Cuticles of Vascular Epiphytes: Efficient Barriers for Water Loss after Stomatal Closure?

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Water permeabilities of astomatous, isolated cuticular membranes of 15 vascular epiphyte species (families: Araceae, Orchidaceae and Piperaceae) from the moist lowland forest of Barro Colorado Nature Monument, Panama, were investigated. Permeances determined at 30°C ranged from $4.38 \times 10^{-8} \text{ m s}^{-1}$ (Asparagaceae) to $6.07 \times 10^{-6} \text{ m s}^{-1}$ (Polystachya foliosa (Hook.) R.E. Schult.) to $6.07 \times 10^{-6} \text{ m s}^{-1}$ (Polystachya foliosa (Hook.) R.E. Schult.) to $6.07 \times 10^{-6} \text{ m s}^{-1}$ (Polystachya foliosa (Hook.) R.E. Schult.) to $6.07 \times 10^{-6} \text{ m s}^{-1}$ (Polystachya foliosa (Hook.) R.E. Schult.). Comparison of these data with permeances of plants from other habitats corroborates the notion that cuticular properties reflect the climatic demands of the growing sites. The studied group of vascular epiphytes, living in a very drought-prone habitat, showed the lowest cuticular permeances to water recorded to date.

Key words: Barro Colorado Island, cuticular transpiration, epiphyte, plant water relations, transport barrier.

INTRODUCTION

Under severe environmental conditions such as water deficiency and high temperatures, stomata will frequently be closed (Lambers et al., 1998). The survival of a plant may then depend strongly upon the residual amounts of water loss from leaf surfaces determined, to varying degrees, by the rates of cuticular transpiration. Crowns of tropical rain forest trees represent an extreme habitat where such a scenario frequently occurs (Benzing, 1990). Drought is particularly pronounced in seasonal forests, but even in wet forests canopy-dwelling plants, such as vascular epiphytes, are only intermittently supplied with moisture from rain, dew or mist. Between these episodic events, high radiation and strong winds lead to a considerable evaporative demand, while the lack of soil reduces available moisture reservoirs to tissue water.

Recently, Schreiber and Riederer (1996) investigated cuticular properties of a large number of species from diverse habitats ranging from the temperate to the tropical zone. Although all leaf samples were obtained from plants growing in the greenhouse, they found a relationship between presumed evaporative demand in the native habitat and the in vitro permeability of cuticular membranes to water. Considering the in situ growing conditions of vascular epiphytes, we hypothesized that cuticles of the species belonging to this plant group should show extraordinarily low permeabilities for water. Pertinent information, however, is rare (see Benzing and Burt, 1970, for a few bromeliad species). Therefore, we tested this notion for a number of species of different taxonomic affinity, growing naturally in a moist tropical forest in central Panama. Moreover, we investigated whether differences in cuticular properties might be detectable on a smaller scale, i.e. between those species growing at less exposed vs. exposed sites of the forest.

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MATERIALS AND METHODS

Fully developed, non-senescent leaves without any visible signs of herbivory or pathogen attack were collected in the Republic of Panama in the Barro Colorado Nature Monument (9°10′N, 79°51′W) in April 1997. The vegetation of this nature reserve, which is located in Lake Gatun, is classified as tropical moist forest, with approx. 200 species of epiphytic and hemiepiphytic plants (Croat, 1978). The climate is characterized by a pronounced dry season from mid-December to late April. The mean annual rainfall is approx. 2700 mm (Croat, 1978).

The epiphyte species included in this study are listed in Table 1. Most samples were from plants growing on Annona glabra L., a tree restricted to the shoreline of Lake Gatun. Leaves of shade-tolerant species were obtained from sites in the forest understory. We tried to cover a large range of the epiphyte taxa present in Barro Colorado Nature Monument, but unfortunately many species proved to be unsuitable for analysis with our method using isolated cuticular membranes. This was not only true for bromeliads with their epidermal trichomes, but also for other taxa because the leaves of many epiphyte species are too narrow. In some cases (e.g. Anthurium clavigerum Poepp., Catasetum viridiflavum Hook., Polypodium crassifolium L.) enzymatic isolation failed.

Astomatous cuticular membranes were isolated from the upper leaf surfaces using methods described previously (Schöherr and Riederer, 1986; Schreiber and Riederer, 1996). The enzymatic solution contained 1% (v/v) Trenolin Super DF (Erbslöhl Geisenheim, Geisenheim, Germany), 1% (v/v) Cellulase Novo Nordisk, Bagsvaerd, Denmark, 0.1% (v/v) NaNO$_3$ in a citric acid buffer adjusted to pH 3.0 with KOH. Samples were kept in the enzymatic solution for about 7 weeks (range 3–11 weeks). After enzymatic isolation, cuticular membranes were stored for 4 weeks before measurements. Storage for several weeks is...
necessary to avoid the influence of the isolation procedure on the permeability of cuticular membranes (Geyer and Schönherr, 1990).

