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Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics of embolism formation and repair

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Abstract Physiological traits related to water transport were studied in *Rhizophora mangle* (red mangrove) growing in coastal and estuarine sites in Hawaii. The magnitude of xylem pressure potential (P_x), the vulnerability of xylem to cavitation, the frequency of embolized vessels *in situ*, and the capacity of *R. mangle* to repair embolized vessels were evaluated with conventional and recently developed techniques. The osmotic potential of the interstitial soil water (π_{sw}) surrounding the roots of *R. mangle* was *c*. $-2.6\pm5.52\times10^{-3}$ and $-0.4\pm6.13\times10^{-3}$ MPa in the coastal and estuarine sites, respectively. Midday covered (non-transpiring) leaf water potentials (Ψ_L) determined with a pressure chamber were 0.6–0.8 MPa more positive than those of exposed, freely-transpiring leaves, and osmotic potential of the xylem sap (π_x) ranged from –0.1 to –0.3 MPa. Consequently, estimated midday values of P_x (calculated by subtracting π_x from covered Ψ_L) were about 1 MPa more positive than Ψ_L determined on freely transpiring leaves. The differences in Ψ_L between covered and transpiring leaves were linearly related to the transpiration rates. The slope of this relationship was steeper for the coastal site, suggesting that the hydraulic resistance was larger in leaves of coastal *R. mangle* plants. This was confirmed by both hydraulic conductivity measurements on stem segments and high-pressure flowmeter studies made on excised leafy twigs. Based on two independent criteria, loss of hydraulic conductivity and proportions of gas- and liquid-filled vessels in cryo-scanning electron microscope (cryo-SEM) images, the xylem of *R. mangle* plants growing at the estuarine site was found to be more vulnerable to cavitation than that of plants growing at the coastal site. However, the cryo-SEM analyses suggested that cavitation occurred more readily in intact plants than in excised branches that were air-dried in the laboratory. Cryo-SEM analyses also revealed that, in both sites, the proportion of gas-filled vessels was 20–30% greater at midday than at dawn or during the late afternoon. Refilling of cavitated vessels thus occurred during the late afternoon when considerable tension was present in neighboring vessels. These results and results from pressure-volume relationships suggest that *R. mangle* adjusts hydraulic properties of the water-transport system, as well as the leaf osmotic potential, in concert with the environmental growing conditions.

Keywords *Rhizophora mangle* · Mangrove · Water relations · Cryo-scanning electron microscopy · Embolism refilling

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Introduction

Large, very negative, xylem pressure potentials (P_x) in the water transport conduits of vascular plants have been frequently inferred from balancing pressures obtained with the pressure chamber on previously transpiring shoots (Scholander et al. 1965; Turner 1981; Sperry et al. 1988b; Lamhamedi et al. 1992; Sun et al. 1995; Kavanagh and

Zaerr 1997). Even though it is not always recognized, it is inappropriate to use transpiring leaves to estimate pre-existing P_x due to non-equilibrium conditions before excision of leaves from the plant. Numerous authors have observed large differences in balancing pressure measurements between adjacent transpiring leaves and covered non-transpiring leaves (Begg and Turner 1970; Ritchie and Hinckley 1971; Turner and Long 1980; Turner 1981). It was found recently that P_x can be correctly estimated using the pressure chamber on leafy twigs that were covered at predawn to avoid transpirational water loss (Melcher et al. 1998a, 1998b; Wei et al. 1999, 2000; Zimmermann et al. 2000). These results are consistent with the suggestion of Passioura (1982) that if the water columns are continuous throughout the plant and if local variations in P_x are negligible, then the covered, non-transpiring leaf should function as a tensiometer, permitting P_x of the stem and remaining nearby leaves to be estimated from its balancing pressure. Substantial hydraulic resistance within leaves causes steep water potential gradients to develop in response to transpiration (Tyree et al. 1974; Turner and Long 1980). Direct measurements of P_x obtained with the xylem pressure probe (Balling and Zimmermann 1990), on the other hand, yielded much less negative values of P_x (within the measuring range of the xylem pressure probe *c*. –1.0 MPa; Thürmer et al. 1999; Wei et al. 1999) than those estimated using a pressure chamber with transpiring leaves (Balling and Zimmermann 1990; Zimmermann et al. 1994). Problems in reconciling measurements made with the xylem pressure probe and the pressure chamber remain in part because the measurement range of the pressure chamber extends beyond that of existing versions of the xylem pressure probe. Correct values of P_x are of great importance for adequate estimates of the driving forces for long-distance water transport in plants.

