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REVIEW PAPER

The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions?

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Abstract

When the stomata are closed under drought, the only route for water loss from the leaf interior to the atmosphere is across the cuticle. Thus, the extent of cuticular transpiration in relation to the reservoirs of water in the plant and the water acquisition from the soil determines the fitness and survival of the plant. It is, therefore, widely assumed that the cuticular water permeability of plants regularly experiencing drought is comparatively low and, thus, adapted to the environment. To test this hypothesis, 382 measurements of cuticular permeability from 160 species were extracted from the literature published between 1996 and 2017. The data had been produced either by using isolated cuticles and astomatous leaf sides or by measuring the minimum leaf conductance under conditions assumed to induce maximum stomatal closure. The species were assigned to 11 life form groups. Except for two particular cases (epiphytes, and climbers and lianas), the cuticular permeabilities of all groups either did not differ significantly or the available data did not allow a statistical test. In conclusion, present knowledge either does not support the hypothesis that ecological adaptions of cuticular water permeability exist or the available data are insufficient to test it.

Key words: Cuticular permeability, ecological adaptation, leaf cuticular transpiration, life form, minimum leaf conductance, permeance

Introduction

Plants experience an inevitable dilemma: when they open their stomata to acquire carbon dioxide, they lose water to the atmosphere. CO_2 uptake and water loss must be optimized to safeguard the survival of the plant also under adverse environmental conditions. Drought induces the stomata to close, thereby minimizing water loss and avoiding water stress. Under such conditions, water loss from the plant to the atmosphere occurs across the cuticle covering the outer cell walls of the epidermis of leaves, fruits, petals, and green stems. Under limited water availability, the extent of cuticular transpiration in relation to the reservoirs of water in the plant and water acquisition from the soil has a major impact on the fitness and survival of a plant.

Plant cuticles consist of a cutin matrix (a polyester of mainly C_{16} and C_{18} hydroxy alkanoic acids and their derivative) and cuticular waxes embedded within the matrix and deposited onto its outer surface (Pollard *et al.*, 2008; Yeats and Rose, 2013). Cuticular waxes almost exclusively make up the barrier against the diffusion of water across the cuticle, contributing 96% (*Prunus laurocerasus* leaf) to 99.5%

Abbreviations: g_{min}, minimum leaf conductance; J, transpiration rate or flow rate of water; p, permeance.

(*Pyrus communis* leaf) of the total resistance (Burghardt and Riederer, 2006). Typical cuticular wax components are very-long-chain aliphatics and cyclic molecules such as pentacyclic triterpenoids (Jetter *et al.*, 2006). The evidence available so far indicates that the aliphatic wax fraction forms the barrier while the contribution of pentacyclic triterpenoids seems to be small or even negligible (Oliveira *et al.*, 2003; Vogg *et al.*, 2004; Leide *et al.*, 2007, 2011; Buschhaus and Jetter, 2012; Jetter and Riederer, 2016). However, as a whole, the relationship between cuticular wax composition and amounts and the cuticular water permeability is not yet sufficiently understood.

Regarding the importance of cuticular transpiration for the fitness and survival of plants under unfavourable conditions of water availability, it is surprising that reliable data on the water permeability of plant cuticles obtained in ecophysiological contexts remain fairly scarce. Kerstiens (1996*a*) had reviewed the state of knowledge on the cuticular water permeability and its physiological relevance. In the meantime, several studies have been published which now allows for an extended view on this topic and a deeper understanding of some of the factors determining cuticular transpiration.

The present review performs a critical meta-analysis of the data on cuticular transpiration published within the last 20 years and assesses them in an ecophysiological context. Plant ecophysiology tries to understand the adaptive value of physiological properties for the fitness of a plant in its natural environment. So, in the present case, the properties of the leaf cuticular transpiration barrier are put into the perspective of the environmental conditions of the natural habitat of the respective plant. The cuticle being a persistent structure is considered not to be subject to major short-term regulatory adjustments. Therefore, adaptive responses can be expected mainly on the phenotypic, epigenetic, and genotypic levels.

Characterizing cuticular barrier properties against water loss

As stated above, the diffusion of water across the cuticle determines the rate and extent of water loss from above-ground primary plant organs. Consequently, the water permeability of the cuticle must be measured rigorously when the physiology behind cuticular transpiration and its ecological implications are to be understood.

The most straightforward way of doing this is to determine the permeability of a carefully isolated, astomatous leaf cuticle under well-defined conditions of temperature and driving force. This approach is preferable whenever a rigorous analysis of the cuticular transport properties in relation to the chemical and physical properties of the transport-limiting barrier is the goal. The permeability parameter obtained from such experiments will be termed 'permeance' p (m s^{-1}) in this review. In many plant species, however, cuticles cannot be isolated, or only stomatous cuticles are present. In these cases, the only transport parameter accessible is the water loss from the intact leaf under conditions known to induce maximum stomatal closure. The corresponding permeability parameter will, in that case, be called 'minimum leaf conductance' g_{min} (m s^{-1} or mmol m^{-2} s^{-1}). The terminology is summarized in Table 1. The term 'cuticular permeability' is used in this review for both permeances and minimum leaf conductances.

