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Sap salinity effects on xylem conductivity in two mangrove species

JORGE LÓPEZ-PORTILLO[1], FRANK W. EWERS[2] & GUILLERMO ANGELES[3]

[1]Departmento de Ecología Funcional and [3]Unidad de Recursos Forestales, Instituto de Ecología, A.C., Apdo. Postal 63, Xalapa 91070 Veracruz, México and [2]Department of Plant Biology, Michigan State University, East Lansing, MI 48824–1312, USA

ABSTRACT

Xylem sap salinity and conductivity were examined in two mangrove ecosystem tree species. For *Avicennia germinans*, extracted xylem sap osmotic potentials ranged from –0.24 to –1.36 MPa versus –0.14 to –0.56 MPa for *Conocarpus erectus*. Xylem sap of *Conocarpus* did not vary in osmotic potential between sites nor between predawn and midday. In *Avicennia*, values were more negative at midday than predawn, and also more negative at hypersaline than hyposaline sites. After removing embolisms, specific conductivity (K_s) was measured as a function of salinity of the artificial xylem sap perfusion. For both species the lowest K_s values, about 70% of the maximum K_s, were obtained when stems were perfused with deionized water (0 mM; 0.0 MPa) or with a 557-mM saline solution (–2.4 MPa). Higher K_s values were obtained in the range from –0.3 to –1.2 MPa, with a peak at –0.82 ± 0.08 MPa for *Avicennia* and –0.75 ± 0.08 MPa for *Conocarpus*. The variations in K_s values with minima both at very low and very high salt concentrations were consistent with published results for swelling and shrinking of synthetic hydrogels, suggesting native hydrogels in pit membranes of vessels could help regulate conductivity.

Key-words: *Avicennia germinans*; *Conocarpus erectus*; halophytes; hydrogels; specific conductivity; xylem sap.

INTRODUCTION

Effects of xylem sap ionic concentrations on the hydraulic conductivity have been reported for glycophytes, that is, plants that grow healthily only in soils with a low salt content (e.g. Zimmermann 1978; Van Leperen, van Meeteren & van Gelder 2000; Zwieniecki, Melcher & Holbrook 2001; De Boer & Volkov 2003). In the present study we examine whether changes in xylem sap salinity can impact hydraulic conductivity in two halophytes, plants that tolerate high salt concentrations in the soil.

According to Zwieniecki *et al*. (2001), the ionic content of the xylem sap has a significant effect on the hydraulic conductivity (K_h) of plants, presumably mediated by the swelling and shrinking of hydrogels in pit membrane microchannels. They reported that the effect was most pronounced at 0–10 mM KCl, with slighter effects at 10–50 mM. However, mangrove plants are reported to have xylem sap salt concentrations ranging from 0.5 to 380 mM (Scholander *et al*. 1962, Ball 1988; Sobrado 2002). Furthermore, both theoretical and experimental studies suggest that synthetic hydrogels can exhibit two peaks of swelling and one peak of shrinkage when bathed in salt solutions ranging from 0.1 to 1000 mM (English *et al*. 1996). If naturally occurring hydrogels in vessel pit membranes impact the conductivity of xylem, halophytes may be the ideal subjects to explore possible hydrogel effects over a wide range of ecologically meaningful salt concentrations, such as may occur within their xylem sap. It is also possible to test for hydrogel effects at a higher salt concentration than would naturally occur in the plant to explore the mechanisms that control conductivity.

Although all mangrove species have the ability to exclude much of the salt in the soil water from entering their xylem sap through root ultrafiltration, they have been classified into two main physiological groups (Scholander 1968), namely, secretors, such as *Avicennia* spp. which have glands on their leaves to remove excess salt from the xylem sap, and non-secretors, such as *Conocarpus erectus*, which lack such glands. Non-secretors appear to be more effective in preventing salts from entering the xylem sap and, reportedly, have lower salt concentrations in their xylem sap when compared with salt secretors (Scholander *et al*. 1962; Tomlinson 1986). Further, in the mangroves *Avicennia marina, A. germinans*, *A. alba*, *Laguncularia racemosa* and *Bruguiera gymnorrhiza* xylem sap salinity varies as a function of substrate salinity (Drennan & Pammenter 1982; Sobrado 2002, 2004; Paliyavuth, Clough & Patanaponpaiboon 2004). In the present study we examine the impact of soil salinity on xylem sap salinity for *A. germinans* and *C. erectus* both at predawn and at midday. Diurnal changes in xylem sap salinity might indicate the potential for metabolic control of xylem conductivity through hydrogel effects.

