



Short Communication

The Effect of Simulated Seawater on Water Permeability of Isolated Leaf Cuticular Layers

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ABSTRACT

Leaf cuticular layer of five plant species; *Vinca major* L., *Prunus laurocerasui* L., *Nerium oleander* L., *Hedera helix* L. and *Ficus benjamina* L. were enzymatically isolated and their water permeabilities were determined. The cuticles were treated with different amounts of this artificial seawater and its effect on cuticular permeability was calculated. The results showed that the effect increased by increasing seawater concentration on cuticle surface. The water permeability varied among the species, and the response of the species was related to their degree of tolerance and lateral heterogeneity of cuticles. Water permeation of *F. benjamina* and *N. oleander* increased. Even though they had low initial water permeation, the effect of seawater on this property of species reached a factor of 2.59 and 3.21, respectively at the highest concentrations of artificial seawater. Washing off the isolated cuticles from seawater residuals indicated that water permeability and seawater effect could be lowered if plants were exposed to rainfall or debris of seawater salts on leaf surface washed from time to time with overhead irrigation.

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Key Words: Plant cuticles; Seawater spray; Salt effect; Ions diffusion

INTRODUCTION

Water plays a major role in physiological processes of the plant cell. Both scarcity and excess of water can cause physiological problems for plants. The cuticle, which is one basic adaptation of plants for their survival on the mainland, covers all above-ground parts of the plants but not woody stems and wounds (Kerstiens, 1996). It forms an effective barrier against desiccation (Marga *et al.*, 2001) and its main function is to minimize water loss from plants when stomata are closed (Schönherr, 1976a). The plant cuticle is a hydrophobic, continuous and flexible thin layer consisting of two lipid fractions; the polymer matrix and cuticular waxes, which are deposited on the outer surface and embedded in the matrix (Luque *et al.*, 1995).

The effects of sea aerosol on coastal vegetation have been reported and some investigations have reported the anatomical and ultra-structural aspects of plants treated with simulated sea aerosol (Sa'nchez-Blanco *et al.*, 2004; Rettori *et al.*, 2005). A number of ornamental species are very interesting options for use in landscaping and gardening projects in coastal regions but their ability to tolerate sea aerosols differ among the species. In view of high mortality in plants exposed to seawater spray in Libya and other Mediterranean countries, we hypothesize that increasing water permeability of plant cuticles when exposed to salt spray and other factors, may play a major role in the damage of coastal vegetation. Purpose of this study was to investigate to what extent seawater can affect water

permeability of isolated cuticular layer. This will improve our understanding of the basis behind damage of coastal vegetation especially some ornamental species used in landscaping.

MATERIALS AND METHODS

Plant material. Fully expanded healthy leaves of *Vinca major* L., *Prunus laurocerasus* L., *Nerium oleander* L., *Hedera helix* L. and *Ficus benjamina* were sampled from mature plants. The leaves were visually investigated to exclude any damages or infections by microorganisms.

Isolation of cuticles. The cuticles were isolated according to the method described by Schönherr and Riederer (1986). The leaves were washed with water, dried with soft tissue and left at room temperature for a few hours until completely dried. Disks of 20 mm diameter were punched out from the leaves and incubated in an aqueous solution containing 2% (v/v) cellulase (Celluclast, Novo Nordisk, Bagsvared, Denmark) and 2% pectinase (Trenolin, Erbslöh, Geisenheim, Germany) in 0.01 M citrate buffer (Merk, Germany; pH 3.0 adjusted with KOH). In order to prevent microbial growth, 1 mL of 1 M sodium azide (Fluka, Neu-Ulm, Germany) was added to 1 L of the enzyme solution. Cuticles from the adaxial leaf sides were separated from the cellular debris and incubated in 0.01 M borax buffer (Fluka, Germany) adjusted to pH 9 for about one week. Later the cuticles were incubated again for about 10 days in deionized water, removed and dried under a stream of pressurised air to flatten and stored in Petri dishes

at room temperature until used.