The possibility that differences in the duration of the enzymatic isolation process could influence subsequent permeability measurements motivated the following experiment. Leaf disks from the hemiepiphyte Clusia uvitana Pittier (Clusiaceae, cultivated in the Würzburg Botanical Gardens), which take about 1 week to yield isolated cuticular membranes (unpubl. res.), were left in enzymatic solution for 1, 2, 4 and 8 weeks, respectively. After each time period, 15 cuticular membranes were removed, dried, flattened, stored for 4 weeks, and then used for permeability measurements. Measurements of cuticular membrane permeabilities were made at 25°C and permeances were calculated. No significant differences between treatments were found (Kruskal–Wallis-ANOVA, d.f. = 3, P > 0.1). This suggests that varying lengths of immersion in enzymatic solution have no significant influence on cuticular membrane permeability in this—and presumably other—species.

Cuticular permeability was determined following the methods of Schreiber and Riederer (1996). Cuticular membranes were mounted between transport chambers made of stainless steel and the time courses of water loss across the cuticular membranes were measured gravimetrically. Linear regression equations were fitted to plots of total amount of water loss vs. time and the flow rates of water loss were obtained from the slopes of the graphs. Permeances (P, m s⁻¹) were calculated as

\[ P = \frac{F}{A \Delta c} \]  

where \( F \) (kg s⁻¹) is the flow rate of water across the cuticular membranes, \( A \) (m²) is the exposed cuticular membrane area (1.13 × 10⁻⁴ m²), and \( \Delta c \) (kg m⁻³) is the gradient of water vapour concentration inside and outside the chamber.

Because the water vapour concentration over silica gel is approximately zero and liquid water was applied in the donor chamber, \( \Delta c \) is equal to the saturation water vapour concentration at the experimental temperature.

All 14 species were measured at 30°C which approximated the in situ microclimatic conditions (Zotz and Winter, 1994). Temperature effects on the permeability of cuticular membranes were only studied on a subset of ten species. Temperatures used were 20°C, and 5 degree increments from 30 to 55°C. In other studies, permeances are frequently determined at 25°C. Although we did not measure permeances at this temperature due to technical problems, estimated values obtained by interpolation from permeances at 20°C and 30°C allow comparison with literature data. In this report, permeances are based on water concentrations in the gas phase, in contrast to the permeances given by Schreiber and Riederer (1996) which were based on liquid water. Conversion of their results to vapour-phase based

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**TABLE 1. Permeances to water of isolated cuticular membranes (P in m s⁻¹ at 30°C) for 14 epiphyte species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Habitat class</th>
<th>n</th>
<th>P × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspasia principissa Rchb. f.</td>
<td>Orchidaceae</td>
<td>Shade</td>
<td>3</td>
<td>0.5 ± 0.10</td>
</tr>
<tr>
<td>Anthurium salviniae Hemsl.</td>
<td>Araceae</td>
<td>Shade</td>
<td>12</td>
<td>0.7 ± 0.13</td>
</tr>
<tr>
<td>Oncidium amplexilatum Lindl.</td>
<td>Orchidaceae</td>
<td>Sun</td>
<td>13</td>
<td>1.0 ± 0.29</td>
</tr>
<tr>
<td>Caudaltron bilamellatum (Rchb. f.) R.E. Schult.</td>
<td>Orchidaceae</td>
<td>Exposed</td>
<td>13</td>
<td>1.0 ± 0.22</td>
</tr>
<tr>
<td>Anthurium brownii Mast.</td>
<td>Araceae</td>
<td>Sun</td>
<td>10</td>
<td>1.2 ± 0.21</td>
</tr>
<tr>
<td>Philodendron radiatum Schott.</td>
<td>Araceae</td>
<td>Sun</td>
<td>14</td>
<td>1.2 ± 0.23</td>
</tr>
<tr>
<td>Philodendron tripartitum (Jacq.) Schott.</td>
<td>Araceae</td>
<td>Sun</td>
<td>11</td>
<td>1.1 ± 0.35</td>
</tr>
<tr>
<td>Notylia pentachne Rchb. f.</td>
<td>Orchidaceae</td>
<td>Sun</td>
<td>14</td>
<td>1.3 ± 0.39</td>
</tr>
<tr>
<td>Sobralia suaveolens Rchb. f.</td>
<td>Orchidaceae</td>
<td>Sun</td>
<td>12</td>
<td>1.7 ± 0.30</td>
</tr>
<tr>
<td>Epidendrum nocturnum Jacq.</td>
<td>Orchidaceae</td>
<td>Sun</td>
<td>12</td>
<td>1.8 ± 0.49</td>
</tr>
<tr>
<td>Trichopilia maculata Rchb. f.</td>
<td>Orchidaceae</td>
<td>Shade</td>
<td>13</td>
<td>2.2 ± 0.48</td>
</tr>
<tr>
<td>Sobralia fenzliana Rchb. f.</td>
<td>Orchidaceae</td>
<td>Shade</td>
<td>12</td>
<td>2.7 ± 0.81</td>
</tr>
<tr>
<td>Peperomia cordulata C. DC.</td>
<td>Piperaceae</td>
<td>Sun</td>
<td>12</td>
<td>4.6 ± 0.83</td>
</tr>
<tr>
<td>Polystachya foliosa (Hook.) Rchb. f.</td>
<td>Orchidaceae</td>
<td>Sun</td>
<td>7</td>
<td>6.1 ± 2.32</td>
</tr>
</tbody>
</table>

