

Protecting against water loss: analysis of the barrier properties of plant cuticles

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Abstract

The cuticle is the major barrier against uncontrolled water loss from leaves, fruits and other primary parts of higher plants. More than 100 mean values for water permeabilities determined with isolated leaf and fruit cuticles from 61 plant species are compiled and discussed in relation to plant organ, natural habitat and morphology. The maximum barrier properties of plant cuticles exceed that of synthetic polymeric films of equal thickness. Cuticular water permeability is not correlated to the thickness of the cuticle or to wax coverage. Relationships between cuticular permeability, wax composition and physical properties of the cuticle are evaluated. Cuticular permeability to water increases on the average by a factor of 2 when leaf surface temperature is raised from 15 °C to 35 °C. Organic compounds of anthropogenic and biogenic origin may enhance cuticular permeability. The pathway taken by water across the cuticular transport barrier is reviewed. The conclusion from this discussion is that the bulk of water diffuses as single molecules across a lipophilic barrier while a minor fraction travels along polar pores. Open questions concerning the mechanistic understanding of the plant cuticular transport barrier and the role the plant cuticle plays in ensuring the survival and reproductive success of an individual plant are indicated.

Key words: Plant cuticles, cuticular waxes, water permeability, cuticular transpiration, adaptation, environmental effects, diffusion.

Introduction

Water is a crucial prerequisite for plant life. Both the shortage and the excess of water may cause severe stress to terrestrial plants, with ultimately lethal outcomes. A suite of physiological, anatomical, morphological, and life-history adaptations ensures that plants are able to maintain a water status suitable for survival and reproduction even under adverse environmental conditions. The macrofossil record of terrestrial plant evolution provides evidence for the critical importance the regulation of plant water status has for life in an atmospheric environment: well-developed cuticles and stomata turn up in fossil specimens of the very earliest terrestrial plants known (Edwards *et al.*, 1996, 1998). Both structures together make up an integrated system of tissues and physiological functions optimizing photosynthetic gas exchange under the inescapable constraint of the continuous loss of water to a dry atmosphere.

A major challenge for plants living in an atmospheric environment is, therefore, to develop a barrier against uncontrolled water loss. The barrier has to be efficient, translucent for photosynthetically active radiation, flexible, and self-healing. The plant cuticle combines all these properties in an economic way. The cuticle is a thin (0.1–10 µm thick) continuous membrane consisting of a polymer matrix (cutin), polysaccharides and associated solvent-soluble lipids (cuticular waxes) (Holloway, 1982a; Jeffree, 1996). Cutin is a three-dimensional polymer of mostly C₁₆ and C₁₈ hydroxy fatty acids cross-linked by ester and other bonds (Kolattukudy, 1980; Holloway, 1982b; Holloway and Wattendorff, 1987; Walton, 1990; von Wettstein-Knowles, 1993). Cuticular waxes is a general term for complex mixtures of homologue series of long chain aliphatics like alkanes, alcohols,

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aldehydes, fatty acids, and esters with the addition of varying proportions of cyclic compounds like pentacyclic triterpenoids and hydroxycinnamic acid derivatives (Kolattukudy *et al.*, 1976; Tulloch, 1976; Kolattukudy, 1980; Baker, 1982; Holloway, 1984; Walton, 1990; von Wettstein-Knowles, 1993, 1995; Kolattukudy and Espelie, 1994; Bianchi, 1995; Riederer and Markstädter, 1996).

While some basic properties of plant cuticles as barriers against the diffusion of water have been elucidated (Schönherr, 1976a, b, 1982; Schönherr *et al.*, 1979; Becker *et al.*, 1986; Kerstiens, 1996) a number of questions have been addressed only recently or still remain open. When it comes to the assessment of the impact of environmental stress factors on the plant water status a major question to be addressed concerns the relationship between the chemical and physical properties of plant cuticles and their transport properties as well as the variability and plasticity, both intra- and interspecific, of cuticular water permeability. From a whole-plant point of view the interplay between stomatal regulation and cuticular water permeability must be analysed for understanding the contribution of the cuticular barrier to the maintenance of plant water status (Raven, 1977). The following paragraphs will address these questions by critically evaluating the current state of knowledge and by exploring directions for future research.

Water permeability of plant cuticles

Plant cuticles control the movement of water between two compartments: (1) the outer cell wall of the epidermis and (2) the atmosphere adjacent to the plant. The mechanism of water transport across the cuticle is a simple diffusion process along a gradient of the chemical potential of water. As a first approximation, the cuticle is considered to behave as a solution-diffusion membrane where the penetrating molecules are sorbed at one interface, diffuse across the barrier and subsequently are desorbed at the other interface (Frisch, 1991; Vieth, 1991).