The current controversies over the magnitude of pressure gradients and the mechanisms for long distance water transport has also triggered a debate on the phenomenon and mechanisms of embolism repair. Cavitation in the xylem occurs at negative pressures much greater than those predicted from the tensile strength of water (Briggs 1950; Gerth and Hemmingsen 1976), and the discrepancy is hypothesized to be caused by properties of the xylem, principally pit-membrane pore diameter (Oertli 1971; Pickard 1981; Zimmermann 1983; Crombie et al. 1985; Sperry and Tyree 1988; Tyree and Sperry 1989; Cochard et al. 1992; Jarbeau et al. 1995). The refilling of embolized vessels has been shown to occur when P_x becomes positive, e.g., from root pressure (Sperry et al. 1987; Tyree and Ewers 1991). Because positive root pressure may not occur in most plants on a regular basis, repair of cavitated vessels by this means was believed to be relatively infrequent. Recent studies, however, have shown that repair of embolized vessels can occur even when transpiration rates are high and Ψ_L is low (Borghetti et al. 1991; Salleo et al. 1996; Canny 1997; McCully et al. 1998; Zwieniecki and Holbrook 1998; Holbrook and Zwieniecki 1999; Melcher 1999; Tyree et al. 1999).

Rhizophora mangle is an ideal plant system to use for addressing questions related to diurnal refilling of embolized vessels as well as other fundamental questions about water relations. *R. mangle* lacks root pressure and unlike other mangrove species, such as *Aegiceras corniculatum* and *Avicennia marina*, *R. mangle* does not actively secrete salt from its leaves (Scholander et al. 1963; Atkinson et al. 1967; Popp 1983a; Waisel et al. 1986). The osmotic potential of the xylem sap (π_x) of *Rhizophora mangle* has been reported to be close to zero (*c*. 0.15 MPa) (Scholander et al. 1966) and the osmotic potential of its leaf tissue is near that of seawater –2.5 MPa (Scholander et al. 1966; Popp 1983a). It has been shown that *R. mangle* desalinates seawater by ultra-filtration at the root level (Scholander 1968). Consequently, P_x must always be lower than –2.5 MPa, at coastal sites, to allow for water uptake during the day and to prevent reverse water flow from the plant at night (Scholander et al. 1965, 1966; Scholander 1968; Waisel et al. 1986; Rada et al. 1989).

In this study we employ several independent approaches to characterize whole-plant water transport properties in coastal and estuarine populations of *R. mangle*. Our objectives were to: (1) assess the frequency of xylem embolism *in situ* and compare this with estimates of xylem vulnerability to cavitation in excised branches, (2) determine whether embolized vessels are repaired diurnally, (3) estimate *in situ* P_x obtained from balancing pressures of non-transpiring leaves, and (4) determine how water-transport properties differ in *R. mangle* trees exposed to markedly different levels of salinity. Simultaneous measurements of transpiration, Ψ_L of covered and freely transpiring leaves, and π_x were obtained. Diurnal variation in the frequency of embolized vessels was evaluated with a cryo-scanning electron microscopy technique in stems previously flash-frozen in liquid N_2 to immobilize the water.

