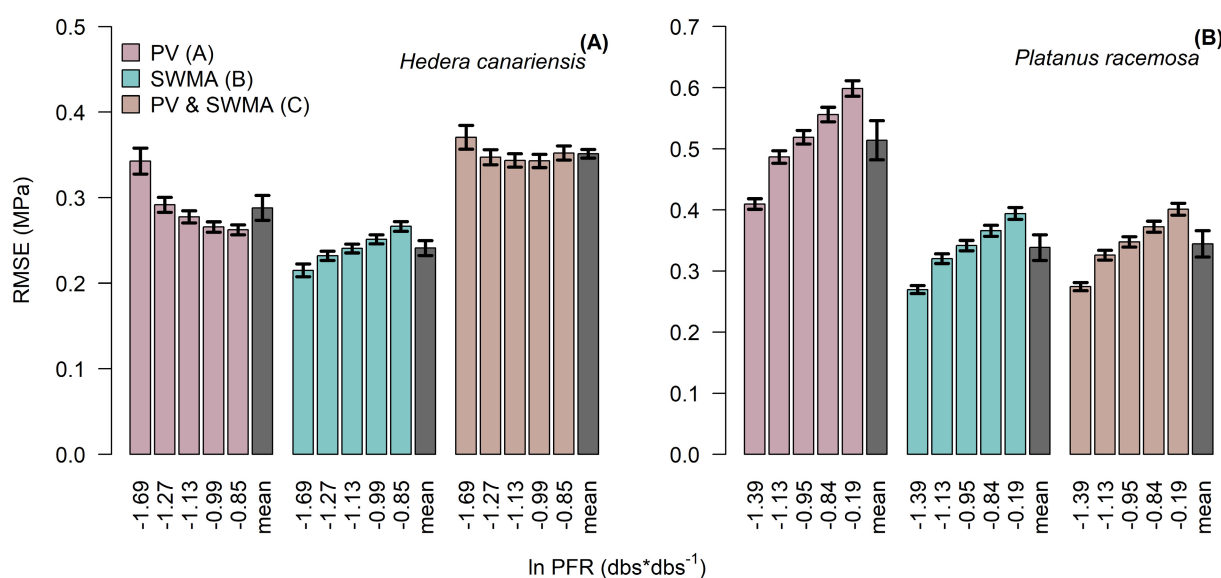


FIGURE 6 Root mean squared error of predictions ($\text{RMSE } \widehat{\Psi}_{\text{leaf}}$) for five values across the typical measured range of the log transformed peak field ratio, $\ln(\text{PFR})$, which relates to water mass per area (WMA) and can thus be scaled up to give $\widehat{\Psi}_{\text{leaf}}$ for *Hedera canariensis* (A) and *Platanus racemosa* (B) (analysis using PFR data from (Browne et al., 2020)). For each $\ln(\text{PFR})$, we predicted 1000 $\widehat{\Psi}_{\text{leaf}}$ values based on the approach presented in Figure 1, and for each term either using the mean value, or adding error by sampling from a normal distribution based on the measured mean and standard deviation (Table 3) to: (simulation A) the pressure volume (PV) curve parameters; (simulation B) the saturated water mass per area (SWMA); and (simulation C) both the PV parameters and SWMA.

mean values for scaling up from spectroscopic measurements for remote sensing from leaves to canopies.

4.1 | Variation in ITV_{ref} among pressure volume curve parameters and other leaf traits

The ITV_{ref} , estimated based on sun-exposed, mature leaves of plants grown under similar conditions, enables the use of ecophysiological and structural composition data collected using typical sampling for comparison of within-species variation in PV parameters relative to other traits. Notably, ITV at this scale provides a “baseline” ITV that minimizes environmental variation and ecotypic variation that would

arise among populations, and emphasizes the developmental plasticity, genetic variation and microclimate disparity (e.g., in irradiance and water status) that arises among sun leaves (Sack et al., 2006). An avenue for future study is the variation in ITV_{ref} for given traits of given species that may arise under different growing conditions.

