Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential*

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Abstract

The water permeability of the leaves of three deciduous plants (Acer campestre, Fagus sylvatica, Quercus petraea) and two evergreen plants (Hedera helix, Ilex aquifolium) was analysed in order to assess its role as a mechanism of drought resistance. Cuticular permeances were determined by measurement of the water loss through adaxial, astomatous leaf surfaces. Minimum conductances after complete stomatal closure were obtained by leaf drying curves. The comparison of the water permeabilities determined with these two experimental systems revealed good agreement in the case of Acer, Fagus, Quercus, and Ilex. For Hedera the minimum conductance was 3-fold higher than the cuticular permeance indicating a significant contribution of residual stomatal transpiration. The leaf water potential was measured as a function of water content and analysed by pressure–volume curves. The influence of water potential as a component of the driving force for transpirational water loss was assessed in order to identify modifications of the cuticular barrier by the leaf water content. The eco-physiological meaning of the water relations parameters describing transpiration under drought conditions (cuticular transpiration, minimum transpiration, residual stomatal transpiration, effect of leaf water content on transpiration) and the water relations parameters derived from pressure–volume curves (osmotic potential at full saturation, turgor loss point, bulk modulus of elasticity) are discussed with regard to adaptations for drought resistance.

Key words: Cuticular permeance, drought resistance, minimum conductance, plant cuticle, pressure–volume analysis, stomatal closure, water potential, water stress.

Introduction

The regulation of water relations is a fundamental prerequisite for the survival of terrestrial plants. Therefore, morphological and physiological adaptations are realized in order to attain drought resistance, which can be divided into the strategies of drought escape, drought tolerance and drought avoidance (Ludlow, 1989).

Leaves represent the main interface between the plant and the atmosphere. Restriction of water loss to a minimum is of decisive importance under conditions of severe drought. This is mainly achieved by the coverage of leaf surfaces with the cuticle which acts as an effective barrier against water loss (Schönherr, 1982). Cuticular transpiration has been studied by the measurement of the water loss through astomatous leaf surfaces. The most prominent approach used isolated cuticular membranes (Riederer and Schreiber, 2001). The number of species suitable for cuticle isolation is limited, but hypostomatous leaf discs were recently used successfully for the measurement of cuticular transpiration (Hoad et al., 1996; Schreiber, 2001). In these systems, any interference from stomata can be completely excluded. Therefore, the water permeability has been described by the term ‘cuticular permeance’ (P) (Kerstiens, 1996). A survey of the cuticular water permeabilities measured so far points in the direction that adaptations of the cuticular barrier are realized depending on life form and growth habitat.
Material and methods

Plant material

The deciduous species *Acer campestre* L., *Fagus sylvatica* L. and *Quercus petraea* L. and the evergreen species *Ilex aquifolium* L. and *Hedera helix* L. were selected. All species exhibit hypostomatus leaves. Fully expanded leaves of similar exposure (south-western) and height (2 m) were harvested in July and August 2001 from plants growing in the Botanical Garden of the University of Würzburg.

Leaf parameters

The degree of sclerophyll (\(S_c\)):

\[
S_c = \frac{DW}{A}
\]  

and the degree of succulence (\(S_a\)):

\[
S_a = \frac{FW_s - DW}{A}
\]

were determined from the saturation fresh weight (\(FW_s\)), the dry weight (\(DW\)) and the leaf area (\(A\)), respectively. The relative water content (\(RWC\)):

\[
RWC = \frac{FW - DW}{FW_s - DW}
\]

and the relative water deficit (\(RWD\)):

\[
RWD = 1 - RWC
\]

were used for characterizing the water status according to the actual fresh weight (\(FG\)). The saturation fresh weight was determined by saturation of the detached leaves in a humid chamber with the cut petioles immersed in water until a constant weight was reached. For all species, full resaturation of the leaves was reached after 2 h. The dry weight was obtained by keeping the leaves at 90 °C until a constant weight was reached.

Sublethal water deficit

The sublethal water deficit was chosen as a parameter to ensure that leaf drying curves and pressure–volume curves were recorded only in the absence of detectable irreversible damage. The leaves were saturated with water and different degrees of water deficits were adjusted by exposing the leaves to transpirational water loss on the laboratory bench. Afterwards the leaves were resaturated. The sublethal water deficit was identified as the relative water deficit at which the resaturation weight was lower than 90% of the initial saturation weight (Weinberger et al., 1972).