Given that control of hydrogel behaviour could be an adaptive response in plants to allow greater hydraulic conductivity when needed, we predicted that hydraulic conductivity in both *Avicennia germinans* and *Conocarpus erectus* would respond to the salinity of the xylem sap, but with increases in hydraulic conductivity at much higher salt con-

Correspondence: Jorge López-Portillo.
E-mail: lopez-p@ecologia.edu.mx

centrations than previously reported in studies with glycophytes. Furthermore, we predicted that when growing at the same site, the secretor, *A. germinans* would have higher concentrations of salt in its sap, and that it would reach its maximum conductivity at higher salt concentrations than the non-secretor, *C. erectus*. Polyampholytic hydrogels (i.e. large molecules which contain many acidic and basic groups) have a peak swelling in salt solutions at both 0.2 and 1000 mM (English *et al*. 1996). We predicted that when stems were perfused either with pure deionized water or in solutions that were significantly more saline than what is normally found in the plants, there would be a decline in conductivity due to swelling of hydrogels at pit membranes. Lastly, if reduced xylem conductivity at extreme salinities were caused by the swelling of hydrogels, it should be completely reversed by perfusions of intermediate salinity solutions.

MATERIALS AND METHODS

Study site

Work was done in the mangrove forests of La Mancha Lagoon in the State of Veracruz, Mexico (19°33′–19°36′ N; 96°22′–96°24′ W), in the Gulf of Mexico. In this area, average annual precipitation is between 1200 and 1500 mm and mean annual temperature is 25 °C with a minimum in January (22 °C) and a maximum in May (28 °C). Measurements were made in May of 2001, at the height of the dry season. This was when water potentials were lowest, but the sampling sites varied in the amount of freshwater versus seawater input and the level of water stress experienced by plants varied accordingly. We worked with two species, the 'button mangrove' *Conocarpus erectus* L. (Combretaceae) and the 'black mangrove' *Avicennia germinans* L. (Avicenniaceae).

To examine relationships between soils, pressure potentials and xylem sap osmotic potentials, we selected extreme sites for the two species in terms of substrate salinity. Site 1 (low salinity) was a large monospecific stand of *Conocarpus*. Site 2 (high salinity) was a mixed forest with a narrow monospecific band of *Conocarpus*. For *Avicennia,* site 3 (low salinity) was a mixed forest with the greatest freshwater input and water level and included *Laguncularia racemosa, Achrostichum aureum* and *Rhabdadenia biflora*, but not *Conocarpus*. Site 4 (high salinity) was a mudflat with two monospecific bands, one of *Avicennia* and one of the extreme halophyte *Batis maritima.* We sampled 12 plants per site, both at predawn and midday and ran two way analyses of variance (ANOVAs) for each species to compare between sites and time of day. Differences between sites in soil water potentials ($n = 6$ samples per site) were tested with one way ANOVAs.

Sites 5 and 6 were adjacent to sites 2 and 1, respectively, but at ecotones between monospecific stands of *Conocarpus* and *Avicennia.* Matched pairs of plants with intermingled canopies were selected for direct comparisons in soil water potential, xylem sap osmolarity, xylem pressure potential, and responses of the stem specific hydraulic conductivity ($K_s = K_h$/sapwood cross-section) to experimental changes in xylem sap osmolarity. Six matched pairs and their associated soils were sampled at midday for each of the three experimental sessions, including a total of 12 pairs of plants at site 5 and six pairs at site 6. We carried out paired *t*-tests for comparisons between the species and also between the soil samples.

Osmotic and pressure potential measurements

Salt in xylem sap of mangroves is composed mostly of Na^+ and Cl^-, the same dominant ions as in seawater (Clough, Andrews & Cowan 1982; Popp, Larher & Weigel 1985; Ball 1996). Consequently, we used sodium chloride solutions to test for possible hydrogel effects in the stem xylem. Since we measured soil osmotic potential and native xylem sap osmotic potential with the psychrometer method, and pressure potentials with a pressure chamber, in the present study we show results in pressure units (MPa) rather than in solute concentrations (mM). Lang (1967) reported 216 mM NaCl per MPa at sea level and 25 °C. Our own calibration with chemically pure NaCl solutions at sea level and 25 °C gave an estimate of 232 ± 14 mM per MPa (C. Paredes López, unpubl. results). We will use this mean value because it was obtained with the same methodology we describe below.

One pair of soil samples were collected about 0.40 m from the stem base at a depth of 10–20 cm, where fine root density was highest. Soil osmotic potential was measured with the psychrometer method (see below), with the two soil sample results averaged for each plant.