We found relatively low ITV_{ref} in PV parameters compared to other leaf morphological traits. PV parameters such as π_o , ϵ , and π_{tip} are theoretically expected to be conservative for individuals of a given species as they either contribute to or directly represent thresholds of dehydration responses (Bartlett et al., 2014; Bartlett, Scoffoni, & Sack, 2012). By contrast, other leaf morphological traits may vary to optimize competitive ability across more axes of performance, including light capture and water and nutrient use efficiencies (Bolnick

et al., 2011; Funk, 2008; Givnish, 1988; Novick et al., 2022). Another explanation of the conservative variation in the PV parameters is that it arises from bulk cell-level traits such as protoplast osmotic concentration and cell wall thickness, which may be more conservative within a species than organ-level morphological traits with higher ITV_{ref} such as LDMC and ρ_{leaf} , which depend on not only cell properties, but also are strongly influenced by variation in cell numbers and sizes in the different tissues (John et al., 2017).

4.2 | Theoretical implications of variation among PV parameters and with leaf traits

In support of established biophysical hypotheses for PV parameters, we confirmed intraspecifically the relationships for the determination of “higher level” traits based on underlying traits, i.e., of π_{tip} , RWC_{tip} , and C_{ft} from ϵ , π_o , and a_f (Figure 3). The π_{tip} and RWC_{tip} could be predicted as explicit functions of π_o , ϵ , and C_{ft} as previously reported across species (Bartlett, Scoffoni, & Sack, 2012). These findings thus supported the causal role of the π_o and ϵ as drivers of π_{tip} , capacitance at full turgor, and the RWC at turgor loss point. For example, the accumulation of solutes within the symplast (i.e., more negative osmotic potential at full turgor) allows for the maintenance of turgor at more negative water potentials, and additionally variation in the extensibility of cell walls (ϵ) influences the water storage of cells, impacting the variation in RWC_{tip} and C_{ft} .

Additionally, we found correlations among traits in their ITV_{ref} across species, such that species with high variation in one trait also varied strongly in others (Figure 4). This pattern is expected given the relationships among PV parameters, and highlights the inter-related functionality of these traits. We note that the correlation of ITV across traits would not necessarily be expected to hold across other sets of traits, contexts or scales of variation generally. Theory for trait plasticity holds that traits may differ in their variation according to species and function (Grime et al., 1986; Grime & Mackey, 2002) such that species specialized in resource-rich environments may show high ITV in organ-level morphological traits that confer competitive ability (e.g., leaf size and leaf density), whereas species of resource-poor conditions may show high ITV in physiological traits that could enable high resource capture during periods of high availability within the lifetime of the organ (e.g., photosynthetic rate; Grubb, 1998). Further, previous studies of intracanopy plasticity in leaf morphological traits (i.e., leaf mass per area, leaf perimeter²/area, sapwood to leaf area ratio and stomatal density) including sun and shade leaves found that trait variation was not correlated across species as was found here for ITV_{ref} for PV traits (Sack et al., 2006). An important avenue for future study is the question of whether the ITV of PV parameters would be correlated across species for other scales of ITV , that is, for plasticity across light or nutrient gradients. Notably, in this study, we focused on the relationships between ITV_{ref} of PV parameters, but not among morphological parameters given the lack of raw individual data for some of the studies; future studies could test the relationship of ITV_{ref} across functional traits more commonly measured (such as, e.g., leaf

mass per area and leaf thickness) would benefit from available large global datasets.

4.3 | Implications of low ITV_{ref} for PV parameters for scaling up from leaf to plant to landscape

Our findings indicate the viability of scaling up spectroscopic measurements of WMA to Ψ_{leaf} using species mean PV curve parameters (Figure 6). The narrow ITV_{ref} of PV parameters results in relatively low error, generally <0.5 MPa in RMSE, such that Ψ_{leaf} estimation is not rendered imprecise by the baseline plasticity of sun leaves. The error in predicting Ψ_{leaf} was greater for both species when varying pressure volume curve parameters (simulation A) than SWMA (simulation B), given our randomly sampling error for two PV parameters simultaneously (π_o and ϵ). The increasing error for *P. racemosa* at more dehydrated states corresponds to the effects of lamina shrinkage during leaf dehydration in an already relative thin-leaved species (Browne et al., 2020; Scoffoni et al., 2014). Our demonstration of the utility of species-mean PV parameters in the estimation of Ψ_{leaf} from ln (PFR) indicates that analyses utilizing species-means for SWMA and PV-parameters can be feasible for estimating Ψ_{leaf} and provides a first estimate of the error expected from this approach.