Cuticular transpiration

Cuticular permeances were determined by the measurement of water loss through adaxial, astomatous leaf surfaces. Leaf discs of 2 cm diameter were punched out. In the case of *Acer campestre*, *Fagus sylvatica* and *Quercus petraea* they were used directly for the experiments. In the case of *Hedera helix* and *Ilex aquifolium* cuticular membranes were isolated by incubation of the leaf discs in an enzymatic solution of cellulase (Cellulast; Novo Nordisk, Bagsvaerd, Denmark) and pectinase (Trenolin Super DF; Erbslöh, Geisenheim/Rhein, Germany). After 3 weeks the isolated cuticles were removed, washed in deionized water and stored until their experimental usage (Schönherr and Riederer, 1986; Schreiber and Riederer, 1996). The cuticular membranes and the leaf discs were mounted in transpiration chambers made out of stainless steel (Schönherr and Lendzian, 1981; Knoche et al., 2000) with the upper side orientated towards the atmosphere. The donor compartment of the transpiration chamber was filled with 1 cm³ water. In order to ensure that the water flow into the leaf discs was not limited, the lower epidermis was disrupted with carborundum powder. The transpiration chambers were placed upside down in plastic boxes containing silica gel stored at 25 °C. The water loss across the exposed area of the transpiration chamber was measured by weighing the chambers at regular time intervals (SBC 21 semi-micro balance; Scetec, Göttingen, Germany; ±0.1 mg). In all cases the plots of the weight loss (\(W\)) versus the time (\(t\)) were linear, as shown previously for this type of experiment (Schreiber and Riederer, 1996; Knoche et al., 2000). The transpiration rate (\(T\)) at...
maximum driving force was calculated from the slope of the regression line divided by the exposed area (A, 1.13 cm²):

\[ T = \frac{\Delta W}{\Delta t \cdot A} \]  

(5)

The cuticular permeance (P) was obtained from the cuticular transpiration rate (Tcut) divided by the concentration difference of water across the cuticular membrane acting as driving force:

\[ P = \frac{T_{cut}}{\rho (\delta_{chamber} - \delta_{air})} \]  

(6)

Since the transpiration chambers were filled with pure water, the corresponding water activity in the chamber (δchamber) is equal to unity. The humidity of the air was controlled by silica gel resulting in a water activity (δair) close to zero (Slavik, 1974). Therefore, the driving force for transpirational water loss is identical to the density of pure liquid water (ρ). Permeances were calculated on the basis of the equivalent vapour-phase density which amounts to 23.07 g m⁻³ at 25°C (Nobel, 1991).

**Leaf drying curves**

The minimum transpiration after complete stomatal closure was measured by the mass loss of detached leaves. The leaves were saturated with water and, afterwards, the cut petioles were sealed with paraffin wax (solidification point 40°C; Fluka, Neu-Ulm, Germany). The leaves were fixed on a holder in order to expose both leaf surfaces to the surrounding air and placed on a balance at 25°C in a climatic chamber. A bonnet containing silica gel ensured that the leaves were kept in an atmosphere of controlled humidity. The balance was connected to a computer which allowed an automatic registration of the fresh weight at periodical time intervals. Since the transpiration rate decreased initially with time, the transpiration rate was calculated according to equation (5) for each time interval separately. After a critical value of the relative water deficit was reached, the water loss decreased linearly with time leading to a constant minimum transpiration rate. Analogous to equation (6) the minimum conductance (gmin) was calculated from the minimum transpiration rate (Tmin) divided by the driving force:

\[ g_{min} = \frac{T_{min}}{\rho (\delta_{chamber} - \delta_{air})} \]  

(7)

The activity of liquid water in the leaf interior (δleaf) was assumed to be unity, following the general proposal in ecophysiological literature (Kerstiens, 1996). The validity of this assumption will be assessed in detail in the discussion section.