Two metre long shoots were cut in the field and immediately bagged to minimize water loss from the leaves. In the air-conditioned laboratory at the field station, twigs were sampled for measurements of xylem pressure potential with a pressure chamber (Scholander *et al*. 1966). As the compensating pressure was reached, a sample of the xylem sap was immediately collected from the twig on a filter disc (Scholander *et al*. 1966; Schurr 1998) and placed in a Wescor C-52 sample chamber to equilibrate before osmotic potentials were measured by psychrometry, using a HR-33T Dew Point Microvoltmeter (Wescor Inc., Logan, UT, USA).

We also measured xylem sap osmotic potential from 10 cm long, 1 cm wide, stem segments that were adjacent to the segments used for K_h measurements. In preliminary experiments we found that extracting xylem sap by chasing it with another solution gave contaminated results, reflecting the chase solutions, even in the first drop. In stems cut in the air, we found that applying 100 kPa gas pressure did not yield enough sap to wet a filter paper disc. The cut vessels must have released all of their water to surrounding tissues before we could extract the sap. Therefore, we cut the 2 m long stem several times under tap water until we obtained the target segment. We reasoned that some water may be drawn into the stem from the water bath with the first underwater cut, but after releasing the xylem tension

with subsequent cuts, no external water entered the segment, and the water bath osmolarity did not impact xylem sap measurements. To test this, 10 additional stems per species were collected and all the segments were cut under a water bath containing 0.1% safranin. The dye reached an average of 18% (SE 8%) of the total target segment length in *Avicennia* and 1 ± 2% in *Conocarpus*. In contrast, the adjacent bottom and top segments were dyed in 48 ± 10 and 79 ± 8% (both statistically different from the target segment) of their length, respectively, in *Avicennia*, and 7 ± 5 and 33 ± 10% (only this value statistically different from the target segment and significantly different from zero) in *Conocarpus*. On the other hand, sequential transverse cuts of the target segment indicated that in the most extreme case in *Avicennia*, less than 50% of the vessels were dyed along 4 cm when adding both cut ends, but that the average sum length was 0.44 ± 0.23 cm in *Avicennia* and 0.04 ± 0.04 cm in *Conocarpus*. Thus, the water bath did not significantly contaminate the xylem sap of the target segments.

We debarked 1–2 cm from both ends of the segment, removed the 10 cm segment from water, shaved the cut ends with a new razor blade, and connected one end to a gas pressure source. In a preliminary study, by applying low gas pressure (10 kPa), we collected the sap of the first six drops on separate filter discs which were used as replicates. Values of osmotic potential were consistent for the first and second drop from the stem end, but not for subsequent drops, which gave more negative values of osmotic potential. Apparently evaporation, aided by gas bubbles forced through the stem, concentrated the sap solution in the later drops, so only the first drop was used for the stem xylem sap measurements. Results thus obtained for 1 cm diameter stems were very similar to those for xylem sap extracted from twigs of the same branch with a pressure chamber.

Hydraulic conductivity versus sap salinity

This experiment consisted of measuring K_h with perfused solutions of different salinities. The experiment was repeated in three trials in order to determine the time needed for a stable effect, which was presumably the time needed for maximum displacement of salt ions at pit membranes after shifting between different salinity solutions. Each trial involved six matched shoot pairs of *Conocarpus* and *Avicennia* collected from the ecotone sites (sites 5 and 6) with only one shoot sampled per plant. Two-metre-long stems were recut under water to arrive at 20 cm long and 1 cm wide segments which were transversely shaved on each end with a fresh razor blade and connected into a manifold (Sperry, Donnelly & Tyree 1988). There were seven reservoirs, each with a solution of different NaCl concentrations, attached by three-way valves to the manifold. Therefore we could switch salt perfusion solutions very rapidly and perfuse all 12 stems at once with the desired solution. Tubing from the downstream end of the stems fed into an electronic balance for flow rate readouts. Three-way valves allowed us to measure one stem at a time with low pressure head (1.5 kPa), or to perfuse all stems at once at a higher pressure of 15 kPa. Preliminary experiments with dyes indicated that when we switched solutions with a pressure head of 15 kPa, some of the new solution would pass through the entire length of the 20 cm stem segments within a few seconds.

The tap water at the research station was saline (Ewers *et al*. 2004), with an osmotic potential of about –0.3 MPa throughout the period of study. The osmotic potential of the xylem sap of the 12 stems used in an experiment, plus six samples of tap water were measured, and the initial perfusion solution was created by adding sodium chloride to tap water to arrive at an osmotic potential intermediate between the xylem osmotic potential of the two sampled species. The mean (± SE) osmotic potential of the initial perfusion solution, filtered through a 0.2-μm in-line filter, was measured as –0.70 ± 0.01, –0.50 ± 0.03 and –0.34 ± 0.01 MPa for the three experiments.