Under steady-state conditions, the amount of water permeated from the leaf tissue to the atmosphere linearly increases with time, and the flow rate J ($\text{g m}^{-2} \text{s}^{-1}$) is given by

$$J = P_c A (C_i - C_o) \quad (1)$$

where C_i and C_o are the vapour-based concentrations of water (g m^{-3}) in the outer epidermal wall and the atmosphere, respectively. The error is negligible when C_i is assumed to equal the saturation concentration of water vapour at the temperature of the leaf surface. A (m^2) is the area of the cuticle exposed and P_c (m s^{-1}) is the permeance (or conductance) of the cuticle (for a vapour-based concentration gradient). P_c is equivalent to a mass transfer coefficient (Cussler, 1997) relating the flux of

water across the cuticle per unit area to the concentration difference.

Numerical values for cuticular permeances can easily be converted to mole fraction-based conductances g' [$\text{mol m}^{-2} \text{s}^{-1}$] which are advantageous for field studies (Hall, 1982; Nobel, 1991) according to

$$g' = P_c \frac{p}{RT} \quad (2)$$

where p , R and T stand for the atmospheric pressure, the gas constant and the absolute temperature, respectively. At standard pressure and 25 °C g' differs from P_c by a factor of 41.

For homogeneous membranes, permeances are directly related to the fundamental transport properties of the membrane by

$$P = \frac{KD}{\Delta x} \quad (3)$$

where K (dimensionless), D ($\text{m}^2 \text{s}^{-1}$) and Δx (m) are the partition coefficient of water between the membrane and the gas phase, the diffusion coefficient of water within the membrane and the thickness of the membrane, respectively. Even though the assumption of homogeneity is not valid for the plant cuticle, equation 3 is a useful tool for analysing and understanding cuticular permeability. In qualitative terms, equation 3 predicts that permeance and, at a given driving force, the flow rate across the cuticle will increase with increasing relative solubility and mobility of water in the membrane (or more precisely: its transport-limiting barrier).

The water permeability of the cuticles has to be measured while strictly avoiding any interference by a residual component of stomatal transpiration when fundamental transport properties of cuticles or the relative importance of the cuticular and stomatal pathway for transpiration are at the focus of interest. Therefore, only astomatous leaf, fruit or stem surfaces are suitable, in principle. Most studies so far have employed cuticular membranes which can be obtained by an enzymatic isolation method (Schönherr and Riederer, 1986). The flux of water across the isolated cuticle can be measured gravimetrically (Schönherr and Lenzian, 1981) and by using ^3H -labelled water (Schönherr, 1976a) or highly sensitive humidity sensors (Becker *et al.*, 1986). The latter method is also suitable for measuring cuticular permeability *in situ* (Kerstiens, 1995). In the majority of the experiments reported, a maximum concentration difference between the inner and the outer side of the cuticle has been applied by using water-saturated and water-free conditions on one and on the other side, respectively.

Permeances for water determined so far with astomatous leaf cuticular membranes or *in situ* leaf cuticles range over 2.5 orders of magnitude from 3.6×10^{-7} (*Vanilla planifolia*) to 1.4×10^{-4} (*Abies alba*) m s^{-1} (Table 1). The 10%, 50% and 90% quartiles of the cuticular permeances

Table 1. Permeances ($\times 10^6$; m s^{-1}) for water of astomatous leaf cuticlesPermeances (P_c) are calculated for a vapour-phase driving force (g m^{-3}).