Materials and methods

Plant material and study sites

Rhizophora mangle L. (Rhizophoraceae), is an important component of mangrove ecosystems, and is found on coastal fringes and along streams of many tropical regions (Chapman 1976). Measurements were made on adult *R. mangle* plants from December 1997 through November 1998. Two study sites, open coastal and estuarine, located on the island of Oahu, Hawai'i, were chosen because previous studies have indicated that they differed in the salinity levels of the soil water. The coastal site was located at Queens Beach on the south-eastern coast of the island and is dry and exposed. The less saline estuarine site, was located near Kailua about 2 km from the ocean on the windward side of the island. The plants studied at this site were rooted in a drainage canal connecting a freshwater marsh with Kailua Bay. At both sites, leaf and air temperature, relative humidity, and photosynthetic photon flux density were monitored with standard micro meteorological sensors connected to a datalogger (CR10X, Campbell Scientific, Logan, Utah).

Field water relations

Leaves were excised at 2-h intervals from dawn until early evening for determination of Ψ_L from their balancing pressures using a pressure chamber. Measurements were made both on leaves that were covered with plastic bags and aluminum foil before dawn (n=5 trees) following the protocol outlined by Turner and Long (1980), and on leaves that were not covered and allowed to transpire freely throughout the day (n=5 trees). Simultaneously, diurnal courses of leaf stomatal conductance were determined with a steady-state porometer (Model 1600, LICOR Inc., Lincoln, Neb.). Transpiration was calculated using simultaneous estimates of leaf-to-air vapor pressure differences (VPD) and stomatal conductance (Jones 1992). Even though this is not a true estimate of transpiration due to partial removal of the boundary layer during measurements, it is accurate for comparative purposes because leaf sizes and shapes of the two populations were similar.

The osmotic potential of the interstitial soil water (π_{sw}) and leaf tissue (π_L) were determined diurnally using a vapor-pressure osmometer (Model 5500 Wescor, Logan, Utah) that was calibrated with standard solutions across a wide range of osmotic potentials. Samples were collected approximately every two hours from dawn to dusk at both sites. The water samples were stored in sealed containers, and the leaves were double-bagged and stored in an ice chest. At the end of the day, the leaves were transported back to the laboratory, where they were stored at –70°C. The leaves were then thawed, equilibrated to room temperature, placed inside sections of Tygon tubing, and crushed with a vise to extract sap for determination of π, and corrected for apoplastic dilution from the symplastic water fractions determined from pressure-volume curves. A 10-µl aliquot of sap was placed on a filter-paper disk and inserted into the osmometer.

Samples for determination of xylem sap osmotic potential (π_x) were obtained by forcing a solution with a known π through recently excised 0.2- to 0.3-m-long stem segments. An applied pressure of 0.01 MPa was sufficient to cause exudation from the downstream ends of the stem segments. The first ten successive drops of exudate were collected in separate conical centrifuge tubes. The tubes were kept tightly sealed, placed in plastic bags, and stored in a cooler until laboratory determinations of π_x could be made (within 3–5 h from the time of collection). In the laboratory, a 10-µl aliquot of fluid from each vial was placed on a filter paper disk for measurement in the vapor pressure osmometer.

Pressure-volume relations

Large leafy branches (*c*. 2 m long) were collected from both the coastal and estuarine sites at dawn and transported promptly to the laboratory. The branches were covered with plastic bags containing moist paper towels prior to excision. In the laboratory, smaller leafy twigs (0.1 m long) were re-cut from the large branches underwater (one twig per branch), and the excised ends were placed in a test tube containing water so that only a few centimeters of the stem ends were in direct contact with the water. During the 2-h hydration period, the distal leafy portions of the twigs were covered with small plastic bags to reduce transpirational water loss. After the hydration period, the portion of the stem end that was in contact with the water was removed, and the stem weight was determined on an analytical balance with a precision of 1 mg. This weight was assumed to be equal to the weight of the leafy twig at full turgor. The leafy twig was then placed into a plastic bag and immediately transferred to a pressure chamber, and the balancing pressure, the pressure required to return water to the cut surface, was obtained. After each determination of Ψ_L, the sample was reweighed. This procedure was repeated at various dehydration intervals to generate a pressure-volume relationship. After the final balancing pressure and fresh weight determinations, the dry weight of the twig was obtained by drying it in an oven at 70°C, for 7 days. Tissue water relation characteristics were analyzed following models presented in detail elsewhere (Tyree and Hammel 1972; Cheung et al. 1976).