Measurement of water permeability. Water permeability (cuticular transpiration) was determined using a gravimetric method as described by Schönher and Lenzian (1981). Stainless steel transpiration chambers were used in this study. The edges of the transpiration chambers in contact with plant cuticle were sealed with high vacuum silicone grease (Wacker Chemie, Burghausen, Germany). The chambers were filled with 900-1000 μL of deionized water that served as a donor solution. Cuticles were mounted on the transpiration chambers with their morphological outer surface facing the atmosphere. These chambers were placed upside down in closed polyethylene boxes above silica gel and incubated in an incubator (Binder, Tuttlingen, Germany) at $25\pm 0.5^\circ\text{C}$ for overnight. Water loss was monitored by weighing the chambers every 24 h for 4 to 5 days with a microbalance (Sartorius Analytic BP 221S, Göttingen, Germany) connected to a personal computer (SartoConnect version 3, 1).

Amounts of water diffused across the membranes were summed up and plotted as a function of time. Rates of water loss (F) were calculated from linear regression lines fitted to the plotted data. The water concentration over silica gel was negligible, therefore water density in the chambers (1000 kg. m^{-3}) was used as driving force for transpiration and the exposed area of the cuticle (A) to the atmosphere was 1.13 cm^2 . Permeance (P) was calculated using the equation:

$$P = F / (A \cdot \Delta c)$$

Seawater effect on water permeability of isolated cuticles. After water permeability of each single layer was measured, 200 μL of seawater solution was applied on the outer surface of the layer. The composition of synthetic seawater solution was: NaCl, Na_2SO_4 , MgCl_2 , CaCl_2 and KCl at concentration of 23.48, 3.92, 4.98, 1.10 and 0.66 g L^{-1} . In parallel, five chambers of each species were treated with 200 μL deionized water as a control. The chambers were left between 6 to 24 h at room temperature until the water of the treating solution had evaporated. They were incubated again at $25\pm 0.5^\circ\text{C}$ and transpiration measured during the next 4 to 5 days as described above. This treatment was repeated again to increase seawater concentration on plant cuticle by increasing the amount of seawater per cuticle area and for other two treatments by adding 300 and 500 μL of seawater subsequently and water transpiration measured again in every treatment. To test whether the salt effect is reversible or there was irreversible damage of the cuticles the salt residues were washed off from the cuticles again and the experiment was continued in order to determine the permeance after washing off the salt from the cuticles.

RESULTS

There were clear differences in the water permeability

of species. The low permeance observed in both *N. oleander* and *F. Benjamina* was 8.13×10^{-11} and 8.67×10^{-11} , respectively (Table I). Treating outer surfaces of cuticular layers with different amounts of seawater showed that water permeability increased by increasing the amounts of applied seawater (Table II). A linear correlation between increase of water permeability and amounts of applied seawater was observed. The effect of seawater on water permeability of isolated cuticular layers of the five species varied depending upon the species (Table II). The effects of increasing cuticular water permeability reached the highest values when cuticular layers treated with high concentration of simulated seawater reached 2.59 and 3.21 for *N. oleander* and *F. Benjamina*, respectively.

The effects were significantly decreased again by washing the cuticular layers with water, although initial low permeances were not fully established again. Treatment of the cuticular membranes with deionized water as a control did not influence cuticular water permeability of all five species tested (Table II).

DISCUSSION

It is well known that cuticular permeability of different plant species varies significantly, which has been interpreted as an adaptation to the natural habitats of the species (Schreiber & Riederer, 1996; Riederer & Schreiber, 2001). Five species used in this study fitted in this classification of cuticular water permeabilities and it was clear with *F. benjamina* and *N. Oleander*.

The size of polar pores in *Citrus* and *Allium* cuticles was estimated to be around 0.45 nm (Schönher, 1976b). Since the hydrated diameter of many ions is below 0.8 nm (Tyree *et al.*, 1990), it is highly probable that ions can sorb and move within these pores by diffusion. From these findings it is clear that polar inorganic ions, which form part of simulated seawater are sorbed to the lipophilic cuticles. Consequently, polarity of the cuticle is increased and increasing amounts of water are sorbed to the cuticular layer. This leads to a swelling of the layer and finally to an increased cuticular transpiration. With increasing amounts of ions, more amounts of water are sorbed, as a consequence of increased cuticular permeability. The difference in increasing water permeability, when treated with simulated seawater, is due to the lateral heterogeneity in cuticle structure and function (Schönher, 2005). Treating the outer