Species	Permeance	Reference
<i>Abies alba</i>	140	(Lendzian <i>et al.</i> , 1986)
<i>Acer pseudoplatanus</i> ^a	23	(Kerstiens, 1994)
<i>Aechmea fasciata</i>	4.2	(Lendzian and Kerstiens, 1991)
<i>Allium cepa</i>	19	(Schönherr and Mérida, 1981)
<i>Anthurium brownii</i>	1.15	(Helbsing <i>et al.</i> , 2001)
<i>Anthurium salviniae</i>	0.68	(Helbsing <i>et al.</i> , 2001)
<i>Aspasia principissa</i>	0.46	(Helbsing <i>et al.</i> , 2001)
<i>Betula pubescens</i> ^a	10	(Kerstiens, 1994)
<i>Camellia sinensis</i>	4.68	(Schreiber and Riederer, 1996b)
	5.78	(M Riederer, unpublished results)
<i>Caularthron bilamellatum</i>	1.13	(Helbsing <i>et al.</i> , 2001)
<i>Citrus aurantium</i>	5.55	(Schreiber and Riederer, 1996b)
	7.10	(Baur, 1997)
	9.52	(M Riederer, unpublished results)
	12	(Becker <i>et al.</i> , 1986)
	13	(Lendzian and Kerstiens, 1991)
	15	(Schönherr and Schmidt, 1979)
	28	(Schönherr and Schmidt, 1979)
	36	(Schönherr and Lendzian, 1981)
	45	(Lendzian <i>et al.</i> , 1986)
	47	(Haas and Schönherr, 1979)
	60	(Schönherr, 1976a)
	69	(Geyer and Schönherr, 1990)
<i>Citrus limon</i>	20.4	(Schreiber and Riederer, 1996b)
<i>Clivia miniata</i>	0.48	(M Riederer, unpublished results)
	1.1	(Becker <i>et al.</i> , 1986)
	5.1	(Mérida <i>et al.</i> , 1981)
	6.81	(Schreiber and Riederer, 1996b)
<i>Clusia flava</i>	2.02	(M Riederer, unpublished results)
<i>Clusia vitana</i>	4.87	(M Riederer, unpublished results)
	13.7	(M Riederer, unpublished results)
<i>Coffea arabica</i>	2.2	(Garrec and Plebin, 1986)
<i>Corynocarpus laevigatus</i>	4.97	(M Riederer, unpublished results)
<i>Cydonia oblongata</i>	10.1	(M Riederer, unpublished results)
	27.3	(Schreiber and Riederer, 1996b)
<i>Delphinium sp.</i> ^a	20	(Kerstiens, 1994)
<i>Epidendrum nocturnum</i>	1.77	(Helbsing <i>et al.</i> , 2001)
<i>Euonymus japonica</i>	7.92	(M Riederer, unpublished results)
	15.5	(Schreiber and Riederer, 1996b)
<i>Fagus sylvatica</i>	37	(Lendzian and Kerstiens, 1991)
<i>Ficus benjamina</i>	5.64	(Schreiber and Riederer, 1996b)
<i>Ficus elastica</i>	1.46	(M Riederer, unpublished results)
	1.8	(Baur, 1997)
	3.95	(M Riederer, unpublished results)
	4.07	(Schreiber and Riederer, 1996b)
	4.3	(Becker <i>et al.</i> , 1986)
<i>Forsythia intermedia</i>	8.62	(M Riederer, unpublished results)
<i>Forsythia suspensa</i>	16.8	(Schreiber and Riederer, 1996b)
<i>Ginkgo biloba</i>	22.6	(Schreiber and Riederer, 1996b)
<i>Garcinia spicata</i>	6.38	(M Riederer, unpublished results)
<i>Hedera helix</i>	0.74	(Baur, 1997)
	2.17	(M Riederer, unpublished results)
	2.47	(Schreiber and Riederer, 1996b)
	2.7	(Becker <i>et al.</i> , 1986)
	3.1	(Lendzian and Kerstiens, 1991)
	4.3	(Schönherr and Lendzian, 1981)
	5.6	(Lendzian and Kerstiens, 1991)
<i>Ilex aquifolium</i>	8	(Garrec and Kerfourn, 1989)
	11	(Lendzian and Kerstiens, 1991)
<i>Juglans regia</i>	19.9	(Schreiber and Riederer, 1996b)
<i>Ligustrum vulgare</i>	18.8	(Schreiber and Riederer, 1996b)
<i>Liriodendron tulipifera</i>	18.2	(Schreiber and Riederer, 1996b)
<i>Maianthemum bifolium</i>	48.1	(Schreiber and Riederer, 1996b)
	77	(Lendzian and Kerstiens, 1991)