Leaf hydraulic resistance

Resistance to flow through petioles and leaves was measured using a high-pressure flow meter (HPFM) filled with a degassed dilute acid solution (10 mmol l^{-1} oxalic acid, pH≅2). The principles behind the operation of the HPFM are given in detail elsewhere (Tyree et al. 1993). Briefly, the HPFM permits rapid measurements of water flow while controlling the water pressure gradient across excised stem segments, twigs, or even intact root systems. Measurements were made on small leafy twigs collected at dawn, kept in plastic bags, and immediately transported back to the laboratory. Before measurement, all but two recently expanded mature leaves were removed and the distal stem end was sealed using a thick walled, snugly fitting section of Tygon tubing that was plugged on one end. To prevent leakage during measurements the tubing was attached to the stem so that it covered all new petiole scars produced from leaf removal. Before attaching the two-leaf stem segment to the HPFM, about 3–5 cm of the upstream end of the stem was re-cut underwater, and 1 cm of the pith was removed and the cavity filled with plasticine (a soft clayey material) to prevent water from moving through the pith. Successive measurements of leaf hydraulic resistance were made by applying a pressure of 0.2 MPa to the upstream end of the stem and determining the flow rate every 3 s. The leaf-bearing stem was kept in a plastic bag during the measurement to reduce water potential gradients resulting from evaporation. Standardizing the number of leaves per stem segment facilitated direct comparisons between measurements by avoiding the need to carry out complex series/parallel resistance analyses.

Stem hydraulic conductivity

The stem hydraulic conductivity (k_h) was determined on segments that were collected at dawn, immediately re-cut under water at both ends, and transported back to the laboratory for measurements. Samples about 0.15 m in length and about 1 cm in diameter were flushed with a degassed 10 mol m^{-3} solution of oxalic acid (pH=2) at a constant pressure of about 0.2 MPa for 20 min in an apparatus similar to that described by Sperry et al. (1988a), which was configured to accommodate six samples simultaneously. The flush ensured that gas emboli within the vessel lumens were removed. After the 20-min flush, k_h was determined using a lower applied pressure of about 0.01 MPa. The leaf specific conductivity (k_L) was calculated by dividing k_h by the total leaf area downstream of the stem segment, and the specific conductivity (k_S) was calculated by dividing k_h by the area of conducting xylem. The cross section of the proximal end of the stem segment was used for k_S calculations.

Xylem vulnerability to cavitation

Cavitation and subsequent embolism formation in stem segments subjected to dehydration-generated tensions were detected by measuring the percent loss of k_h. Briefly, about 60 large leafy branches (*c*. 2 m long) were collected from the estuarine and coastal site at dawn and were immediately doubled-bagged with moist paper towels located between the two plastic bags to prevent transpirational water loss during transport from the field to the laboratory. They were then removed from their bags and allowed to transpire freely while held at *c*. 20°C and 50% relative humidity (RH) for varying intervals (0–9 days). At the end of each dehydration time interval, entire leafy twigs were re-bagged overnight for 12 h, to ensure Ψ equilibration throughout the branch. Balancing pressures of a distal leafy twig was measured using the pressure chamber. The chamber pressure was increased at a rate of 0.005 MPa s^{-1}, and the pressure at which water first appeared at the cut surface was taken to be the balancing pressure, which was considered to be equal and opposite in sign to the total leaf water potential Ψ_L. Paired measurements of Ψ_L and percent loss of k_h were used to generate a vulnerability curve (Sperry and Tyree

1988a). Briefly, at each dehydration interval, a 10-cm-long stem segment was excised under deionized water, cleanly shaven at both ends with a sharp Teflon-coated razor blade, and attached to a five-way-manifold hydraulic measuring apparatus (Tyree and Sperry 1988). Stem k_h was initially measured using a degassed 10 mmol l^{-1} oxalic acid solution (pH≈2) at a hydraulic head of 0.01 MPa. Gas emboli that formed during stem dehydration were then removed by applying a series of 25-min high-pressure (0.175 MPa) flushes until k_h remained constant between flushes. The final value of k_h was assumed to be equal to maximum k_h and the percentage loss of k_h was determined.