For *Hedera helix*, leaf drying curves were also measured under the condition that water loss solely occurred through the adaxial, stomatous side of the leaves. After harvesting the abaxial, stomatous side was coated with paraffin wax. The subsequent procedures were carried out as for the uncoated leaves. The dry weight was determined after removal of the paraffin wax by washing the leaves in warm water. Both leaf sides were coated with paraffin wax in order to measure the effectiveness of the sealing. In addition, the water release from the sealant was assessed by coating an artificial surface (aluminium foil) with paraffin wax. In both control experiments the amount of water loss per unit of time and of area was one order of magnitude lower than the transpiration rate of the leaves of *Hedera helix* and can be considered as negligible.

**Pressure–volume curves**

The water potential of the leaves was measured with a pressure bomb. Different levels of water deficits were adjusted by exposing the leaves to transpirational water loss on the laboratory bench. Pressure–volume curves were constructed by plotting the reciprocal of the water potential (Ψ) versus the relative water deficit (RWD). Regression analysis was applied to find the best fit to the linear portion of the curve representing the osmotic potential term (Ψsat):

\[ \frac{1}{\Psi_s} = \frac{1}{\Psi_{sat}} - \frac{RWD}{X \Psi_{sat}} \]  

(8)

From the regression line, the turgor loss point, the osmotic potential at full saturation (Ψsat) and the symplastic fraction of the total water content (X) were determined. The bulk modulus of elasticity (ε) was obtained from the initial part of the curve:

\[ \varepsilon = \frac{d\Psi_s}{dRWC} \cdot RWC_{sym} \]  

(9)

describing the decrease of turgor potential (Ψs) with the relative symplastic water content (RWCsym) (Tyree and Jarvis, 1982).

**Statistics**

All measurements are based on at least 10 replicates and are given as means with 95% confidence intervals. Cuticular permeances and minimum conductances follow a log-normal distribution (Baur, 1997). Therefore, statistical treatment and significance tests were carried out after logarithmic transformation of the data.

**Results**

**Leaf parameters**

The evergreen leaves of *Hedera helix* and *Ilex aquifolium* distinctly differ from the leaves of the deciduous species *Acer campestre*, *Fagus sylvatica* and *Quercus petraea* by their higher degree of sclerophyll and higher degree of succulence (Table 1).

**Sublethal water deficit**

The sublethal water deficit as determined by the dehydration–resaturation experiments indicates that irreversible damage of the leaves occurs for all species, if roughly half of the saturation water content is lost (Table 1).

**Cuticular transpiration**

The cuticular transpiration rates at maximum driving force of the evergreen species are about one order of magnitude lower than for the deciduous species. The corresponding cuticular permeances, based on vapour-phase concentration differences, fall for the evergreen leaves in the range of 10⁻⁶ m s⁻¹ and for the deciduous leaves in the range of 10⁻⁵ m s⁻¹ (Table 2).

**Minimum transpiration**

The leaf drying curves are characterized by an initial decline of the transpiration rate followed by a plateau with a minimum and constant transpiration rate (Figs 1A, 2A). In the cases of *Acer campestre*, *Fagus sylvatica*, *Quercus petraea*, and *Ilex aquifolium* the minimum conductance after the point of inflection is not significantly different.
from the cuticular permeance (Student’s t-test of the logarithmic transformed data, \(P > 0.05\)). In the case of *Hedera helix* the 3-fold higher value of the minimum conductance is significantly different from the cuticular permeance (\(P=10^{-16}\)). For the abaxially coated leaves of *Hedera helix* the leaf drying curve also exhibits a point of inflection, though the initial decline of the transpiration rate is not as pronounced as for the uncoated leaves (Fig. 1B). The minimum conductance after the point of inflection is not significantly different from the cuticular permeance (\(P=0.2\)) (Table 3).

**Pressure–volume analysis**

The water relations parameters obtained from pressure–volume curves (Figs 1C, 2B) reveal that the evergreen leaves exhibit a lower osmotic potential at full saturation, a lower water potential at the turgor loss point, a higher bulk modulus of elasticity, and a lower symplastic portion of the total water content than the deciduous leaves (Table 4).