Table I. Water permeances (P) of CMs of five different species, the values are means \pm 95% confidence intervals

Species	P (m s^{-1}) \pm ci
<i>Hedera helix</i>	$4.33 \cdot 10^{-11} \pm 1.75 \cdot 10^{-11}$
<i>Ficus benjamina</i>	$8.67 \cdot 10^{-11} \pm 4.04 \cdot 10^{-11}$
<i>Nerium oleander</i>	$8.13 \cdot 10^{-11} \pm 1.26 \cdot 10^{-11}$
<i>Vinca major</i>	$1.09 \cdot 10^{-10} \pm 1.89 \cdot 10^{-11}$
<i>Prunus laurocerasus</i>	$2.49 \cdot 10^{-10} \pm 5.43 \cdot 10^{-11}$

Table II. Effect of simulated sea water on water permeability of five cuticular membranes, the concentrations of sea water were compensated by dose per area and the values are means \pm 95% confidence intervals

Species	Effect ($P_{\text{aft.}}/P_{\text{bet.}}$) \pm c.i				
	mol.m ⁻²				
	0	0.85	2.11	4.23	After washing
<i>Hedera helix</i>	1.00 \pm 0.40	1.20 \pm 0.21	1.85 \pm 0.33	2.32 \pm 1.07	0.98 \pm 0.16
<i>Ficus benjamina</i>	1.00 \pm 0.47	0.81 \pm 0.06	1.22 \pm 0.28	2.59 \pm 0.51	2.29 \pm 0.35
<i>Nerium oleander</i>	1.00 \pm 0.16	0.95 \pm 0.07	1.35 \pm 0.13	3.21 \pm 0.85	2.50 \pm 0.23
<i>Vinca major</i>	1.00 \pm 0.22	1.28 \pm 0.10	1.43 \pm 0.09	1.82 \pm 0.17	1.52 \pm 0.14
<i>Prunus laurocerasus</i>	1.00 \pm 0.17	1.55 \pm 0.14	2.37 \pm 0.35	3.63 \pm 0.76	2.48 \pm 1.12

sides of the cuticles of with increasing amounts of seawater, despite increasing sodium concentration in seawater, resulted in enhanced effects on water permeability of all species studied (Table II).

In order to be able to sorb to the cutin polymer, the salts deposited on the cuticle surface have to be in a liquid state (Schönherr, 2001; Schlegel & Schönherr, 2002). Upon drying they will crystallize and render them completely immobile. Hydration and dissolution of salts is determined by their point of deliquescence (POD), which refers to the humidity over a salt solution containing solid salts (Schönherr, 2001). When the humidity is above the POD, the salt residue on the cuticle sorbs water from the atmosphere (Schlegel & Schönherr, 2002) dissolves and the ions of the salt are mobile and can diffuse into the cutin polymer, while below the POD this process stops. The POD of most salts form the artificial seawater was relatively high, while KCl had a POD of 86% and NaCl had 95%. This implied that both salts were in a liquid state at much high humidities.

In the nature, air humidity, which almost in all cases is high in coastal regions, is a limiting factor for salt penetration through cuticles of the coastal plants and water permeability is increasing by increasing humidity (Schreiber *et al.*, 2001). This effect can be significantly enhanced, accumulating salts of seawater on leaf surface when seawater spray occurs at faster rate.

In all cuticles treated with seawater an increase in cuticular water permeability occurs. These effects were again reduced by washing the cuticles. Although, original low permeabilities were never established again (Table II), the fact that washing reduces cuticular permeability indicates that increase in cuticular water permeability is at least partially reversible.

In the Mediterranean areas, coastal storms in winter are usually accompanied with rain, which reduce the effectiveness of the spray. In the absence of rainfall, as in almost all the time during the year, especially summer, water loss from the plants may increase. Damage the coastal vegetation resulting from salt spray may occur, leading to plant death. This damage might be reduced by washing the plants used in landscaping, like *Nerium* and *Ficus*.

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(Received 13 May 2009; Accepted 09 July 2009)