Table 1. Continued

Species	Permeance	Reference
<i>Monstera deliciosa</i>	1.86	(Schreiber and Riederer, 1996b)
	2.43	(M Riederer, unpublished results)
<i>Nerium oleander</i>	3.3	(Becker <i>et al.</i> , 1986)
	4	(M Riederer, unpublished results)
	22.6	(Schreiber and Riederer, 1996b)
<i>Notylia pentachne</i>	1.27	(Helbsing <i>et al.</i> , 2001)
<i>Olea europaea</i>	5.46	(Schreiber and Riederer, 1996b)
<i>Oncidium ampliatum</i>	0.95	(Helbsing <i>et al.</i> , 2001)
<i>Peperomia cordulata</i>	4.61	(Helbsing <i>et al.</i> , 2001)
<i>Philodendron ilsemanii</i>	1.04	(M Riederer, unpublished results)
<i>Philodendron radiatum</i>	1.18	(Helbsing <i>et al.</i> , 2001)
<i>Philodendron selloum</i>	2.86	(Schreiber and Riederer, 1996b)
<i>Philodendron tripartitum</i>	1.12	(Helbsing <i>et al.</i> , 2001)
<i>Polygonatum multiflorum</i>	72	(Lendzian and Kerstiens, 1991)
<i>Polystachya foliosa</i>	6.07	(Helbsing <i>et al.</i> , 2001)
<i>Potamogeton lucens</i>	110 000	(Schönherr, 1976a)
<i>Prunus avium</i> ^a	5.9	(Kerstiens, 1994)
<i>Prunus laurocerasus</i>	5.77	(Schreiber and Riederer, 1996b)
	17	(Lendzian and Kerstiens, 1991)
<i>Pyrus communis</i>	6.34	(M Riederer, unpublished results)
	8.29	(M Riederer, unpublished results)
	12	(Becker <i>et al.</i> , 1986)
	21	(Schönherr and Lendzian, 1981)
	67	(Baur, 1997)
<i>Schefflera actinophylla</i>	0.82	(Becker <i>et al.</i> , 1986)
<i>Sobralia fenzliana</i>	2.66	(Helbsing <i>et al.</i> , 2001)
<i>Sobralia suaveolens</i>	1.69	(Helbsing <i>et al.</i> , 2001)
<i>Stephanotis floribunda</i>	33	(Baur, 1997)
<i>Trichopilia maculata</i>	2.17	(Helbsing <i>et al.</i> , 2001)
<i>Vanilla planifolia</i>	0.36	(M Riederer, unpublished results)
	0.74	(Schreiber and Riederer, 1996b)
<i>Vinca minor</i>	7.5	(Lendzian and Kerstiens, 1991)

^aExperiments were performed with intact leaves; all other data were obtained from isolated cuticular membranes.

reported are at 1.1×10^{-6} , 5.8×10^{-6} and $3.7 \times 10^{-5} \text{ m s}^{-1}$, respectively. The central 50% of all permeance data fall within a range from 2.2×10^{-6} and $1.8 \times 10^{-5} \text{ m s}^{-1}$ thus varying at the most by a factor of 8. Cuticular water permeances determined so far for fruit cuticular membranes fall into a range from 2.2×10^{-5} (*Solanum melongena*) to $2 \times 10^{-4} \text{ m s}^{-1}$ (Table 2). The median fruit cuticular permeance of $8.6 \times 10^{-5} \text{ m s}^{-1}$ is almost one order of magnitude higher than the respective value for leaf cuticles.

Interpreting the variability of cuticular water permeances in terms of adaptation to different habitats and stressful environmental conditions is an ecophysiological issue discussed for a considerable time (Stålfelt, 1956). There is no experimental evidence so far that phenotypic plasticity allows plants to adapt cuticular permeance to changes in evaporative demand. An extensive study on the water permeability of leaf cuticles from *Citrus aurantium* leaves grown under varying environmental conditions was unable to detect any significant effects (Geyer and Schönherr, 1990).

Explaining interspecific variation in terms of ecophysiological adaptations proved to be more successful.

Table 2. Permeances ($\times 10^6$; m s^{-1}) for water of astomatous fruit cuticles

Permeances are calculated for a vapour-phase driving force (g m^{-3}). All data were obtained from isolated cuticular membranes.

Species	Permeance	Reference
<i>Capsicum annuum</i>	58.3	(Schreiber and Riederer, 1996b)
	93.0	(Becker <i>et al.</i> , 1986)
	126	(Baur, 1997)
	200	(Lendzian and Kerstiens, 1991)
<i>Lycopersicon esculentum</i>	27.0	(Schreiber and Riederer, 1996b)
	53.0	(Lendzian and Kerstiens, 1991)
	82.0	(Schönherr and Lendzian, 1981)
	140	(Becker <i>et al.</i> , 1986)
<i>Malus sylvestris</i>	90.0	(Schreiber and Riederer, 1996b)
<i>Solanum melongena</i>	22.0	(Becker <i>et al.</i> , 1986)
	78.0	(Schönherr and Schmidt, 1979)
	120	(Schönherr and Schmidt, 1979)

The water permeances of leaf cuticular membranes from 21 plant species tended to cluster according to life-forms and climate of origin (Schreiber *et al.*, 1996a). The lowest water permeabilities were observed with evergreen leaves from epiphytic or climbing plants naturally growing in a tropical climate. The next group in the order of increasing cuticular permeance comprised xeromorphic plants typically growing in a Mediterranean-type climate. The group with the highest water permeances combined deciduous plant species with mesomorphic leaves growing in temperate climates (Fig. 1A).