Irrespective of the approach, under steady-state conditions, the flow of water across a cuticle follows the first Fick's law. The transpiration rate or flow rate of water J (g m^{-2} s^{-1}) across the cuticle is equal to the amount of water lost W (g) per unit of exposed area A (m^2) and time t (s):

$$J = \frac{W}{\Delta t \cdot A} \quad (1)$$

The permeance p or the minimum leaf conductance g_{min} can be obtained from the transpiration rate J and the driving force Δc of water across the cuticle according to:

$$p = g_{min} = \frac{J}{\Delta c} \quad (2)$$

The driving force is either the difference between the concentration of water vapour in the leaf interior and the surrounding atmosphere (g m^{-3}) or the difference in the mole fraction of water vapour in both compartments (mol mol^{-1}) resulting in permeability parameters with either units m s^{-1} or mmol m^{-2} s^{-1}. Since in cuticular transpiration the diffusion of water occurs in the solid phase of the cuticle, atmospheric pressure has no influence and, consequently, the concentration-based driving force is the appropriate one. The resulting permeability parameters have the units m s^{-1}. Such permeability parameters have the additional advantage that they can be mechanistically interpreted regarding the mobility

Table 1. *Definition of parameters used to describe the permeability of leaf cuticles measured with different materials and using different driving forces for calculation*

For unit conversion see Equation 3.

Term	Symbol	Material	Driving force	Units
Permeance	p	Isolated cuticles or astomatous leaf sides with stomatous surfaces sealed	Difference in water vapour concentration (g m^{-3})	m s^{-1}
Minimum leaf conductance	g_{min}	Whole detached leaves under conditions of maximum stomatal closure	Difference in water vapour concentration (g m^{-3})	m s^{-1}
Minimum leaf conductance	g_{min}	Whole detached leaves under conditions of maximum stomatal closure	Difference in water vapour mole fraction (mol mol^{-1})	mmol m^{-2} s^{-1}

and solubility of water in the transport-limiting barrier. The mole fraction-based driving force must be used in cases when the diffusion occurs in the vapour phase as in stomatal transpiration. Mole fraction-based permeability parameters can be converted to concentration-based parameters when the atmospheric pressure and the temperature during the experiment are known. Mole-based and concentration-based permeability parameters are related according to:

$$\frac{\text{Permeability}_{\text{mole fraction}}}{\text{Permeability}_{\text{concentration}}} = \frac{P}{\text{RT}} \quad (3)$$

where P is the atmospheric pressure, R the gas constant, and T the absolute temperature. So, a mole fraction-based permeability as very often given in the ecological literature can be converted into a concentration-based one by multiplying it by a factor of 2.45×10^{-5} when the measurement was performed at standard atmospheric pressure and 25 °C.

Measuring the permeability of leaf cuticles

Different methods for measuring cuticular permeability must be employed depending on the availability of isolated cuticles. Schönherr and Lendzian (1981) introduced the now commonly used method for measuring the permeance of isolated cuticles, p. In brief, enzymatically isolated, astomatous plant cuticles are mounted on small transpiration chambers filled with water. The chambers are placed into an environment of controlled relative humidity and temperature, and the loss of water across the cuticle is measured gravimetrically (Fig. 1A; Schreiber and Schönherr, 2009). The slope of the curve is the flow rate of water J (Equation 1) from which the permeance p (m s^{-1}) can be calculated according to Equation 2.

However, intact cuticles suitable for permeability measurements can be isolated only from a limited number of plant species. If the leaves are hypostomatous, the cuticular permeance can still be measured for the adaxial, astomatous leaf surface by sealing the stomatous leaf surface, for example with paraffin wax (Burghardt and Riederer, 2003; Brodribb *et al.*, 2014). The measurement of the cuticular permeance of amphistomatous leaves (many herbaceous species and grasses), however, is not possible with this method.

For amphistomatous leaves, g_{min} is measured by a simple gravimetric method [Minimum epidermal conductance (g_{min}, a.k.a. cuticular permeability) Prometheus Wiki http://prometheuswiki.org/tiki-index.php?page=Minimum+epidermal+conductance+%28gmin%2C+a.k.a.+cuticular+conductance%29, last accessed 9 September 2017]. In short, intact detached leaves are exposed to dry air, and leaf drying curves are measured gravimetrically (Fig. 1B). During the drying of the leaf, the stomata close progressively, and the rate of water loss from the leaf decreases until a constant rate J is reached when the stomata are maximally closed. g_{min} is calculated from J and the driving force according to Equation 2.

g_{min} is not equal to 'low conductances' measured in the field such as night-time conductances (Caird *et al.*, 2007). Field measurements are commonly done with porometers or gas exchange systems with, in many cases, insufficient sensitivities to record true g_{min} values. The 'low conductance' values from the field are often considerably higher than g_{min}, indicating that most species can further close the stomata under severe desiccation stress (Howard and Donovan, 2007; Walden-Coleman *et al.*, 2013).

Potential sources of error when measuring minimum leaf conductances

Detached leaves are much more complicated systems than isolated cuticles and, consequently, deducing g_{min} from leaf drying curves is prone to several errors which must be carefully dealt with. Potential sources of error are changes of the leaf area and the driving force during desiccation.

Depending on the specific anatomy of a leaf, its surface area may decrease during the dehydration process induced