Initial K_h was measured at a low pressure head of about 1.5 kPa. The 12 stems were then perfused simultaneously with the initial perfusion solution at a hydrostatic pressure of 150 kPa to remove air emboli. Hydraulic conductivity was re-measured at low pressure and the high pressure perfusion was repeated until maximum K_h was reached, confirming that all air emboli were removed (Sperry *et al*. 1988). We then shifted to the different reservoirs of sodium chloride solutions, which had osmotic potentials of 0, –0.15, –0.3, –0.6, –1.2 and –2.4 MPa (which corresponded to solutions prepared with 0, 1.8, 3.7, 7.4, 14.8 and 29.6 g NaCl per litre of deionized water). Measurements of K_h were made at low pressure (1.5 kPa) and were later followed by perfusions of the new sodium chloride solutions (or distilled water) at 15 kPa to begin a new measurement session. Measurements at low pressure were more accurate in our system. The high pressure perfusions were used to shorten the time needed to completely saturate the stems with the new solution.

The specific conductivity (K_s) was determined as K_h divided by the xylem area of the stem segment. Since K_s varied with the osmotic potential of the perfusion solution, maximum K_s was determined for each stem, and the values at the other perfusion concentrations were expressed as a percentage of that maximum. The statistical analysis was a repeated measures ANOVA with arcsine transformed data on percent of maximum K_s to compare within species since in this experiment various solutions were perfused through the same stems. The means and standard errors obtained from the model were back transformed to the original linear scale.

RESULTS

Interspecific xylem sap osmotic potentials

Although the ranges overlapped, the osmotic potentials of the extracted xylem sap of *Avicennia* tended to be more negative than those of *Conocarpus* (Fig. 1). In *Conocarpus* the osmotic potentials ranged from –0.14 to –0.56 MPa

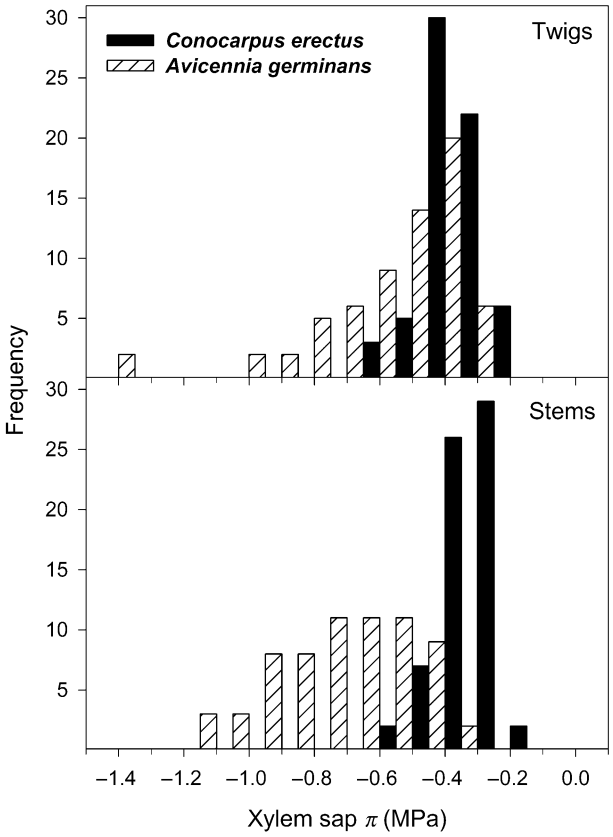

Figure 1. Xylem sap osmotic potentials (π) in twigs and stems of *Conocarpus erectus* (solid bars) and *Avicennia germinans* (hatched bars). Each species is represented by a sample of 66 twigs and stems corresponding to different individuals.

(32–130 mM) in twigs and from –0.16 to –0.56 MPa (37–130 mM) in stems. For *Avicennia* the range was from –0.24 to –1.36 MPa in twigs (56–316 mM) and from –0.21 to –1.05 MPa (49–244 mM) in stems (Fig. 1).

Diurnal changes in salinity at extreme sites

For *Conocarpus,* both at sites 1 and 2 there was no significant difference in stem xylem sap osmotic potentials in predawn and midday collections (Table 1). Likewise, the two *Conocarpus* sites were not significantly different in stem pressure potential at midday and in soil water potential. In contrast, *Avicennia* xylem sap osmotic potentials were significantly less negative at predawn than at midday, both at sites 3 and 4. The soil water potential from site 4 was one order of magnitude more negative than Site 3 and there were significant differences in pressure potential both between sites and times of day (Table 1). At predawn and midday there were, respectively, 0.25 and 0.24 MPa lower stem sap osmotic potentials at site 4 than site 3, indicating greater xylem sap salinity at the more saline site (Table 1).