It is illustrative to compare water permeances of plant cuticles to water-vapour barriers encountered in everyday life. The water permeabilities of cuticles from deciduous and Mediterranean evergreen leaves are comparable to those of polymer films (of approximately equal thickness) used for food packaging (Fig. 1B). The permeances for water of some evergreen and tropical leaf cuticles reach values maximally one and a half orders of magnitude lower than conventional polymer films and are still less permeable to water than advanced synthetic materials like liquid-crystal polymers (Langowski, 1997).

Correlation with chemical and physical properties

The variation of cuticular water permeability observed among different plant species and organs has stimulated speculation on the underlying physical and chemical causes. Intuitively, one might assume that, according to Fick's first law, differences in cuticular permeability should be directly related to a variation in the thickness of the cuticular membrane. Indeed, this is the explanation offered by the classical and the majority of the modern textbooks on plant physiology or ecology especially when discussing adaptation of xerophytes to

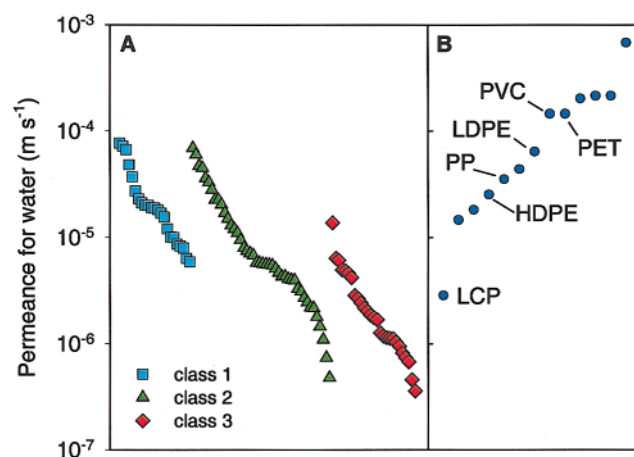


Fig. 1. Range of permeances for water of leaf cuticular membranes (vapour-based driving force). The data from Table 1 are classified according to leaf anatomy and habitat: class 1, deciduous species with mesomorphic leaves growing in temperate climates; class 2, xeromorphic plant typically growing in a Mediterranean-type climate; class 3, evergreen leaves from epiphytic or climbing plants naturally growing in a tropical climate (A). Permeances for water of synthetic polymer films 1 μm thick (B). (Data recalculated from Langowski, 1997.)

their habitat. Despite the obvious plausibility of this argument, there is no experimental evidence supporting it. An early study had demonstrated that there was no correlation between cuticular thickness and cuticular transpiration (Kamp, 1930). Rigorous experiments with astomatous cuticular membranes under controlled conditions (Schönherr, 1982; Becker *et al.*, 1986; Lendzian and Kerstiens, 1991; Schreiber and Riederer, 1996b) were unable to detect any positive relationship between water permeance and the thickness of isolated leaf or fruit cuticles (Fig. 2A).

This result is not as surprising as it may appear at first sight. The cuticular waxes have been identified as the actual barrier of plant cuticles against the diffusion of water or solutes (Schönherr, 1982; Schönherr and Riederer, 1989). This barrier, however, is not equally distributed across the thickness of the cuticular membrane. Actually, waxes are accumulated at the outer surface of the cuticle as studies using polarization microscopy (Meyer, 1938; Roelofsen, 1952; Sitte and Rennie, 1963) and bi-directional desorption kinetics (Schönherr and Riederer, 1988) have demonstrated. However, the hypothesis that the amount of cuticular waxes in a cuticle determines its permeance for water has also been falsified by experimental evidence (Schreiber and Riederer, 1996b). Estimates for the thickness of the cuticular wax layer ranged from 0.1 to 5 μm in a sample of cuticular membranes from 23 plant species but did not explain the variation in cuticular permeance (Fig. 2B).