**Discussion**

**Classification of the leaves**

The leaves of the deciduous and evergreen species can be classified as mesomorphic and scleromorphic, respectively. The mesomorphic leaves of trees and shrubs in Central Europe are characterized by a mean degree of sclerophyll of 39 g m\(^{-2}\) and a mean degree of succulence of 56 g m\(^{-2}\) (Stocker, 1976). The three deciduous species fit to these general values quite well, while the two evergreen species have significant higher degrees of sclerophyll and succulence (Table 1). Scleromorphic leaf structures are often associated with plants growing under drought-prone conditions. This implies that sclerophyllous leaves exhibit characteristic features of xeromorphic leaves and develop effective mechanisms of drought resistance (Groom and Lamont, 1997; Bussotti *et al*., 2002). For the species selected in the present study these mechanisms will be outlined in the following sections. In Central Europe drought occurs mainly during the winter and is caused by a restriction of the water uptake from frozen soil (Larcher, 1972). Evergreen leaves have to be better adapted to the drought period while deciduous leaves escape this situation by the loss of leaves. Therefore, pronounced differences of the drought resistance can be expected.

**Drought tolerance**

The sublethal water deficit indicates the occurrence of irreversible damage. Dehydration–resaturation experiments are only one method among many to assess drought tolerance (Slavik, 1974). Though the sublethal water deficit is only a rough parameter which has to be used carefully as a descriptor of drought tolerance (Larcher, 1975), the limited ability of both the evergreen and the deciduous species to tolerate dehydration is demonstrated. Accordingly, the measurements of transpiration and water potential were carried out in the range of relative water deficits below the sublethal value in order to ensure that the water relations parameters are representative for intact vital leaves. However, if leaf drying curves were carried out over the whole range of water deficits, the appearance of the sublethal water deficit did not influence the course of the curve (Fig. 2A).

**Effect of leaf water content and water potential on leaf drying curves**

Leaf drying curves can be separated into the following stages: the stomatal phase, the closing phase and the phase of minimum transpiration after complete stomatal closure (Hygen, 1951; Cape and Percy, 1996). The decline of the

<table>
<thead>
<tr>
<th>Species</th>
<th>(S_c) (g m(^{-2}))</th>
<th>(S_u) (g m(^{-2}))</th>
<th>(RWD_{sld})</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer campestre</em></td>
<td>25 (23–27)</td>
<td>56 (48–64)</td>
<td>0.55 (0.46–0.64)</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>32 (29–35)</td>
<td>58 (52–64)</td>
<td>0.48 (0.29–0.66)</td>
</tr>
<tr>
<td><em>Quercus petraea</em></td>
<td>33 (30–36)</td>
<td>68 (61–75)</td>
<td>0.56 (0.34–0.61)</td>
</tr>
<tr>
<td><em>Ilex aquifolium</em></td>
<td>67 (64–70)</td>
<td>100 (95–105)</td>
<td>0.46 (0.28–0.63)</td>
</tr>
<tr>
<td><em>Hedera helix</em></td>
<td>53 (48–58)</td>
<td>89 (82–96)</td>
<td>0.52 (0.35–0.68)</td>
</tr>
</tbody>
</table>

**Table 2. Cuticular transpiration rate (\(T_{cut}\)) at maximum driving force and cuticular permeance (\(P\)) determined with isolated cuticular membranes (*Hedera helix* and *Ilex aquifolium*) and astomatous leaf discs (*Acer campestre*, *Fagus sylvatica* and *Quercus petraea*)**

The results are given as means with 95% confidence intervals calculated on the basis of logarithmically transformed data.

<table>
<thead>
<tr>
<th>Species</th>
<th>(T_{cut}) (\times 10^3) (g m(^{-2}) s(^{-1}))</th>
<th>(P) (\times 10^3) (m s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer campestre</em></td>
<td>5.3 (4.2–6.9)</td>
<td>2.3 (1.7–3.0)</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>10 (7.5–13)</td>
<td>4.3 (3.3–5.6)</td>
</tr>
<tr>
<td><em>Quercus petraea</em></td>
<td>12 (8.6–17)</td>
<td>5.3 (3.8–7.4)</td>
</tr>
<tr>
<td><em>Ilex aquifolium</em></td>
<td>1.4 (1.0–2.2)</td>
<td>0.63 (0.42–0.94)</td>
</tr>
<tr>
<td><em>Hedera helix</em></td>
<td>0.56 (0.42–0.78)</td>
<td>0.24 (0.18–0.33)</td>
</tr>
</tbody>
</table>