Means ± 95% confidence intervals and sample sizes (n) are given. Species names follow nomenclature of d’Arcy (1987). Assignment of the species to different habitat classes determined by Croat (1978) and personal observations.
permeances was achieved by multiplying their values with the ratio of liquid water densities and water vapour at saturation at the temperatures of measurement.

Results were analysed with STATISTICA software (STATISTICA 5.1, StatSoft Inc., Tulsa, OK, USA). In most cases, strong deviation from normality only permitted the use of non-parametric statistics. Phase transitions were analysed with breakpoint regressions.

RESULTS

The cuticular membranes isolated from 14 vascular epiphyte species showed water permeances (at 30°C) which varied by more than one order of magnitude (Table 1): they ranged from $0.46 \times 10^{-8}$ m s$^{-1}$ (Aspasia principissa) to $6.07 \times 10^{-6}$ m s$^{-1}$ (Polystachya foliosa). Interspecific differences did not correlate with the habitat of a given species. Comparing the species growing in the forest understorey or the inner canopy of trees (shade habitat, Table 1) with those found at more exposed sites (sun habitat) yielded no significant differences in permeances (Mann–Whitney U test, $P = 1.00$). Remarkably, the two drought-deciduous species (Croat, 1978; pers. obs.) investigated had substantially higher cuticular permeances than the evergreen species included in this study (Table 1).

The permeance to water of isolated cuticular membranes increased with temperature. The mean permeances measured at 55°C were up to 28-fold higher than those for 30°C. Cuticular permeances increased steeply with temperature in the high-temperature range, while at lower temperatures moderate temperature dependencies were observed. The transition points, which were determined by breakpoint regressions ($r^2$ between 0.95 and 0.99), ranged from 39 to 45°C for the ten species investigated in this respect (Table 2).

<table>
<thead>
<tr>
<th>Species Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthurium brownii</td>
</tr>
<tr>
<td>Cauldron bilamellatum</td>
</tr>
<tr>
<td>Epidendrum nocturnum</td>
</tr>
<tr>
<td>Sobralia fenzliana</td>
</tr>
<tr>
<td>Trichopilia maculata</td>
</tr>
<tr>
<td>Philodendron radiatum</td>
</tr>
<tr>
<td>Anthurium salviniae</td>
</tr>
<tr>
<td>Philodendron tripartitum</td>
</tr>
<tr>
<td>Sobralia suaveolens</td>
</tr>
<tr>
<td>Encyclia chimboraensois</td>
</tr>
</tbody>
</table>

Table 2. Transition points (°C) of the temperature dependence of the permeance to water of isolated cuticular membranes from ten species of vascular epiphytes

FIG. 2. Permeances (P, at 25°C) for cuticular membranes of different plant groups. Given are means ± 95% confidence intervals, sample size (n) is shown on top of the bars. From left to right: tropical, non-impounding epiphytes (this study); tropical hemiepiphytes and climbing plants (Schreiber and Riederer, 1996); terrestrial plants from mediterranean-type climate (Schreiber and Riederer, 1996); epiphytic bromeliads (Kerstiens, 1996, after Benzing and Burt, 1970); terrestrial plants from temperate climates (Schreiber and Riederer, 1996). Statistical differences between plant groups were highly significant (Kruskal–Wallis-ANOVA, d.f. = 4, $P < 0.001$).