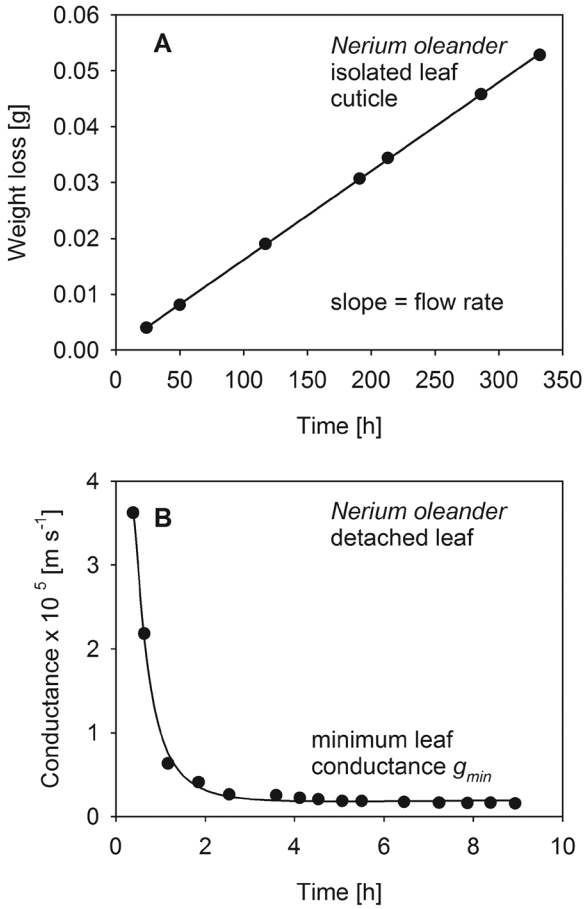

Fig. 1. Representative results obtained from two experimental set-ups to determine the cuticular permeability of *Nerium oleander* leaves. (A) The linear loss of water across an isolated adaxial cuticle inserted in a transpiration chamber. The slope is the flow rate of water J from which the permeance p can be calculated according to Equations 1 and 2. The resulting permeance at 25 °C was 1.77×10^{-5} m s^{-1} (n=10; Schuster, 2016). (B) Leaf drying curve of a leaf exposed to dry air at 25 °C. The conductance reaches a plateau after ~3 h when maximum stomatal closure is achieved. This value is the minimum leaf conductance g_{min} and was 2.16×10^{-5} m s^{-1} (n=5). The permeability parameters obtained by the two experimental set-ups did no differ significantly (Mann–Whitney U-test; P=0.12; Schuster, 2016).

for measuring leaf drying curves. According to Pérez-Harguindeguy *et al.* (2016), the area of completely dehydrated leaves may be up to 80% smaller than that of fully saturated leaves. The percentage loss of area in dry leaves was analysed by Scoffoni *et al.* (2014) for 14 plant species with diverse leaf forms, leaf textures, and drought tolerances. The average shrinkage detected ranges from 4.9% to 69.0%. Schuster *et al.* (2016) reported a shrinkage of 4.5% for *Rhazya stricta* leaves at the critical relative water deficit (i.e. when damage to the photosynthetic apparatus occurs). This rather small decrease in leaf surface area did not influence the transpiration rate significantly. However, for leaves with higher degrees of shrinkage, g_{min} has to be corrected accordingly by using the actual leaf surface at each dehydration level.

The second potential source of error when measuring g_{min} from leaf drying curves is the change of the driving force for the diffusion from the leaf interior to the atmosphere. The driving force Δc is the difference between the concentration of water vapour in the leaf intercellular air space ($c_{wv\ leaf}$) and the surrounding atmosphere ($c_{wv\ air}$):

$$\Delta c = c_{wv\ leaf} - c_{wv\ air} = a_{leaf} \times c_{wv\ sat\ leaf} - a_{air} \times c_{wv\ sat\ air} \quad (4)$$

The activity of the water vapour in the atmosphere surrounding the leaf (a_{air}) is obtained from the relative humidity and the saturated air water vapour concentration ($c_{wv\ sat\ air}$) from tabulated values for the given temperature. In the same way, the water activity in the intercellular air space (a_{leaf}) and the water vapour saturation concentration at leaf temperature ($c_{wv\ sat\ leaf}$) must be known. The water activity in the leaf for each dehydration level can be deduced from a pressure–volume analysis of leaf water potential. The leaf water potential at a given dehydration step ψ_{leaf} can be converted into the corresponding leaf water activity a_{leaf} according to:

$$\Psi_{leaf} = \frac{R \times T}{V_w} \times \ln\ a_{leaf} \quad (5)$$

where R is the gas constant, T the absolute temperature, and V_w the molar volume of water (Nobel, 2009). When the permeability of the cuticle is relatively high, the resistance of the boundary layer on top of the leaf may also influence g_{min}. It acts as an additional resistance in series, and g_{min} must be corrected accordingly (Slavic, 1974).

A further critical issue when evaluating leaf drying curves is to determine at which time point or level of dehydration the stomata are maximally closed (Grace, 1990; Burghardt and Riederer, 2003; Walden-Coleman *et al.*, 2013). Stomata are at least partially open in the initial phase at full leaf saturation, and the conductance is high. Upon dehydration, stomata close progressively during ongoing leaf dehydration, leading to a decline of total conductance. The conductance reaches a constant and minimum value at maximum stomatal closure in the final phase, the g_{min}. The time to reach maximum stomatal closure may vary between species and, therefore, complete leaf drying curves must be measured and displayed whenever values of g_{min} are reported. Some authors merely assume that maximum stomatal closure is reached after an arbitrary period of dehydration time (e.g. 30 min) without providing any experimental evidence for this. This approach, however, may lead to erroneous (overestimated) values.

In some cases, a slight decrease of the minimum transpiration rate may be observed even after the point of maximum stomatal closure. This has led to speculations that the cuticular permeability to water may decline with decreasing leaf water content due to a deswelling of the cuticle (Pisek and Berger, 1938; Van Gardingen and Grace, 1992; Anfodillo *et al.*, 2002). Such an effect was not observed for *R. stricta*, with g_{min} remaining constant at a low value even under prolonged periods of dehydration (Schuster *et al.*, 2016). This is probably due to the protocol applied to measuring leaf drying curves with *R. stricta* which is improved in comparison with the standard protocol used so far. In the improved protocol, transpiration rates are corrected for leaf area shrinkage, and conductances are calculated using driving forces, taking into account the decline of the water potential during leaf dehydration and the actual leaf temperatures. Neglecting leaf area shrinkage and assuming that the water activity in the leaf interior is equal to unity over the whole period of leaf dehydration, as generally practised, leads to an apparent, but erroneous, small decline of g_{min} which is due to an insufficient experimental protocol and not to cuticle deswelling.