The results are given as means with 95% confidence intervals. The results are given as means with 95% confidence intervals.
transpiration rate after initiation of the experiment indicates the immediate closure of the stomata and with the onset of desiccation the hydroactive stomatal closing proceeds until a constant and minimum level is reached (Figs 1A, 2A). The appearance of the minimum transpiration rate is closely correlated with the turgor loss point (Figs 1C, 2B) which can be considered as a main indicator for maximum stomatal closure. This finding corresponds with the response of stomata to a critical value of water potential which was found to be identical with the turgor loss point (West and Gaff, 1976; Hinckley et al., 1983).
After complete stomatal closure the maintenance of a constant minimum transpiration rate over a wide range of water deficits may be surprising, since the water potential declines continuously during leaf drying. The alteration of the driving force for transpirational water loss becomes evident when the leaf water potential derived from pressure±volume analysis is converted into water activity according to:

\[ a = e^{\frac{-\psi_v}{RT}} \]

where \( V_w \) is the molar volume of water (1.80 \times 10^{-5} \text{ mol m}^{-3}), \( R \) the gas constant (8.314 J mol K\(^{-1}\)) and \( T \) the absolute temperature. In terms of water activity the decline of water potential is not as dramatic as it seems (Fig. 2C). For example, the leaves of Acer campestre at full saturation exhibit a water potential of zero corresponding to a water activity of unity. When the water potential sinks to a value of –1.9 MPa at the turgor loss point the activity is equal to 0.99 and a further drop to –3.4 MPa at a relative water deficit of 0.5 would result in a water activity of 0.98. Consequently, the comparably small decline of water activity will not cause a significant reduction in the minimum transpiration rate. Therefore, the calculation of minimum conductances according to equation (7) under the assumption that water activity is equal to unity introduces only a negligible error. A dramatic decline of water activity occurs only when the relative water deficit reaches the range of the symplastic portion of the water content. For example, at a relative water deficit of 0.8 the water activity is equal to 0.91, while at a deficit of 0.85 the water activity has declined to 0.66 (Fig. 2C). Accordingly, the reduction of the driving force is reflected by a gradual decline in the transpiration rate which finally reaches zero (Fig. 2A).

**Effect of water potential on the leaf drying curve of abaxially coated leaves**

The leaf drying curve for the abaxially coated leaves of Hedera helix shows an initial 2-fold decline in the transpiration rate (Fig. 1B). This phenomenon, which is also known as ‘incipient drying’ in the earlier literature (Stålfelt, 1956), is assumed to be associated with a change of cuticular permeability caused by dehydration of the cuticular membrane as the leaf water content declines (van Gardingen and Grace, 1992). However, the agreement of the cuticular transpiration rate and the minimum transpiration rate after that initial decline contradicts this hypothesis.

### Table 3. Transpiration parameters obtained from leaf drying curves: relative water deficit at minimum transpiration (RWD\(_{\text{mt}}\)), minimum transpiration rate (T\(_{\text{min}}\)) and minimum conductance (g\(_{\text{min}}\)), and ratio between minimum conductance and cuticular permeance (g\(_{\text{min}}/P\))

<table>
<thead>
<tr>
<th>Species</th>
<th>RWD(_{\text{mt}})</th>
<th>T(_{\text{min}}) (g m(^{-2}) s(^{-1}))</th>
<th>g(_{\text{min}}) (m s(^{-1}))</th>
<th>g(_{\text{min}}/P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer campestre</td>
<td>0.16 (0.14–0.18)</td>
<td>6.4 (5.6–7.5)</td>
<td>2.8 (2.4–3.3)</td>
<td>1.2</td>
</tr>
<tr>
<td>Fagus sylvatica</td>
<td>0.13 (0.11–0.15)</td>
<td>9.2 (7.5–11)</td>
<td>3.9 (3.2–4.8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Quercus petraea</td>
<td>0.12 (0.10–0.14)</td>
<td>13 (11–17)</td>
<td>5.8 (4.7–7.2)</td>
<td>1.1</td>
</tr>
<tr>
<td>Ilex aquifolium</td>
<td>0.067 (0.054–0.080)</td>
<td>1.6 (1.4–1.9)</td>
<td>0.70 (0.59–0.82)</td>
<td>1.1</td>
</tr>
<tr>
<td>Hedera helix</td>
<td>0.11 (0.09–0.13)</td>
<td>1.7 (1.5–1.9)</td>
<td>0.73 (0.66–0.81)</td>
<td>3.0(^a)</td>
</tr>
<tr>
<td>Hedera helix</td>
<td>0.065 (0.057–0.072)</td>
<td>0.64 (0.56–0.75)</td>
<td>0.28 (0.24–0.32)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^a\) In the case of Hedera helix the minimum conductance is significantly higher than the cuticular permeance. In all other cases no significant differences were detected.