Comparisons between species at the ecotones

There were no significant differences between species in the water potentials of the soils collected at the base of paired plants at the ecotones (sites 5 and 6). However, the twig and stem xylem sap osmotic potentials were, respectively, 86 and 111% more negative in *Avicennia* than in *Conocarpus* (Table 2). Furthermore, twig xylem pressure potentials in these sites were 34% more negative in *Avicennia* than in *Conocarpus.*

Hydraulic conductivity versus sap salinity

In the first two experiments we found that the salinity of the perfusion solutions influenced the K_h values, and experimented with the time needed for full effect of a solution. We found that 18 min at 15 kPa were needed when shifting from a saline solution to deionized water, whereas only 6 min at that pressure were needed for full effect when shifting to more saline solutions. We consistently used those perfusion times and pressures for the third experiment, the results of which are shown in Fig. 2.

After $K_{s\,max}$ was obtained in stem segments following high pressure perfusion, changes in salinity concentrations of the solutions resulted in changes in specific conductivity (K_s) for both *Conocarpus* and *Avicennia*. There was great variability in the K_s values among stems in both species, but within a species there was a similar pattern of response to the shifts in the osmotic potential of the solution

Table 1. Extreme sites: soil water potential (Ψ), predawn (PD) and midday (MD) stem pressure potential, and stem sap osmotic potential (π) in a non-secretor (*Conocarpus erectus*) and a salt secretor (*Avicennia germinans*)

			Stem pressure potential (MPa)		Stem sap π (MPa)	
Species	Site	Soil Ψ (MPa)	PD	MD	PD	MD
Conocarpus erectus	1	–1.72 (0.46) a	–1.34 (0.13) c	–2.48 (0.13) a	–0.31 (0.02) a	0.31 (0.02) a
	2	–2.63 (0.46) a	–1.98 (0.13) b	–2.78 (0.13) a	–0.30 (0.02) a	–0.30 (0.02) a
$F_{d.f.}$		$1.97_{1,10}$ ns	$12.7_{3,44}$		$0.20_{3,44}$ ns	
Avicennia germinans	3	–0.40 (0.20) b	–2.53 (0.08) d	–3.83 (0.08) c	–0.40 (0.04) d	–0.53 (0.04) c
	4	–4.93 (0.20) a	–4.56 (0.08) b	–5.93 (0.08) a	–0.65 (0.04) b	–0.77 (0.04) a
$F_{d.f.}$		$262_{1,10}$	$351_{3,44}$		$20_{3,44}$	

Values are means (SE). *F*-values of the one-way ANOVA for soil Ψ and the two-way ANOVAs (site and time of day as factors) for stem pressure potential and sap osmotic potential are all significant at $P < 0.001$, except when marked ns (non-significant). Same letters indicate no significant differences between or among means in each measurement block. Subscripts indicate the degrees of freedom for each ANOVA.

	Soil Ψ (MPa)	Twig xylem Ψ P (MPa)	Twig xylem Ψ π (MPa)	Stem xylem π (MPa)
Conocarpus erectus	–3.40 (0.30)	–4.13 (0.32)	–0.35 (0.02)	–0.35 (0.02)
Avicennia germinans	–3.24 (0.29)	–5.55 (0.20)	–0.65 (0.08)	–0.69 (0.06)
t	0.95	5.55	4.15	6.98
P	0.72	< 0.001	< 0.001	< 0.001

Values are means (SE), $n = 18$.

Table 2. Ecotone sites: paired *t*-tests for soil water potential (Ψ), midday twig pressure potential (P), xylem sap osmotic potential (π) and stem xylem sap π in paired individuals of the non-secretor (*Conocarpus erectus*) and the salt secretor (*Avicennia germinans*) in two combined ecotone sites (sites 5 and 6)

(Fig. 2). In experiments 2 (not shown) and 3 (Fig. 2), the –0.6 MPa treatment was repeated twice on the same stems, with both higher (up to –2.4 MPa in Fig. 2) and then lower (0 MPa) osmotic potentials between the two –0.6 MPa treatments.