Xylem embolism was also assessed visually using a cryo-scanning electron microscopy (cryo-SEM) technique on branches collected at the coastal site. After selected dehydration intervals, leafy twigs attached to the same branches used to generate vulnerability curves were plunged into liquid nitrogen (LN_2) for several min. The twigs were then quickly removed, and 3-cm segments were placed in vials pre-cooled with LN_2. The samples were stored in a cryo-shipper filled with LN_2 until the cryo-SEM analyses were performed.

Cryo-scanning electron microscopy

Attached shoots of plants growing in the field were immersed in LN_2, *in situ*, for approximately 1 min before they were excised. The frozen stems (*c*. 1 cm in diameter) were then rapidly re-cut into smaller, 3-cm pieces, which were placed in pre-frozen ventilated plastic vials and stored in LN_2. This procedure was repeated five times between *c*. 0600 and 2000 hours in both the estuarine and coastal sites during the course of the same day. Balancing pressures were determined with a pressure chamber on both exposed leaves and leaves that had been covered to prevent transpiration immediately prior to collection of each set of branch samples for cryo-SEM analyses. The frozen stem segments were stored in a cryo-shipper that maintained the temperature at –170°C during shipment to the Science Technology Center at the Carleton University, Ottawa, Canada. Upon arrival, the stem sections were prepared for imaging. They were fastened onto stubs with Tissue Tek and conveyed under LN_2 to a cryo-microtome (CR 2000, Research and Manufacturing, Tucson, Ariz., USA) under LN_2. Transverse faces of the stem were planed, first roughly with a glass knife, and finally very smoothly with a diamond knife, all at –80°C (Huang et al. 1994). The specimens were transferred under LN_2 to a cryo-transfer system (Oxford CT 1500, Oxford Instruments, Eynsham, Oxford, UK) and finally to the cryo-stage in a scanning electron microscope which was kept at –170°C (JSM 6400, JEOL Ltd., Tokyo, Japan).

The faces of the specimens were etched slightly by warming them slowly to –90°C while observing them at 1 kV. Etching was stopped and the specimens re-cooled as soon as cell outlines began to appear. They were then coated with aluminum (100 nm) in the preparation chamber, returned to the sample stage (–170°C) and observed at 7 kV. Micrographs were recorded as video prints or on Kodak TMax 100, 120 roll film. Further details on these procedures are available in Hopkins et al. (1991) and Huang et al. (1994). In the micrographs of each stem segment, the total numbers of gas-filled (cavitated or embolized) and water-filled (ice-filled) vessels were counted.

Results

Typical daily courses of VPD, PPFD, and Ψ_L for each site are shown in Fig. 1. Differences in Ψ_L of covered and transpiring leaves were larger at midday than in the early morning and late afternoon. Values of Ψ_L for both covered and transpiring leaves were substantially lower (more negative) at the coastal site than at the estuarine site. The Ψ_L difference between covered and transpiring