We recommend that this approach to testing the importance of ITV_{ref} in the prediction of scaled up leaf water status from spectroscopic measurements be applied to studies of other species and spectroscopic approaches, to enable the application of mean PV parameters in these applications. Spectroscopic assessment of leaf water content can be applied at leaf, canopy, or landscape scale (Asner et al., 2016; Hunt et al., 1987; Li et al., 2018; reviewed by Browne et al. (2020)). Notably, the application tested here was for the prediction of Ψ_{leaf} at the scale of individual sun leaves using transmittance spectroscopy. Indeed, several studies matching Ψ_{leaf} for sun leaves with airborne spectroscopic reflectance measurements indicate strong predictive power in estimated Ψ_{leaf} among sun leaves (Momen et al., 2017). Yet, for other spectroscopic measurements at canopy and landscape scales, such as using vegetative optical depth for the estimation of WMA, involves disentangling the effects of shade leaves and branches, which will have different PV parameters than sun leaves. For instance, X-band microwave detection represents attenuation from water in leaves and tissues at the top of the canopy whereas L-band microwave detection may include water throughout a canopy (Konings et al., 2021). Thus, these approaches to scale to whole canopies may need to account for ITV that includes environmental-driven trait variation within canopies to achieve strong predictive power for estimates of landscape Ψ_{leaf} (Holtzman et al., 2021; Konings et al., 2021).

Approaches to the sensing of Ψ_{leaf} based on estimates of WMA have great urgency and importance across scales. At leaf scale, Ψ_{leaf} measurements that can be made in vivo, in situ within a gas exchange system, would contribute strongly to the determination of photosynthetic drought responses, and our findings indicate that species-mean PV parameters could be used for such an application. At larger scales,

measurements from a mounted in situ or remote system will improve estimates of drought-induced physiological responses at ecosystem scale (Konings et al., 2021; Momen et al., 2017; Rao et al., 2019), and enable the development and refinement of “need-based” irrigation systems of crops and urban ecosystems (Jepsen et al., 2011). Coupled with eddy-flux tower or spectroscopic measurements of canopy evapotranspiration and temperature (Fisher et al., 2020), remotely sensed Ψ_{leaf} measurements would enable a higher resolution of the control of canopy fluxes by leaf water status and hydraulic conductance (Anderegg et al., 2017; Novick et al., 2022). While analysis of the ITV_{ref} leads to important insights and applications at leaf scale, the range of applications at larger scales shows the increasingly need to quantify the ITV of pressure-volume parameters across a yet wider range of contexts.

AUTHOR CONTRIBUTIONS

Marvin Browne and Lawren Sack conceived and designed the study. Marvin Browne, Megan K. Bartlett, Christian Henry, Grace John, Christine Scoffoni, and Nezh Tolga Yardimci collected experimental data. Marvin Browne processed the experimental data and performed analyses. Marvin Browne and Lawren Sack interpreted the results, wrote the manuscript, and designed the figures. All authors discussed the results and commented on the manuscript.

ACKNOWLEDGMENTS

We are grateful to A. Baird, A. Ongjoco, M. Ochoa and N. Vinod for discussion. This work was supported by the U.S. National Science Foundation (grants 1951244, 2017949, and 1735325), and the Anthony and Jeanne Pritzker Family Foundation.

DATA AVAILABILITY STATEMENT

Data used in our analyses are provided within Table S2.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Browne, M., Bartlett, M.K., Henry, C., Jarrahi, M., John, G., Scoffoni, C. et al. (2023) Low baseline intraspecific variation in leaf pressure-volume traits: Biophysical basis and implications for spectroscopic sensing. *Physiologia Plantarum*, 175(4), e13974. Available from: <https://doi.org/10.1111/ppl.13974>