Minimum leaf conductance and cuticular permeance

The g_{min} at maximally closed stomata is often assumed to be essentially equal to the cuticular permeability (Nobel, 2009). However, it is a matter of debate whether indeed all stomata are closed and whether they are closed as tightly as possible under severe desiccation stress. For describing this phenomenon, the terms 'leaky stomata' (Brodribb *et al.*, 2014; Scoffoni *et al.*, 2014) or residual stomatal transpiration after maximum stomatal closure (Burghardt and Riederer, 2003) have been used. Unfortunately, so far, there is no reliable method to detect to what extent potentially incomplete stomatal closure may affect g_{min}. A good approximation is the method described by Santrůcek *et al.* (2004) which is based on the principle that the diffusion of water vapour in the gas phase can be manipulated by using different gases (such as helium, nitrogen, or carbon dioxide), while the diffusion of water vapour in the solid phase of the cuticle is not affected. The differences in the water vapour diffusion coefficients in the different gases can be used to discriminate between the gas phase diffusion of water vapour across the stomatal pores and the solid phase diffusion in the cuticle.

There are only two studies which rigorously tested whether permeances and minimum conductances of the identical leaf material significantly differ. A direct comparison of cuticular permeability determined with isolated cuticles and g_{min} (Burghardt and Riederer, 2003) showed that in four (*Acer campestre*, *Fagus sylvatica*, *Ilex aquifolium*, and *Quercus petraea*) out of five plant species both permeability parameters were equal. Residual stomatal transpiration or leaky stomata did not affect g_{min}. The one exception was *Hedera helix*

where g_{min} was higher than cuticular permeability by a factor of three. Another study comparing the cuticular permeance determined with leaf discs inserted in transpiration chambers and g_{min} of *Teucrium chamaedrys* leaves also revealed no significant differences between the two permeability parameters (Burghardt *et al.*, 2008). The data set used in this meta-analysis (Supplementary Table S1 at *JXB* online) contains a total of nine species (*Acer campestre*, *Fagus sylvatica*, *Ficus benjamina*, *Hedera helix*, *Ilex aquifolium*, *Nerium oleander*, *Prunus laurocerasus*, *Quercus petraea*, and *Teucrium chamaedrys*) for which both permeance and minimum leaf conductance values are available, sometimes published by different authors. Both parameters for cuticular permeability are linearly related (Fig. 2), and the intercept and the slope of the regression line are not significantly different from 0 and 1, respectively. The regression line is also not significantly distinct from a one to one relationship. The data for *F. sylvatica* were not included in this analysis because they are highly variable. In conclusion, there is strong evidence that minimum conductance is a good proxy for cuticular permeability. However, it cannot be excluded *a priori* that the g_{min} measured by the leaf drying method may overestimate in some species the actual permeability of the cuticle of stomatous leaf surfaces because of incomplete stomatal closure. Therefore, cuticular permeance should not uncritically be equated to g_{min} as long as residual stomatal contributions to the total transpirational water loss of a leaf were not quantified experimentally.

Evaluating literature data for cuticular permeability

Cuticular permeability is measured by a variety of methods and in contexts ranging from biophysics to field plant ecology.

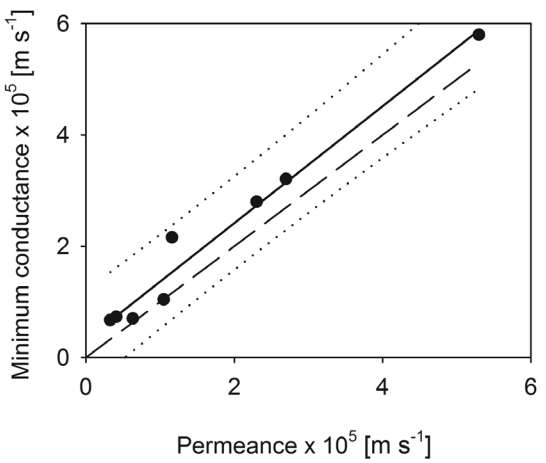

Fig. 2. Linear regression of minimum conductances g_{min} versus permeances p measured for *Acer campestre*, *Ficus benjamina*, *Hedera helix*, *Ilex aquifolium*, *Nerium oleander*, *Prunus laurocerasus*, *Quercus petraea*, and *Teucrium chamaedrys*. Regression equation: g_{min}=3.18 × 10^{-6} (SE 1.70 × 10^{-6})+1.05 (SE 7.27 × 10^{-2})×p (adj. r^2=0.967). The regression line is not significantly different from a one to one relationship (dashed line). Consequently, for these species, means comprising both minimum conductances and permeances were calculated and used for further analysis.

The questions asked also vary and, consequently, the methodologies and the plant species investigated are distinct. The reports are widely dispersed in the literature of many fields, and they are disparate in reliability. Therefore, a compilation and critical evaluation appear to be advantageous for promoting a better understanding of the ecophysiology of cuticular transpiration and permeability.

Values of cuticular permeances p and minimum leaf conductances g_{min} were compiled from reports published from 1996 to 2017. Earlier data had been reviewed by Kerstiens (1996*a*). Both p and g_{min} (Table 1) were included under the assumption that both are comparable and that the influence of potentially leaky stomata is absent or small. It is understood that this assumption may lead to the inclusion of overestimated values of g_{min} in the present analysis.

Surprisingly, over the last 20 years, only 42 and 17 publications reported values of g_{min} and p, respectively (Supplementary Table S1). In total, 382 individual data including in some cases multiple data for a single species and in three cases means for groups of species (eight *Eucalyptus* species, seven terrestrial *Cymbidium*, and 13 epiphytic *Cymbidium* species) were evaluated. If necessary, g_{min} values were converted from mmol m^{-2} s^{-1} to m s^{-1} and, for illustrative purposes, values in m s^{-1} were also converted to mmol m^{-2} s^{-1}. For conversion, standard atmospheric pressure and 25 °C were assumed as experimental conditions. Only the p and g_{min} values with the unit m s^{-1} were used for further analysis. A critical issue when evaluating the literature values was the fact that in ecophysiology the projected leaf area is used for calculating transpiration rates and subsequently g_{min}. Therefore, if not indicated otherwise, the projected leaf area was doubled for obtaining genuinely leaf surface-based cuticular permeabilities (accounting for both the adaxial and abaxial leaf sides) which can be compared with values of p. In some reports, only transpiration rates are given. These data were not included in this survey because frequently the experimental conditions (air and leaf temperature, relative humidity, and light) are not indicated with sufficient precision. Also, transpiration rates relating to the (fresh) weight rather than the surface area were not considered.