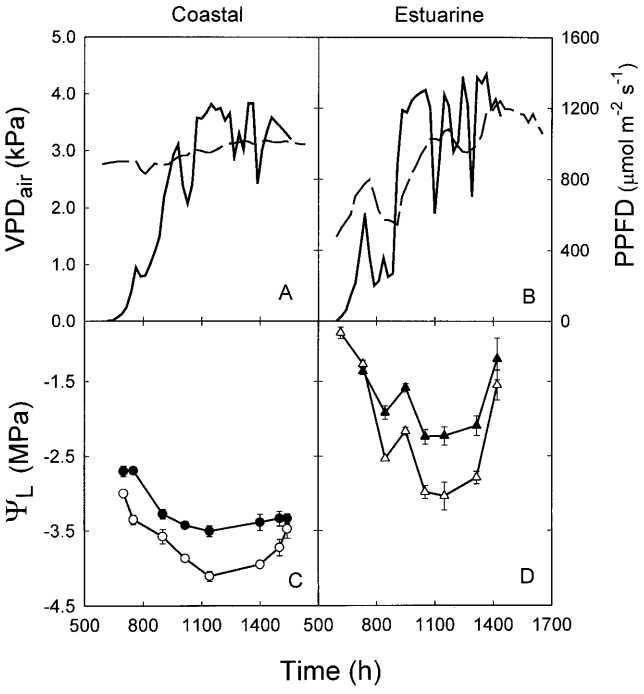

Fig. 1 A,B Representative daily courses of vapor pressure deficit (*VPD, dashed line*), photosynthetic photon flux density (*PPFD, solid line*) and **C,D** water potential (Ψ_L) of covered (*solid symbols*) and freely transpiring (*open symbols*) leaves of *Rhizophora mangle* plants growing in coastal (*circles*) and estuarine sites (*triangles*)

leaves was linearly dependent on the transpiration rate, with transpiration explaining 80 and 90% of the variation in $\Delta\Psi_L$ for the coastal and estuarine site, respectively (Fig. 2). The range of $\Delta\Psi_L$ values was larger for the estuarine site, but the slope of the relationship was steeper for plants growing at the coastal site suggesting, according to the Ohm's law analogy, a higher resistance to water flow in coastal *R. mangle* leaves. Consistent with these site-specific differences, the coastal plants had significantly lower k_L and k_S measured with excised branches, than estuarine plants (Table 1). Although apparent leaf hydraulic resistance determined with the HPFM tended to increase as the tissue became increasingly infiltrated (Fig. 3), leaf hydraulic resistance of coastal plants was always greater than that of estuarine plants. The steady increase in resistance with time made it difficult to determine average leaf resistance in fully infiltrated leaves because the resistance did not approach an asymptotic value, even after 300 min (data not shown) of continuous measurements.

The osmotic potential of the interstitial sea water (π_{sw}) and the osmotic potential of the leaf tissue (π_L) was more negative at the coastal site than at the estuarine site, and the water potential (Ψ) of covered and freely-transpiring leaves were more negative at the coastal site than at the estuarine site at midday (Table 2). Calculated P_x were more negative than π_{sw} at both sites. P_x was calculated by subtracting the osmotic potential of the xylem

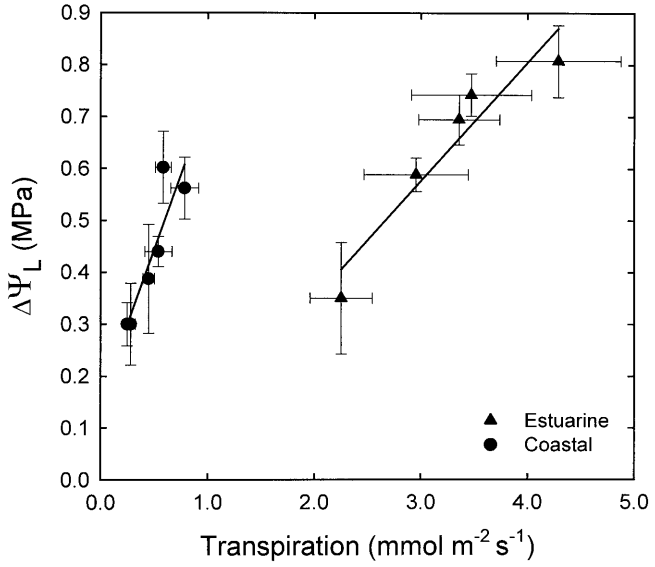

Fig. 2 The water potential difference between covered and uncovered leaves ($\Delta\Psi_L$) as a function of transpiration rate in coastal and estuarine *R. mangle* plants. Transpiration was calculated by multiplying stomatal conductance and leaf-to-air vapor pressure difference. *Values* are means±1 SE (n=5). *Lines* are linear regressions fitted to the data: coastal site y=0.578x-0.1539, r^2=0.81; estuarine site y=0.229x-0.110, r^2=0.90