Similarly, all attempts to correlate water permeance with the qualitative or quantitative composition of plant cuticular waxes have failed so far. In hindsight, these

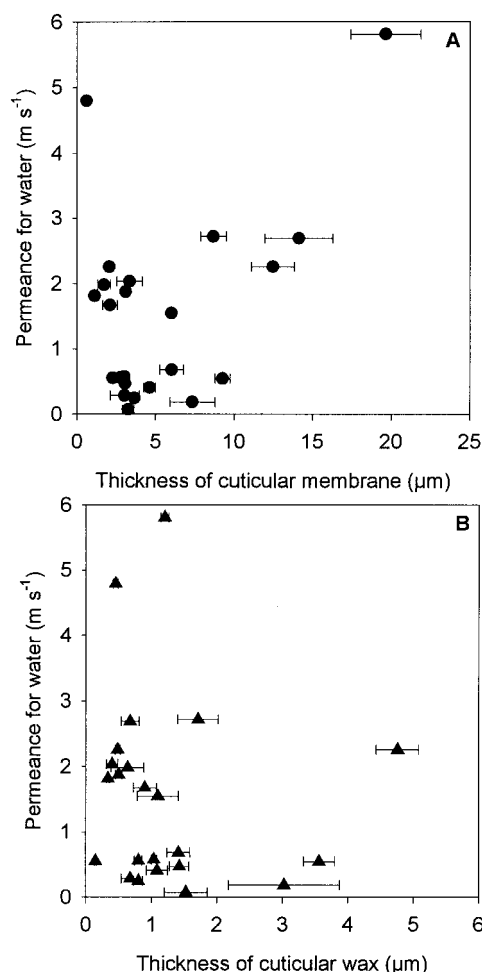


Fig. 2. Plots of vapour-based permeances of cuticular membranes isolated from leaves (21 species) and fruits (2 species) versus the thickness of the cuticle (A) and the thickness of cuticular wax (B). Thickness was estimated from mass-per-unit-area data using specific masses of 1.1 and 0.8 g cm⁻¹ for cuticular membranes and waxes, respectively. (Data were recalculated from Schreiber and Riederer, 1996b.)

attempts appear also to be somewhat naïve as (1) the current knowledge on the chemical composition of cuticular waxes is arbitrarily restricted and biased toward their aliphatic components (Riederer and Markstädter, 1996) and, (2) there is evidence for discrete layers of cuticular wax with highly divergent chemical composition (Jetter *et al.*, 2000). As long as the transport-limiting wax layer of a plant cuticle can not be identified and analysed thoroughly, correlating bulk chemical composition of cuticular waxes with water permeance makes little sense.

There are, however, significant relationships between cuticular water permeance and cuticular transport parameters determined for solutes. The penetration of water and small organic acids (benzoic, salicylic and 2,4-dichlorophenoxy acetic acid) across the cuticular membranes from several plant species was highly

correlated (Niederl *et al.*, 1998). The same was true for water permeance of cuticular membranes from 24 plant species and the diffusion coefficients of octadecanoic acid in the reconstituted cuticular waxes of these species (Schreiber and Riederer, 1996a). The quantitative property–property relationships obtained for both systems are predictive tools for the estimation of cuticular permeance in species without astomatous cuticles. The success of this approach also implies that the permeance for small organic solutes or the properties of cuticular waxes as typified by the diffusion coefficient of a mobility probe reflect physical characteristics of the transport-limiting barrier of the cuticle common to both water and solute diffusion.

Environmental effects on cuticular permeability

Under natural conditions, leaf surfaces are damaged either by wind or abrasion (Pitcairn *et al.*, 1986; Hoad *et al.*, 1992) or by herbivores and microbial pathogens resulting in leaky cuticles. But even when such ‘brute force’ attacks are disregarded the permeability of a barrier membrane is no static property. Rather, it is subject to changes according to physical or chemical effects from its surroundings. This is equally true for, for example, synthetic polymer membranes and lipid bilayers and obviously also applies to plant cuticles.

Temperature is the predominant physical factor influencing the permeance of a barrier. Two terms contributing to permeance (see equation 3) are temperature-dependent: the diffusion coefficient of a molecule diffusing in the membrane increases with temperature while its partition coefficient between the membrane and the adjacent phases (generally) decreases. Temperature has a stronger effect on diffusion than on partitioning; that is why permeances generally increase with temperature.