The range of all data was from 2.55 × 10^{-7} (*Zamioculcas zamiifolia*; Karbulková *et al.*, 2008) to 4.00 × 10^{-3} m s^{-1} (*Ipomoea batatas*; Zobayed *et al.*, 2000). The distribution has an extreme positive skew, and 347 out of 382 individual values are in the first bin from zero to 20 × 10^{-5} m s^{-1} (Fig. 3A) which is a clear indication of the presence of a few extreme outliers. Tukey's boxplot rule yielded a value of 3.1 × 10^{-4} above which data can be considered as outliers. This reduced the number of data considered in the further analysis to 367. A total of 109 data (30%) permeance p values were measured with isolated cuticles or astomatous leaf surfaces, while the rest were derived from leaf drying curves. The distribution of all data still has a prominent positive skew but is much more even (Fig. 3B). From this set of data, species means were calculated where appropriate. So, data for 157 species and three groups of species were subjected to further analysis.

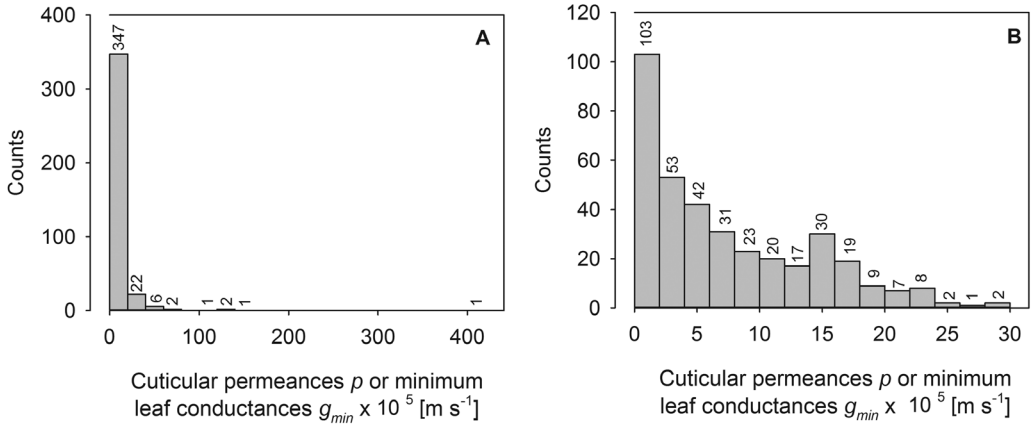

Fig. 3. Distribution of all cuticular permeabilities collected from the literature published from 1996 to 2017 (see Supplementary Table S1). (A) Distribution of all data. (B) Distribution after the exclusion of 15 outliers.

Cuticular transpiration in relation to life form groups

It is striking that despite the removal of outliers, the species values range over a factor of 1122 from 2.55×10^{-7} (*Zamioculcas zamiifolia*) to 2.86×10^{-4} m s^{-1} (*Pinus pumila*). The overall median is 3.60×10^{-5} m s^{-1} and the central 50% of the species have cuticular permeabilities ranging from 1.02×10^{-5} m s^{-1} to 7.20×10^{-5} m s^{-1}. The upper and lower limits of the interquartile range (IQR) differ only by a factor of 7.1, which is comparatively small regarding the total variability of cuticular permeabilities.

Nevertheless, it is intriguing to ask whether the variability in cuticular permeability is related to plant properties of ecological relevance. Intuition and textbook knowledge lead to the common assumption that plants living under conditions where the availability of water is limited or extremely variable should have lower cuticular permeabilities as compared with those living in humid conditions. Earlier attempts to explain differences compared cuticular permeabilities of evergreen with those of deciduous leaves (Kerstiens, 1996*b*; Schuster *et al.*, 2016). Kerstiens (1996*b*) claimed that there is a tendency for evergreen leaves to have lower values of cuticular permeance or minimum leaf conductance than deciduous leaves. However, it is unclear which plant species were included in this comparison. Schuster *et al.* (2016) compared cuticular permeabilities of 12 deciduous and 13 evergreen woody species. The median value of the deciduous species was 2.10×10^{-5} m s^{-1}, and the median value of the evergreen species was 1.05×10^{-5} m s^{-1}, but both groups show a broad range of overlapping values.

Two other studies categorized cuticular permeabilities according to leaf anatomy and habitat. The lowest permeabilities were found for evergreen leaves from epiphytic or climbing plants from the tropics, followed by evergreen plant species typically growing in Mediterranean-type climates. The highest permeabilities were found for deciduous plant species with mesomorphic leaves growing in temperate climates (Schreiber and Riederer, 1996; Riederer and Schreiber, 2001). Again, a broad range of overlapping values was detected.

In the present review, we include all values for permeances and minimum leaf conductances published during the last 20 years, group them into life form groups, and subject them to statistical analyses. The species considered here were assigned to 11 life form groups which were derived from the 'life forms of world terrestrial vegetation' as proposed by Box (2012). In some cases, several life forms according to Box had to be assigned to one life form group because of an insufficient amount of data or imprecise information on the life form of a species (Supplementary Table S2). The life form approach for classification was chosen since it is commonly accepted that plant traits are also determined by numerous factors other than climate and habitat. For instance, leaf longevity has pronounced effects on many leaf properties.