DISCUSSION

Permeances to water of isolated cuticular membranes from the adaxial leaf surfaces of vascular epiphytes ranged from $0.5 \times 10^{-6}$ to $6.1 \times 10^{-6}$ m s$^{-1}$. Values for the subset of evergreen epiphyte species ranged from $0.5 \times 10^{-6}$ to $2.7 \times 10^{-6}$ m s$^{-1}$ (Table 1). Thus, the results of the present study support the notion put forward by Schreiber and Riederer (1996) that since cuticular properties become increasingly important in more arid habitats, there should be a negative relationship between cuticular permeability and water supply and/or evaporative demand. Since the growing sites of most vascular epiphytes are characterized by fluctuating water supply and frequent drought (Benzing, 1980; Zotz and Thomas, 1999). Thus, following recalculated from Schreiber and Riederer, 1996). Primary hemiepiphytes such as Clusia sp. or Ficus sp. go through an epiphytic stage with much reduced availability of water (Holbrook and Putz, 1996). It would be interesting to determine whether differences in cuticular properties can be found between ontogenetic stages of such species. Most terrestrial plants show much higher permeabilities to water further indicating the importance of water in the epiphytic habitat.

Remarkably, on a lower spatial scale, a correlation between relative water availability and cuticular properties could not be found. Epiphytes growing at exposed sites did not feature leaf cuticles with extraordinarily low permeability. The species with the lowest permeance (Aspasia principissa) is normally restricted to the shaded understorey (Zimmerman and Aide, 1989).

Many members of the Bromeliaceae have gained a certain independence from intermittent water supply by evolving water-impounding foliage, so-called ‘tanks’ (Benzing, 1980; Zotz and Thomas, 1999). Thus, following
temperatures (Schoenherr et al., 1979; Schoenherr and Meberda, 1981; Riederer et al., unpubl. res.). The ecological relevance of this finding depends both upon the temperature at which a phase transition occurs and the maximum leaf temperatures reached under natural conditions. Phase transitions in the species of this study were observed at temperatures ranging from 39 to 44.5°C (Table 2), comparable to values reported for other, terrestrial plants investigated in this work (Fig. 2). Although consistent with our notion, the use of different methodologies (original data in Benzing and Burt, 1970) should lead to caution in our interpretation until further experiments with similar methodology allow an unambiguous comparison.

Temperature-dependent changes in the properties of plant cuticles lead to strongly increased water loss at high temperatures (Schonherr et al., 1979; Schonherr and Merida, 1981; Riederer et al., unpubl. res.). The ecological relevance of this finding depends both upon the temperature at which a phase transition occurs and the maximum leaf temperatures reached under natural conditions. Phase transitions in the species of the study were observed at temperatures ranging from 39 to 44.5°C (Table 2), comparable to values reported for other, terrestrial plants (Schonherr et al., 1979; Schonherr and Merida, 1981; Riederer et al., unpubl. res.). In nature, however, leaf temperatures of this magnitude have rarely been observed in vascular epiphytes. Even at exposed growing sites in the outer canopy on emergent trees on Barro Colorado Island, leaf surface temperatures normally range from approx. 24°C (night) to approx. 35°C (early afternoon) (Zotz and Winter, 1994; Zotz and Tyree, 1996). When stomata are completely closed due to prolonged drought, leaf temperatures may further increase, although strong trade winds may allow considerable convective heat loss. For example, in the dry season, fronds of extremely drought stressed Polypodium crassifolium rarely reached maximum temperatures of 39°C, and if so only for periods of a few minutes (Zotz and Winter, 1994; and unpubl. res.).

The efficiency of leaf cuticles in ensuring plant survival during times of drought can be illustrated for Caularthron bilamellatum and Aspasia principissa. The two orchids show contrasting habitat preferences with Caularthron bilamellatum growing at very exposed microsites (Zotz and Ziegler, 1997) and Aspasia principissa preferring the understory and lower canopy strata (Zimmerman and Aide, 1989). For both species supplementary information on water storage is available. For illustrative purposes, the critical minimum level of tissue water content is assumed to be 25% of the value at saturation (Killian and Lemeé, 1956; Stocker, 1956). For many epiphyte species very low critical minimum leaf water contents have been reported (e.g. Andrade and Nobel, 1997).

For a scenario assuming perfect stomatal closure, 50% relative humidity and 30°C leaf temperature throughout the day, daily water losses of approx. 3.0 g m⁻² and approx. 1.2 g m⁻² can be estimated for C. bilamellatum and A. principissa, respectively (Table 3). Consequently, it would take approx. 268 and 192 d, respectively, until 75% of the leaf water content at saturation was lost across the adaxial and abaxial cuticles of a leaf. Such prolonged desiccation periods exceed the length of the entire dry season in central Panama (Windsor, 1990). Evidently, these estimates are partially conservative as in situ the assumption of complete stomatal closure under drought conditions is probably not met under all circumstances.

Considering that most tropical epiphytes live in a drought-prone habitat, cuticular properties are of utmost importance in avoiding desiccation once stomata close. We conclude that the cuticular membranes studied in this report are clearly very efficient in this function.

### ACKNOWLEDGEMENTS

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### LITERATURE CITED