### Table 4. Water relations parameters derived from pressure±volume curves: turgor loss point (RWD\(_{\text{tp}}\)), osmotic potential at full saturation (\(\Psi_p^{\text{sat}}\)), osmotic potential at the turgor loss point (\(\Psi_p^{\text{tp}}\)), bulk modulus of elasticity (\(e\)) and symplastic water fraction (\(X\))

The results were obtained from pressure±volume analysis according to the equations (8) and (9) by calculation of the respective regression lines with 95% confidence intervals.

<table>
<thead>
<tr>
<th>Species</th>
<th>RWD(_{\text{tp}})</th>
<th>(-\Psi_p^{\text{sat}}) (MPa)</th>
<th>(-\Psi_p^{\text{tp}}) (MPa)</th>
<th>(e) (MPa)</th>
<th>(X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer campestre</td>
<td>0.16 (0.14–0.18)</td>
<td>1.5 (1.4–1.6)</td>
<td>1.9 (1.7–2.1)</td>
<td>7.9 (7.1–8.7)</td>
<td>0.90 (0.81–0.99)</td>
</tr>
<tr>
<td>Fagus sylvatica</td>
<td>0.14 (0.11–0.17)</td>
<td>1.4 (1.3–1.6)</td>
<td>1.8 (1.5–2.0)</td>
<td>10.7 (9.7–11.7)</td>
<td>0.75 (0.65–0.92)</td>
</tr>
<tr>
<td>Quercus petraea</td>
<td>0.13 (0.10–0.16)</td>
<td>1.6 (1.5–1.7)</td>
<td>1.9 (1.7–2.2)</td>
<td>11.1 (9.9–12.4)</td>
<td>0.76 (0.68–0.87)</td>
</tr>
<tr>
<td>Ilex aquifolium</td>
<td>0.091 (0.07–0.11)</td>
<td>2.3 (2.2–2.4)</td>
<td>2.7 (2.5–3.0)</td>
<td>20.7 (18.7–22.7)</td>
<td>0.60 (0.54–0.68)</td>
</tr>
<tr>
<td>Hedera helix</td>
<td>0.12 (0.09–0.15)</td>
<td>1.8 (1.7–2.0)</td>
<td>2.3 (2.0–2.7)</td>
<td>14.8 (13.1–16.5)</td>
<td>0.62 (0.55–0.73)</td>
</tr>
</tbody>
</table>
since the cuticles in the transpiration chambers were in contact with pure water, equivalent rather to the state of cuticular hydration at full saturation of the leaves. The decline of the cuticular permeability occurs within the range of water deficits below the turgor loss point. Therefore, a mechanical effect on the cuticular barrier may be responsible for the decline of the cuticular permeability by means of stretching of the wax layer at full saturation and tightening of the wax structure as the turgor releases (Boyer et al., 1997). This implies that the decline of cuticular transpiration only occurs for non-isolated cuticles of intact leaves.

Effect of residual stomatal transpiration on minimum conductance

The cuticular water permeability can only be considered as an adaptation to drought under the assumption that residual stomatal transpiration after complete closure is negligible in comparison to cuticular transpiration. This assumption is not necessarily valid. Residual stomatal transpiration has been identified as the major determinant of epidermal transpiration among genotypes of Sorghum bicolor under conditions of drought (Muchow and Sinclair, 1989). A review of the cuticular permeances and minimum conductances available in the literature revealed large discrepancies for some species (Kerstiens, 1996). However, since the data were mainly collected from different references, a final conclusion based on statistical analysis was not possible. Therefore, the present study aims at a more precise analysis. In the case of Acer campestre, Fagus sylvatica, Quercus petraea, and Ilex aquifolium no significant differences between the minimum conductance and the cuticular permeance were detected and the residual stomatal transpiration after complete closure can be considered as negligible. In the case of Hedera helix the minimum conductance is significantly higher than the cuticular permeance. The influence of residual stomatal transpiration after complete closure of the stomata is demonstrated by coating of the abaxial, stomatous side of the leaves with paraffin wax resulting in a minimum conductance equal to the cuticular permeance (Fig. 3). The minimum transpiration \( g_{min} \) results from the water loss via the stomatal pathway \( g_{sto} \) and the cuticular pathway \( P \) representing two resistances connected in parallel:

\[
g_{min} = g_{sto} + P \tag{11}
\]

Accordingly, for Hedera helix the residual stomatal transpiration amounts to \( 4.9 \times 10^{-6} \) \( \text{m s}^{-1} \) and thus contributes 67% to the total minimum transpiration. The agreement between cuticular permeance and minimum conductance for the other species is not necessarily evidence for a more perfect stomatal closure. Since Hedera helix exhibits the lowest cuticular permeance, the

\[
I = \frac{(RWD_{sl} - RWD_{mt})}{T_{min}} S_u
\tag{12}
\]

At a relative humidity of 50% and at a temperature of 25 °C the survival time amounts to 12 h (Fagus sylvatica), 13 h (Quercus petraea) and 19 h (Acer campestre) for the deciduous species, and to 130 h (Ilex aquifolium) and 120 h (Hedera helix) for the evergreen species. In this model calculation possible changes of the environmental conditions like relative humidity and temperature are not taken
into account. Therefore, on a quantitative level the survival time should not be overrated. On a qualitative level it demonstrates the ability of evergreen leaves to withstand longer periods of drought without the requirement of water uptake from the soil. The residual stomatal transpiration of the leaves from Hedera helix does not restrict this basic tendency, since the contribution is not so dramatic as to bring the minimum conductance into the range of the deciduous species.

Ecophysiological implications of leaf water potential

The analysis of leaf water potential was mainly carried out in order to describe its role as a component of the driving force for transpirational water loss. In addition, pressure–volume curves have been used in ecophysiological studies to assess the ability of leaves to maintain a favourable water status, even at low water potentials, by means of an efficient water recovery from the soil (Cheung et al., 1975). The water uptake from a dry soil is favoured, if a comparable low decline of the water content is accompanied by a steep decline of the leaf water potential, leading to the development of a sufficient water potential gradient between soil and plant. This mechanism is supported by a low osmotic potential at full saturation, a low water potential at the turgor loss point and a high bulk modulus of elasticity corresponding to a low cell wall elasticity. The ability to maintain a favourable water status even at low leaf water potentials has been found for evergreen species in a number of vegetation types (Roberts et al., 1981; Sobrado, 1986; Salleo and Lo Gullo, 1990) and also appears in the evergreen species growing in the temperate climate of Central Europe (Table 4).

The role of sclerophyll for drought resistance

Scleromorphic leaves have received much attention, especially with respect to evergreen plants as typical representatives of the Mediterranean vegetation. Though sclerophyllous plants cannot be considered as a homogenous group (Lo Gullo and Salleo, 1988), rigid cell walls of low elasticity have been proposed as one of the most important physiological mechanisms of drought resistance linked with the morphological leaf structure (Groom and Lamont, 1997). This finding is emphasized in the present study by the strong correlation ($r^2=0.97$) between the degree of sclerophyll and the bulk modulus of elasticity (Fig. 4A).

The scleromorphic leaf structure is often associated with the development of thick cuticles. However, any attempts have so far failed to relate cuticular permeances with cuticle thickness or thickness of the cuticular wax layer (Riederer and Schreiber, 2001). Accordingly, only a weak correlation ($r^2=0.52$) between the degree of sclerophyll and the cuticular permeance exists (Fig. 4B). Therefore, the role of sclerophyll for the development of an effective cuticular transport barrier is not evident. This finding is reasonable since the cuticular water permeability is mainly determined by the transport properties of the cuticular waxes (Schreiber and Riederer, 1996) and ecophysiological adaptations may rather be found on a molecular level in terms of the chemical composition and the physical arrangement of the cuticular waxes.

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References