The cuticular permeabilities within each life form group are not normally distributed and, therefore, non-parametric measures are used to characterize the groups (Table 2). The life form group of deciduous woody plants (trees and shrubs) includes cold-deciduous, temperate plant species such as *Acer*, *Populus*, and *Quercus*, as well as drought-deciduous plant species. A total of 39 plant species represented by 77 individual measurements were included in this group. The group median is 4.72×10^{-5} m s^{-1} and the IQR containing the central 50% of all species values is from 1.93×10^{-5} m s^{-1} to 7.40×10^{-5} m s^{-1}. A total of 54 species were assigned to the life form group of evergreen woody plants (trees and shrubs) as well as a mean permeability value from eight *Eucalyptus* species (81 permeability data). Among others, different *Acacia*, *Banksia*, and *Eucalyptus* species are included. In the cases of *Ruscus aculeatus* and *Ruscus microglossum*, g_{min} had been measured for the phylloclades. The group median is 3.16×10^{-5} m s^{-1}, and the IQR ranges from 9.90×10^{-6} m s^{-1} to 5.79×10^{-5} m s^{-1}. The life form group of evergreen conifers includes six species and is represented by 35 individual data.

For the life form group of dwarf shrubs, only data for *Helianthemum apenninum* and *Teucrium chamaedrys* were available. Three *Vitis* species (*V. labrusca*, *V. vinifera*, and *V. berlandieri*×*V. rupestris*) with 16 individual values were assigned to the life form group of broad-summergreen vines. The life form group of evergreen vines and lianas comprises 15 species with 27 individual data. This group consists of evergreen climbers such as *Hedera helix* and *Vanilla planifolia* having very low cuticular permeabilities and hemiepiphytes

Table 2. *Number of species and genus means assigned to the different life form groups (see Supplementary Table S2) and the medians of the cuticular permeability in the respective groups*

Values in the units of mmol m^{-2} s^{-1} are given for illustrative purposes only and, where appropriate, were calculated assuming standard pressure and a temperature of 25 °C. Significant pairwise differences between the life forms groups according to Dunn's method are indicated by equal numbers.

Life form group	Number of species and genus means	Median of cuticular permeability		Significant differences (P<0.050)
		(m s^{-1})	(mmol m^{-2} s^{-1})	
Deciduous woody plants (trees and shrubs)	39	4.72×10^{-5}	1.93	
Evergreen woody plants (trees and shrubs)	54	3.16×10^{-5}	1.29	
Evergreen conifers	6	4.94×10^{-5}	2.02	
Dwarf shrubs	2	6.52×10^{-5}	2.67	
Broad-summergreen vines	3	9.57×10^{-5}	3.91	3
Evergreen vines and lianas	15	5.15×10^{-6}	0.21	4; 6
Epiphytes	13	1.70×10^{-6}	0.07	3; 5; 7
Non-evergreen forbs	18	1.04×10^{-4}	3.76	2; 4; 5
Evergreen forbs	3	6.81×10^{-6}	0.28	1; 2
Graminoids	3	6.51×10^{-5}	2.66	
Herbaceous crop plants	4	1.73×10^{-4}	7.09	1; 6; 7
Total	160	3.60×10^{-5}	1.47	

from the genera *Ficus* and *Philodendron*. The group median is 5.15×10^{-6} m s^{-1} (IQR from 7.03×10^{-6} m s^{-1} to 9.77×10^{-6} m s^{-1}). The life form group with the lowest median (1.70×10^{-6} m s^{-1}; IQR from 1.73×10^{-6} m s^{-1} to 3.19×10^{-5} m s^{-1}) is the epiphytes represented by 12 species (e.g. from the genera *Anthurium* and *Sobralia*) as well as a mean value of 13 epiphytic *Cymbidium* species.

The life form group of non-evergreen forbs includes a total of 18 herbaceous plant species, such as *Helianthus*, wild *Glycine*, and *Solanum* species, with a group median of 1.04×10^{-4} m s^{-1} (IQR from 1.34×10^{-5} m s^{-1} to 1.10×10^{-4} m s^{-1}). The life form group of evergreen forbs is represented only by *Clivia miniata* and *Zamioculcas zamiifolia*, as well as the mean cuticular permeability of seven terrestrial *Cymbidium* species. With *Eragrostis lehmanniana*, *Digitaria californica*, and *Sesleria albicans*, a very small number of species also belong to the graminoid life form group. Herbaceous crop plants (*Glycine max*, *Oryza sativa*, *Oryza glaberrima*, and *Hordeum vulgare*) were assigned to a separate group because, over several thousands of years, human selection and breeding priorities may have led to cultivars whose cuticular permeability does not have a specific ecological relevance. A total of 77 individual permeability values were available for this group.

Visualizing the data as Tukey's boxplots (Fig. 4) demonstrates that the data for cuticular permeability in most life form groups are distributed over a wide range, with large IQR and outliers outside the 90th and 10th percentiles. In most cases, the medians are much lower than the means, showing that the data do not follow normal distributions and have a positive skew, meaning that within groups most values are in the lower range.

The species data were assigned to life form groups under the assumption that cuticular permeability is an adaptation specific for a given life form to its environmental conditions and life strategy. This hypothesis was tested by a Kruskal–Wallis one-way ANOVA on ranks. This analysis showed that the differences in the median values among the groups are greater than would be expected by chance. So, there are group medians which are statistically significantly different (P<0.001) from each other, supporting the hypothesis that cuticular permeability varies with life form. An all pairwise multiple comparison procedure (Dunn's method) was used to isolate the life form group or groups that significantly differ from the others. Only in 7 out of 55 pairs were significant differences (P<0.05) detected. The median cuticular permeability of both the non-evergreens forbs and the herbaceous crop plants significantly differ from that of the epiphytes, the evergreen vines and lianas, and the evergreen forbs. The broad-summergreen vines and the epiphytes also significantly differ in median cuticular permeability. So, a clear finding of this study is that evergreen plants with an epiphytic or climbing habit have significantly lower cuticular permeabilities as compared with all other life form groups in this study. This may be explained by the absence of a soil water reservoir in epiphytes and a limited water transport capacity in climbers and lianas. No significant differences were found between the graminoids and the epiphytes, the herbaceous crop plants and the evergreen woody plants, and the broad-summergreen vines and the evergreen forbs. The remaining 45 pairs could not be tested due to insufficient numbers of data.