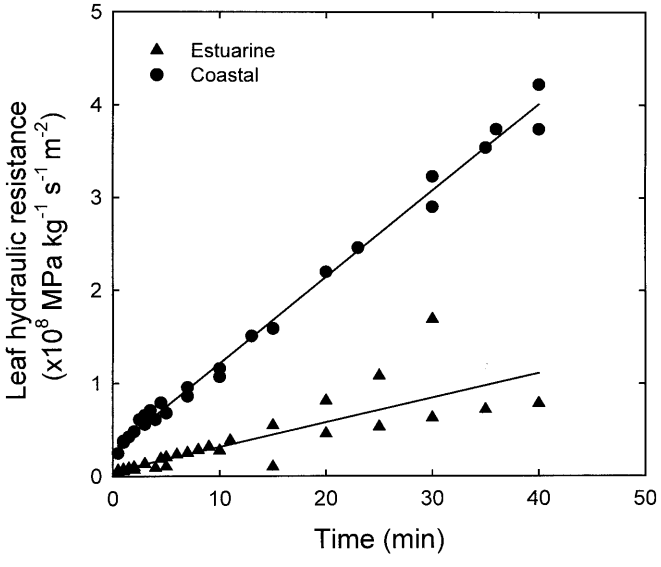

Fig. 3 Leaf hydraulic resistance on an area basis during infiltration of leaves attached to stem segments connected to a high-pressure flowmeter. *Lines* are linear regressions fitted to the data: coastal site y=9.32×10^6x+2.8×10^7, r^2=0.99; estuarine site y=3.04×10^6x+7.06×10^6, r^2=0.64

Table 1 Hydraulic parameters measured on excised stems of *Rhizophora mangle* plants growing at the coastal and estuarine sites. Values are means±1 SE (n=5). *Values* followed by *different letters* within a column are significantly different at P<0.001

	Hydraulic conductivity k_h	Leaf-specific conductivity k_L	Specific conductivity K_S
Site	(×10^{-6} kg m s^{-1} MPa^{-1})	(×10^{-4} kg m^{-1} s^{-1} MPa^{-1})	(kg m^{-1} s^{-1} MPa^{-1})
Coastal	7.67±2.4	1.89±0.33a	0.39±0.11c
Estuarine	23.3±6.9	5.38±0.70b	1.44±0.22d

Table 2 Field water relation parameters for *R. mangle* plants growing at coastal and estuarine sites: interstitial soil water osmotic potential (π_{sw}), bulk leaf osmotic potential (π_L, "corrected" for apoplastic dilution, see Materials and methods), covered leaf water potential (Ψ_L), xylem sap osmotic potential (π_x), and xylem pressure potential (P_x). Values are means±1 SE (n=5). P_x was calculated by subtracting π_x from Ψ_L. Xylem pressure potential is equivalent in magnitude, but usually with opposite sign, to other terms used currently in the literature (e.g., tension and xylem pressure). *Dashes* indicate data not available

Site	Time	π_{sw}	π_L	Ψ_L	π_x	P_x
Coastal	Dawn	–2.57±0.01	–3.22±0.10	–2.70±0.04	–0.18±0.02	–2.52±0.04
	Midday	–	–	–3.51±0.07	–0.16±0.04	–3.35±0.07
Estuarine	Dawn	–0.44±0.01	–1.88±0.04	–0.86±0.08	–0.14±0.09	–0.72±0.08
	Midday	–	–	–2.23±0.12	–0.12±0.09	–2.11±0.09