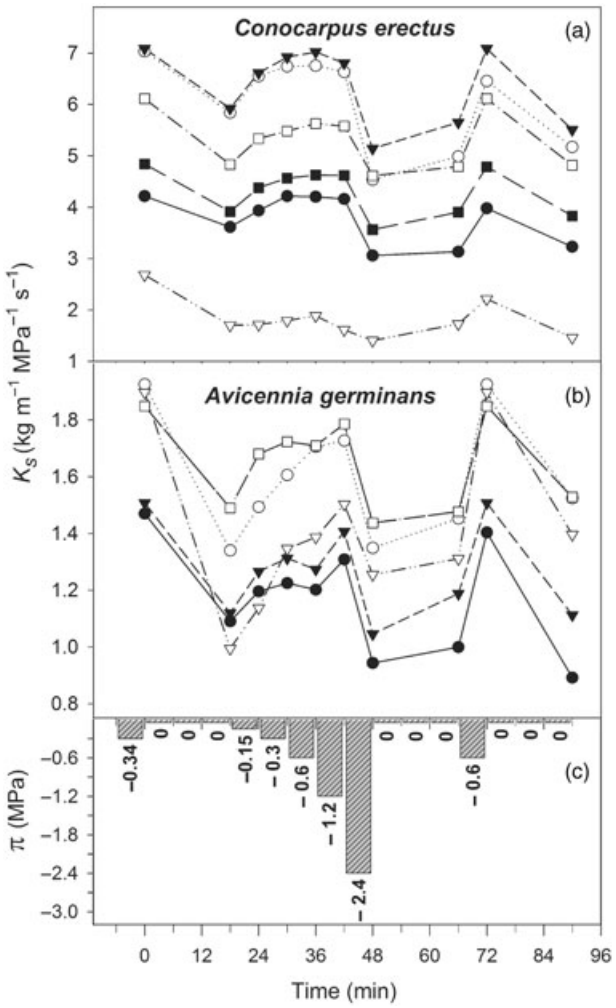

Figure 2. Specific conductivity (K_s) of non-secretor *Conocarpus erectus* (a) and the salt secretor *Avicennia germinans* (b) as a function of NaCl concentration shown in MPa (c). Each symbol in (a) and (b) indicates a different stem. In (c), each bar indicates one 6-min period; numbers below bars show the osmotic concentration (π) of the solution infused into the stems.

The lowest K_s values were obtained when stems were perfused with deionized water (0 MPa) and with the solution with the highest salinity strength (–2.4 MPa). Such low K_s values were reversible when the stems were perfused with solutions of intermediate salinity. Maximum K_s values were maintained in the range from –0.3 to –1.2 MPa. The conductivity of most segments was greater for the initial measurement, made at –0.3 MPa, than at higher concentrations of NaCl (–0.6 or –1.2 MPa) after perfusion of stems with deionized water, but was greatest with the second –0.6 MPa treatment. One possible explanation for this outcome is that some foreign particles were introduced and the alternate shrinking and swelling of hydrogels had the effect of cleaning them out from the pit membranes.

Figure 3 shows the mean percentage of maximum K_s in relation to the osmotic potential of the experimental sap solutions for both species. The data were fitted to a three-parameter Gaussian model (using SigmaPlot; SPSS Inc., Chicago, IL, USA), which accounted for 71 and 65% of the variance in *Conocarpus* and *Avicennia*, respectively (Fig. 3). Of the three parameters in both models, *a* corresponded to maximum K_s in the function, and x_o indicated the osmotic potential of the solution at maximum K_s. According to these models, maximum conductivity occurred at values near –0.8 MPa both in *Conocarpus* and *Avicennia*, which would correspond to a 186-mM solution of sodium chloride. At extreme values (0 and –2.4 MPa), only about 70% of maximum K_s was obtained.

DISCUSSION

Sap osmotic potentials in secretors versus non-secretors

The osmotic potentials found in the xylem sap, corresponding to NaCl concentrations of 32 to 130 mM in *Conocarpus* and 49 to 316 mM in *Avicennia*, were within the range of xylem sap concentrations previously reported for mangroves (Scholander *et al*. 1962; Tomlinson 1986). The first authors to note a positive relation between substrate salinity and xylem sap osmotic potential were Drennan & Pammenter (1982) and this finding has been confirmed for other mangroves (Sobrado 2002, 2004; Paliyavuth *et al*. 2004). At our study site, *Avicennia* tolerates a wider range of substrate salinities than *Conocarpus*, and this trend may apply to secretors versus non-secretors in general.