A surprising finding is that, based on the data presently available, the cuticular water permeability of leaves of deciduous and evergreen plants does not differ significantly (Mann–Whitney rank sum test, P=0.119). Looking more closely at the data in both groups reveals some very high values of cuticular permeabilities (Fig. 4) which, in all cases, were measured by the leaf drying method and, thus, potentially may be caused by a leaky stomata error. So, the test was repeated with all

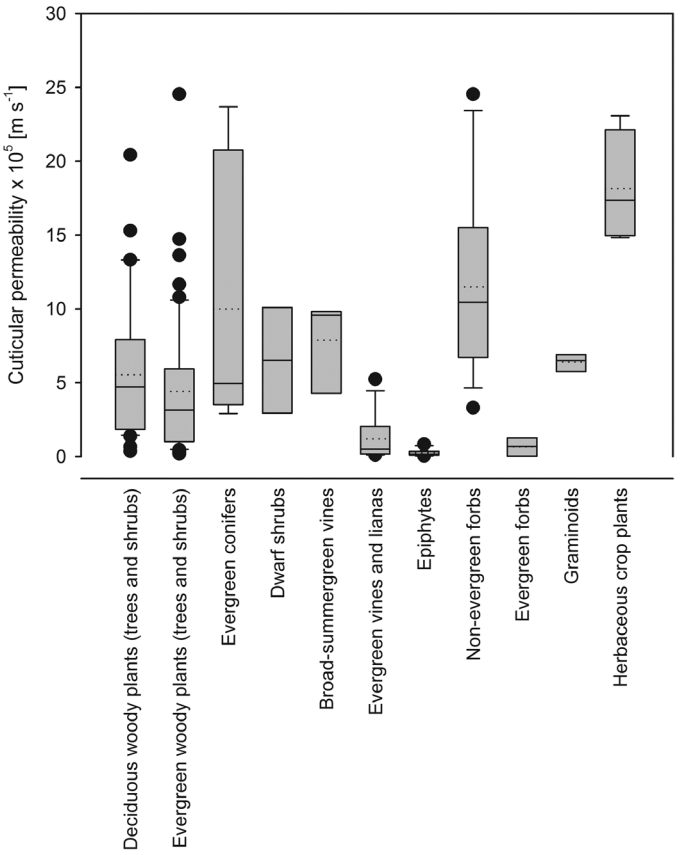

Fig. 4. Tukey's boxplots of the cuticular permeabilities of 160 species or species groups (see text for further explanation). The species were assigned to the life form groups defined in Supplementary Table S2. The boundary of the box closest to zero indicates the 25th percentile, a solid line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots represent outlying points, and the dotted line within the box the mean. Note that some life form groups contain only very small numbers of species.

data which were higher than the 90th percentile in each group excluded. Again, no significant difference between deciduous and evergreen woody plants could be detected (P=0.063), indicating that based on present evidence in woody plants the cuticular permeability is not related to the life span of the leaves.

Conclusions

This comprehensive meta-analysis of data on the cuticular permeability of leaves published during the last 20 years leads to the following conclusions.

(i) The epiphytes and the evergreen climbers and lianas are the only groups which significantly differ in cuticular permeability from the remaining life form groups studied here.

(ii) All other groups do not differ from each other or differences cannot be tested due to insufficient data availability.

(iii) In certain cases, the variability of the data within groups is very high. Most prominent are some very high permeabilities which may be the result of experimental insufficiencies when measuring minimum leaf conductances.

(iv) The high variability may also be due to the definition of the life form groups used in this study. Broad definitions had to be adopted for sufficiently populating the different groups with data, but they may lead to artificial heterogeneities.

As a whole, the present data show that except for two special cases (epiphytes, and climbers and lianas) which, however, only have minor importance for global vegetation cover, the available data are not sufficient to elucidate ecological adaptations regarding cuticular permeability—if these adaptations do exist at all. Much more data must be collected from species carefully chosen for this purpose before the widespread notion of the existence of ecological adaptions of cuticular permeability can be tested based on reliable experimental evidence. Moreover, methodological progress for quantifying the contribution of residual transpiration from not completely closed stomata must be achieved when measuring minimum leaf conductances.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Leaf cuticular permeabilities from the literature published from 1996 to 2017.

Table S2. Life form groups used in this overview and their relationship to the life forms of world terrestrial vegetation as defined by Box (2012).

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References

Anfodillo T, Pasqua di Bisceglie D, Urso T. 2002. Minimum cuticular conductance and cuticle features of *Picea abies* and *Pinus cembra* needles along an altitudinal gradient in the Dolomites (NE Italian Alps). Tree Physiology **22,** 479–487.

Box EO. 2012. Macroclimate and plant forms: an introduction to predictive modeling in phytogeography. Dordrecht: Springer Science & Business Media.

Brodribb TJ, McAdam SAM, Jordan GJ, Martins SCV. 2014. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. Proceedings of the National Academy of Sciences, USA **111,** 14489–14493.

Burghardt M, Burghardt A, Gall J, Rosenberger C, Riederer M. 2008. Ecophysiological adaptations of water relations of *Teucrium chamaedrys* L. to the hot and dry climate of xeric limestone sites in Franconia (Southern Germany). Flora **203,** 3–13.