Leaf surface temperatures may vary over a range of up to 100 K primarily depending on air temperature, irradiation and transpiration. Even under temperate climatic conditions the annual variation of leaf surface temperature may range from –20 °C to 50 °C (Huber, 1959; Nito *et al.*, 1979; Kuraishi and Nito, 1980). The concomitant changes in cuticular permeance for water are pronounced (Schönherr *et al.*, 1979; Schönherr and Mérida, 1981). In a recent comparative study involving leaf cuticles from 12 plant species cuticular permeance for water increased by roughly a factor of 2 in the temperature range from 15 °C to 35 °C (Fig. 3A). Higher temperatures reaching up to 50 °C enhanced cuticular water permeability by approximately one order of magnitude (Fig. 3B). A strong dependence of cuticular permeability on temperature has also been reported for the penetration of organic solutes across plant cuticular

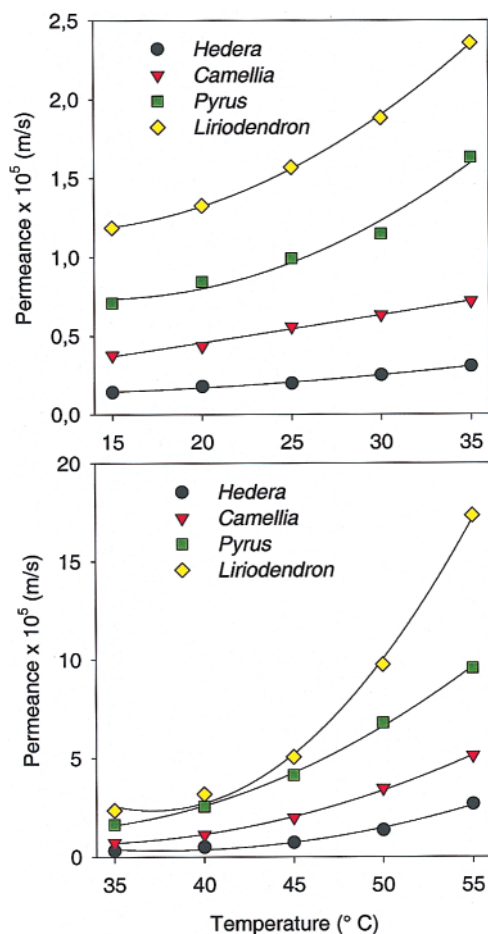


Fig. 3. Dependence on temperature of the permeance for water of leaf cuticles from selected plant species. Temperature courses in the low (A) and high-temperature range (B) are shown for leaf cuticles from *Hedera helix*, *Camellia sinensis*, *Pyrus communis*, and *Liriodendron tulipifera* (M Riederer, unpublished data).

membranes (Baur and Schönherr, 1995, 1998; Schönherr and Baur, 1996; Baur *et al.*, 1997).

It should be noted that these data are corrected for the temperature dependence of the water saturation deficit and thus describe the temperature effect on cuticular transport properties exclusively. Under real conditions, the combination of decreasing cuticular resistance and increasing driving force will lead to drastically elevated flow rates of water across the cuticle.

The physical properties of the cuticular transport barrier may also be altered by chemical impacts. Due to its interfacial nature the plant cuticle is exposed to both biogenic and anthropogenic chemicals reaching it either via the apoplast or the atmosphere. Numerous studies have dealt with the effects that organic chemicals used in pesticidal formulations exert on the cuticular permeabilities for water and solutes (Riederer and Schönherr, 1990; Schönherr, 1993; Schönherr and Baur, 1996, 1997; Schreiber *et al.*, 1996b; Burghardt *et al.*, 1998; Baur and Schönherr, 1998; Baur, 1999). In the presence of

such penetration enhancers cuticular permeabilities may (reversibly) increase by more than one order of magnitude. Even though a strictly mechanistic explanation of this phenomenon is still outstanding, evidence has been accumulated indicating a plasticizing action of certain organic compounds on the physical structure of plant cuticular wax. Some data even suggest that compounds of biogenic origin may have similar effects on cuticular water permeability (Schönherr, 1993) which, finally, might bring cuticular transpiration at least to a certain degree under metabolic control.

The pathway of water diffusing across the cuticle

The simplest conceptual model for the penetration of the plant cuticle by any uncharged molecule is that of a sorption-diffusion membrane. The permeating molecules are sorbed by the membrane on the one side, diffuse across it dissolved as single particles in the membrane phase and subsequently are desorbed on the other side (Barrie, 1968; Vieth, 1991). This model perfectly explains the permeability of the plant cuticle for lipophilic organic non-electrolytes like the active ingredients of pesticidal formulations or pollutants. In these cases, variation in cuticular permeance between two aqueous compartments can primarily be explained by differences in the solubility of the permeant in the cuticle (cuticle/water partition coefficient) and, to a minor degree, by varying molecular size which influences the diffusion coefficient (Schönherr and Riederer, 1989; Riederer, 1990, 1995; Baur *et al.*, 1996, 1997).