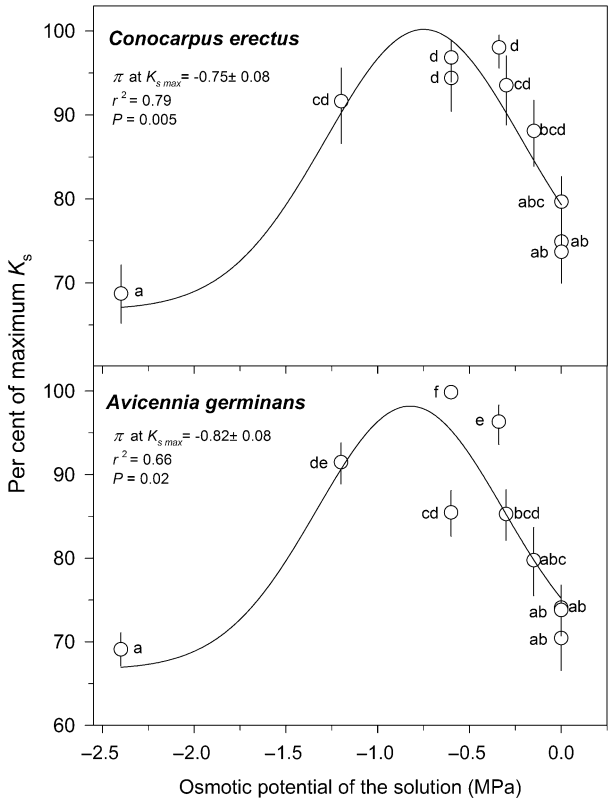

Figure 3. Percentage of maximum xylem specific conductivity (K_s) in stems of the mangroves *Conocarpus erectus* and *Avicennia germinans* as a function of the experimental changes of the salt solution osmolarity (π). Each symbol is the average of six stems. Similar letters beside each symbol indicate no significant differences between average K_s values. Vertical bars indicate ± one standard error. A three-parameter Gaussian function ($y = a \times \exp(-0.5 \times ((x - x_0)/b)^2)$ was fitted to the data, where a and x_0 indicate the maximum K_s and the π at which such value was obtained. The coefficients of determination and P-values are also shown.

As we predicted, even in the site where both species were neighbours, the salt concentration in the xylem sap was higher in the secretor (*Avicennia*) than in the non-secretor (*Conocarpus*), evidencing their distinctive ability to regulate xylem sap osmotic potential relative to the osmotic potential of the substrate. Xylem sap salinity for both species was much lower than the soil salinity. However, whereas *Conocarpus* xylem osmotic potentials were similar between sites and times of day, those of *Avicennia* were quite variable. This indicated that, consistent with their characterization, the non-secretor was more efficient in excluding salts from its xylem sap through root ultrafiltration (Scholander 1968) than the secretor. Previous workers have also highlighted the importance of ion uptake by leaf cells to maintain turgor and to keep up with the water demands due to transpiration (e.g. Popp *et al*. 1985; Flowers & Yeo 1986; Flowers, Hajibagheri & Clipson 1986; Rada *et al*. 1989). In this context, *Avicennia* would be more effective than *Conocarpus* since it can tolerate much lower water potentials.

Hydraulic conductivity versus xylem sap salinity

Our experimental results support the prediction that conductivity decreases if stems are perfused with saps of extreme salinities (either deionized or highly saline solutions) and that such decreases are reversible with solutions of intermediate salinities. These shifts in K_s values with changes in salinity of the xylem sap are consistent with the concept of swelling and shrinking of pectin-based hydrogels in the pit membranes (Zwieniecki *et al*. 2001). Synthetic polyampholytic hydrogels had peaks in swelling at salt concentrations of about 0.1 mM and at 1000 mM, and a peak of shrinkage at about 20 mM (English *et al*. 1996). Those molarities would correspond to the osmotic potentials of essentially 0.0 and –4.3 MPa for maximum swelling. We did not test osmotic solutions as low as –4.3 MPa, but the lowest K_s values were at 0.0 and –2.4 MPa. The 20 mM solution for maximum shrinkage would correspond to about –0.09 MPa and the osmotic potential for peak K_s values that we found was –0.75 MPa in *Conocarpus* and –0.82 MPa in *Avicennia.* Such qualitative similarity between the behaviour of a synthetic polyampholytic hydrogel and plant tissue hydrogels, with more than one peak in swelling and one peak in shrinking may not be a coincidence. A Flory-type swelling model with Coulombic interactions between fixed ions may apply to hydrogels in general and to pectin-based hydrogels synthesized by plants in particular, although salt concentrations for maximum swelling and shrinking may be different, as our data suggest.

At all sites, *Avicennia* appeared to operate with a xylem sap osmolarity closer to its optimum ionic concentration for flow enhancement (–0.82 ± 0.08 MPa) than did *Conocarpus,* which had a less negative native xylem sap osmolarity (–0.35 ± 0.02 MPa) than its optimum ionic concentration for flow enhancement (–0.75 ± 0.08 MPa). Therefore, our hypothesis that the secretor would reach its conductivity peak at higher salinities than the non-secretor was rejected, because the values in both species overlap when considering the standard errors.