Burghardt M, Riederer M. 2003. Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. Journal of Experimental Botany **54,** 1941–1949.

Burghardt M, Riederer M. 2006. Cuticular transpiration. In: Riederer M, Müller C, eds. Biology of the plant cuticle. Oxford: Blackwell Publishing, 293–310.

Buschhaus C, Jetter R. 2012. Composition and physiological function of the wax layers coating *Arabidopsis* leaves: β-amyrin negatively affects the intracuticular water barrier. Plant Physiology **160,** 1120–1129.

Caird MA, Richards JH, Donovan LA. 2007. Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. Plant Physiology **143,** 4–10.

Grace J. 1990. Cuticular water loss unlikely to explain tree-line in Scotland. Oecologia **84,** 64–68.

Howard AR, Donovan LA. 2007. *Helianthus* nighttime conductance and transpiration respond to soil water but not nutrient availability. Plant Physiology **143,** 145–155.

Jetter R, Kunst L, Samuels AL. 2006. Composition of plant cuticular waxes. In: Riederer M, Müller C, eds. Biology of the plant cuticle. Oxford: Blackwell Publishing, 146–181.

Jetter R, Riederer M. 2016. Localization of the transpiration barrier in the epi- and intracuticular waxes of eight plant species: water transport resistances are associated with fatty acyl rather than alicyclic components. Plant Physiology **170,** 921–934.

Karbulková J, Schreiber L, Macek P, Santrucek J. 2008. Differences between water permeability of astomatous and stomatous cuticular membranes: effects of air humidity in two species of contrasting drought-resistance strategy. Journal of Experimental Botany **59,** 3987–3995.

Kerstiens G. 1996*a*. Cuticular water permeability and its physiological significance. Journal of Experimental Botany **47,** 1813–1832.

Kerstiens G. 1996*b*. Diffusion of water vapour and gases across cuticles and through stomatal pores presumed closed. In: Kerstiens G, ed. Plant cuticles an integrated functional approach. Oxford: BIOS Scientific Publishers Limited, 121–134.

Leide J, Hildebrandt U, Reussing K, Riederer M, Vogg G. 2007. The developmental pattern of tomato fruit wax accumulation and its impact on cuticular transpiration barrier properties: effects of a deficiency in a beta-ketoacyl-coenzyme A synthase (LeCER6). Plant Physiology **144,** 1667–1679.

Leide J, Hildebrandt U, Vogg G, Riederer M. 2011. The positional sterile (ps) mutation affects cuticular transpiration and wax biosynthesis of tomato fruits. Journal of Plant Physiology **168,** 871–877.

Nobel PS. 2009. Physicochemical and environmental plant physiology. San Diego: Elsevier Academic Press.

Oliveira AFM, Meirelles ST, Salatino A. 2003. Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. Annals of the Brazilian Academy of Sciences **75,** 431–439.

Pérez-Harguindeguy N, Díaz S, Garnier E, *et al*. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany **61,** 167–234.

Pisek A, Berger E. 1938. Kutikuläre Transpiration und Trockenresistenz isolierter Blätter und Sprosse. Planta **28,** 124–155.

Pollard M, Beisson F, Li Y, Ohlrogge JB. 2008. Building lipid barriers: biosynthesis of cutin and suberin. Trends in Plant Science **13,** 236–246.

Riederer M, Schreiber L. 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. Journal of Experimental Botany **52,** 2023–2032.

Santrůcek J, Simánová E, Karbulková J, Simková M, Schreiber L. 2004. A new technique for measurement of water permeability of stomatous cuticular membranes isolated from *Hedera helix* leaves. Journal of Experimental Botany **55,** 1411–1422.

Schönherr J, Lendzian K. 1981. A simple and inexpensive method of measuring water permeability of isolated plant cuticular membranes. Zeitschrift für Pflanzenphysiologie **102,** 321–327.

Schreiber L, Riederer M. 1996. Ecophysiology of cuticular transpiration: comparative investigation of cuticular water permeability of plant species from different habitats. Oecologia **107,** 426–432.

Schreiber L, Schönherr J. 2009. Water and solute permeability of plant cuticles, measurement and data analysis. Berlin: Springer.

Schuster AC. 2016. Chemical and functional analyses of the plant cuticle as leaf transpiration barrier. PhD thesis, Würzburg University.

Schuster AC, Burghardt M, Alfarhan A, Bueno A, Hedrich R, Leide J, Thomas J, Riederer M. 2016. Effectiveness of cuticular transpiration barriers in a desert plant at controlling water loss at high temperatures. AoB Plants **8,** plw027.

Scoffoni C, Vuong C, Diep S, Cochard H, Sack L. 2014. Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. Plant Physiology **164,** 1772–1788.

Slavic B. 1974. Methods of studying plant water relations. Berlin: Springer.

Van Gardingen PR, Grace J. 1992. Vapour pressure deficit response of cuticular conductance in intact leaves of *Fagus sylvatica* L. Journal of Experimental Botany **43,** 1293–1299.

Vogg G, Fischer S, Leide J, Emmanuel E, Jetter R, Levy AA, Riederer M. 2004. Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid beta-ketoacyl-CoA synthase. Journal of Experimental Botany **55,** 1401–1410.

Walden-Coleman AE, Rajcan I, Earl HJ. 2013. Dark-adapted leaf conductance, not minimum leaf conductance, predicts water use efficiency of soybean (*Glycine max* L. Merr.). Canadian Journal of Plant Science **93,** 13–22.

Yeats TH, Rose JK. 2013. The formation and function of plant cuticles. Plant Physiology **163,** 5–20.

Zobayed SMA, Afreen F, Kubota C, Kozai T. 2000. Water control and survival of *Ipomoea batatas* grown photoautotrophically under forced ventilation and photomixotrophically under natural ventilation. Annals of Botany **86,** 603–610.