This model reaches its limits when polar compounds are considered. Extrapolating the quantitative property-property relationships established for predicting cuticular permeances of lipophilic compounds to substances with 1-octanol/water partition coefficients <1 leads to values much lower than the rates of uptake observed for this type of substances. The transfer of inorganic ions and polar organics across plant cuticles is considerable as exemplified by the substantial rates of leaching (Tukey, 1970) and the practical importance of foliar fertilization. The efficacy and commercial success of certain water-soluble herbicides applied to the foliage (e.g. glyphosate) is also in contradiction to predictions derived from the permeability of lipophilic substances.

This contradictory evidence relates to a long-standing debate whether some kind of polar pores may contribute to cuticular permeability to water and polar solutes (Lyshede, 1978; Maier-Maercker, 1979; Hoch, 1979; Hallam, 1982). Electron micrographs depicting strands of polysaccharide material stretching over the whole thickness of cuticles were considered as evidence for the chemical nature of the postulated pores (Jeffree, 1996).

New experimental evidence helped to substantiate the two-pathway hypothesis for the diffusion of solutes and water across the plant cuticle (Schönherr, 2000; Schreiber *et al.*, 2001): One pathway passing through the amorphous phase of cuticular wax is accessible only to lipophilic solutes. The second pathway is made up by pores of molecular dimensions filled with water which can be travelled by water-soluble organic compounds and by inorganic ions. The presence of such pores has been demonstrated experimentally for de-waxed plant cuticles (Schönherr, 1976b) and postulated from observations that, at least in some plant species, the water permeability of isolated cuticles (Schönherr and Schmidt, 1979; Schönherr and Mérida, 1981) or cuticular transpiration (van Gardingen and Grace, 1992; Kerstiens, 1996) depend on relative humidity.

The humidity effect on cuticular water permeability, however, is small in comparison to that on polar polymer films (Schönherr, 1982) indicating that the polar pathway across the plant cuticle is only of minor importance for overall permeability. Consequently, the bulk of water molecules diffusing across a plant cuticular membrane is expected to do so dissolved in the amorphous phase of the cuticular wax, i.e. the so-called lipophilic pathway. Water is an uncharged small molecule that readily penetrates lipid monolayers (LaMer *et al.*, 1964), biological membranes (Sha'afi, 1981) and semi-crystalline aliphatic polymers like polyethylene (Barrie, 1968). The importance of the lipophilic pathway for the diffusion of water across plant cuticles is further emphasized by two empirical findings: (1) the large effects de-waxing has on cuticular water permeability (Schönherr, 1982) and (2) the good correlation between cuticular water permeabilities and the diffusion coefficients of lipophilic mobility probes in the reconstituted waxes of different plant species (Schreiber and Riederer, 1996a). In summary, the small size and the lack of a charge allow water to cross the cuticle predominantly via the lipophilic pathway while a minor fraction of the water may diffuse through polar pores.

Outlook

The application of quantitative physical and chemical methods for characterizing cuticular properties and functions has considerably advanced our knowledge on this important interface between the plant and the surrounding atmosphere. The effects that physical and chemical factors like temperature and biogenic or anthropogenic organics may exert on cuticular water permeability can now be incorporated into transpiration models or working hypotheses for further investigation. A much clearer picture of the pathways water molecules diffuse along within the plant cuticle is emerging from

the comparative study of water and solute permeability of isolated plant cuticles.

However, major questions both on the molecular and the organismic level of the problem remain unanswered so far. Despite considerable effort the relationship between the water permeability of a cuticle and its chemical composition and physical structure is not well understood. Further work will have to deal with the localization and analysis of that fraction of total cuticular waxes that actually determines the barrier properties of the cuticle. Progress on this route will also depend on the elucidation of the physical structure of cuticular waxes and how this property is influenced by the mixing behaviour of the wax constituent.

In terms of whole-plant performance under stress and ecophysiological adaptations to stressful conditions the role the plant cuticle plays in ensuring the survival and reproductive success of an individual plant has yet to be studied experimentally. It is known, of course, that plant cuticular permeability contributes to minimize uncontrolled water loss at stomatal closure. However, the available quantitative data do not allow a comparative assessment of the relative contributions of strictly cuticular and residual stomatal transpiration, respectively, to total water loss from leaves under drought stress.

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