Glycophytes versus mangroves

For glycophytes, the xylem sap tends to have higher salt concentrations at predawn than at midday (e.g. 10–20 mM versus 1–2 mM, Tyree & Zimmermann 2002; see also Petritschek 1953 and Klepper & Kaufmann 1966 in Kramer & Kozlowski 1979). Therefore, the predicted K_s values would be lowest at midday. However, the reverse may be true for halophytes that are salt secretors, such as *Avicennia germinans*. In these plants, salt secretion by leaf glands reduces the xylem sap salinity, and water uptake during the day overwhelms salt secretion rates, with the result that xylem sap salt concentrations are highest around midday (Scholander *et al*. 1962; Drennan & Pammenter 1982; Sobrado 2002). This would add to a 2.4% increase per °C in whole shoot conductance due to changes in sap viscosity (cf. figure 6.21 in Tyree & Zimmermann 2002) as air temperature rises from predawn to midday.

How important is xylem sap salinity to conductivity in plants? Based on the mean percentage difference in K_s between the maximum and minimum values (calculated as $(1 - (K_{s\,min}/K_{s\,max})) \times 100$) for the full range of experimental solutions, the possible impact on K_s would be 34% (range, 22–48%) in *Avicennia* and 31% (range, 25–48%) in *Conocarpus*. Within the range of native bulk xylem sap osmotic potentials, the mean maximum impact on K_s would be 21% (range, 9–40%) in *Avicennia* and 13% (range, 7–36%) in *Conocarpus.* Lastly, xylem sap in *Avicennia* was on average 0.13 MPa more negative at midday compared to predawn, and in *Conocarpus* there was no difference between time periods. However, it should be emphasized that the osmotic potentials within individual xylem vessels may be much more variable than the bulk xylem sap osmolarities we measured, so there could be significant localized impacts of xylem sap salinity on conductivity in different plant parts. Furthermore, the variability of the data set may be more important than the means of xylem sap osmolarity and K_s for each species, because the variation between twigs or stems within a single plant has the potential to effect a redistribution of resistances in the crown and the delivery of water to areas subject to higher transpiration rates.

Our prediction that hydraulic conductivity in both *Avicennia germinans* and *Conocarpus erectus* would respond to the salinity of the xylem sap, but with increases at much higher salt concentrations than previously reported in glycophytes, was supported by the experimental data. The differential response of conductivity to salinity strengths in several taxa (Van Leperen *et al*. 2000; Zwieniecki *et al*. 2001) and new data and evidence, suggest that the ability to respond to changes in the ionic content of xylem sap may depend on the fine scale distribution of hydrophobic lignin and hydrophilic polysaccharides (with hydrogels such as pectins) on the compound primary wall (Boyce *et al*. 2004). There must be a fairly low lignin content in the mangrove pit membranes, or else the hydrogel effect would not occur.

The relation between diel stem conductivity, sap salinity and gas exchange should be investigated in whole plants to understand the relative contribution of hydrogels. If sap salinity is greater at midday in salt secretors, higher stomatal conductivity should be expected if all else were held constant. However, Becker *et al*. (1997) found the highest sap flow rates (900–1000 $mm^3\ s^{-1}$) from 0900 to 1500 h in whole trees of the salt secretor *Avicennia* cf. *alba*, while stomatal conductances were fairly constant (approximately 400 $mmol\ m^{-2}\ s^{-1}$) and water potentials decreased. In the non-secretor *Rhizophora apiculata*, sap flow rates increased from 300 to 600 $mm^3\ s^{-1}$ in the same period and g_s (which was 80% lower than in *Avicennia*) was not significantly different from zero by midday. It is not clear whether sap salinity could account for some of the enhanced midday sap flow in *Avicennia* relative to *Rhizophora,* since the water potentials and stomatal responses were also quite different between those two species. One possibility would be that greater K_s at midday in salt secretors could help maintain stomatal conductance relatively constant even if air saturation deficit and plant water tension increase, but further evidences are needed.

If the predawn versus midday differences in sap ionic content are adaptive, the nature of the adaptation must be different in glycophytes when compared to halophytes, and a continuum of responses should be expected. In the extreme cases, hydrogels in salt-secreting plants may help maximize conductivity by day and growth at night by allowing for more turgor pressure to build up in meristematic tissues. In glycophytes, hydrogels may maximize conductivity in early morning and at night, but their swelling at midday should reduce the size of pores in pit membranes and thus minimize embolism risk. Capacitance might then compensate for the K_s reduction at midday.

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