sap (π_x) from Ψ_L values of covered leaves (Table 2). When solutions with known osmotic potential were forced through recently excised stem segments in the field, the osmotic potential of the first few drops of extruded exudate was assumed to reflect that of the xylem sap before excision. Similar initial values of π_x were obtained when two solutions, one with relatively high (–0.25 MPa) and the other with more negative (–2.4 MPa) osmotic potentials were forced through the stem segments. The values changed with each successive drop of exudate, gradually approaching the osmotic potential of the solution being forced through the stem (results not shown). Average π_x ranged from –0.12±0.09 to –0.18±0.02 MPa (Table 2), and no significant site-specific differences were observed. Pressure volume analysis indicated that the π_L at full turgor were –3.8 and –2.8 MPa, the π_L at the turgor loss points were –4.5 and –3.3 MPa, and the symplastic water fractions were 0.81 and 0.91 for leafy twigs collected from *R. mangle* plants growing in coastal and estuarine sites respectively.

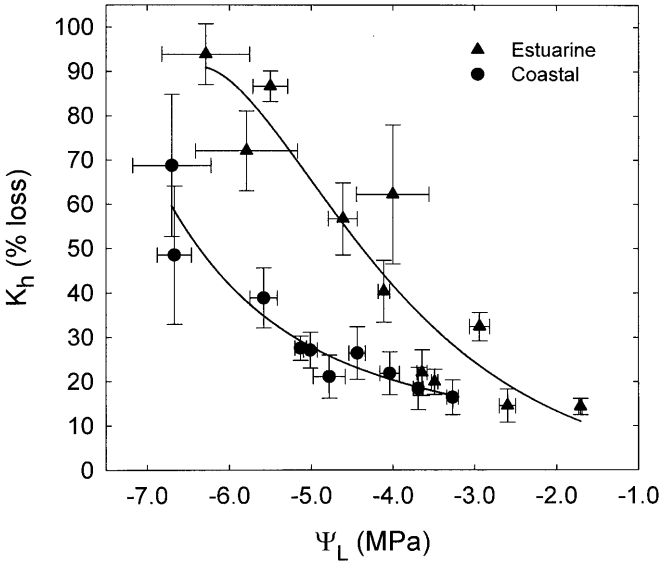

Fig. 4 Loss of stem hydraulic conductivity (k_h) in relation to the water potential of leaves (Ψ_L) in excised air-dried branches of coastal and estuarine *R. mangle* plants. *Values* are means±1 SE (*n*=5)

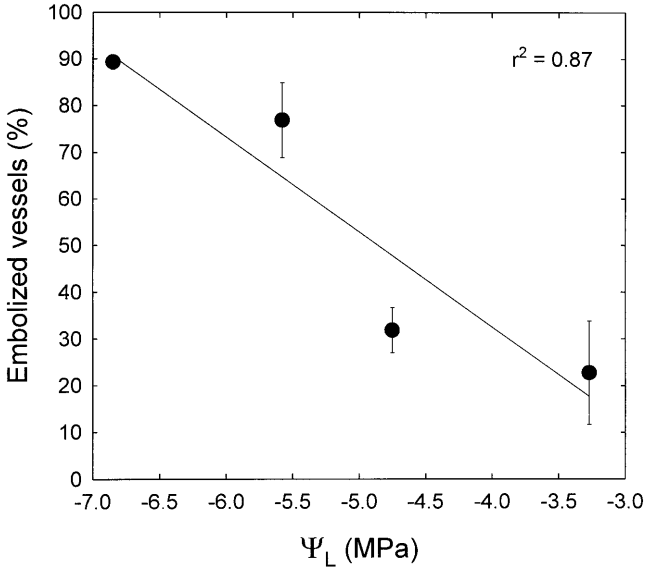

Fig. 5 The percentage of embolized vessels in cryo-scanning electron microscope (cryo-SEM) images of excised, air-dried branches of coastal *R. mangle* plants in relation to water potential of leaves (Ψ_L). *Values* are means±1 SE (*n*=5)

Vulnerability curves generated from coastal and estuarine *R. mangle* plants exhibited different percent loss of k_h for a given Ψ_L value (Fig. 4). A 50% reduction in k_h was observed near –6.5 MPa (near the working capacity of our pressure chamber which can withstand pressures up to 7.0 MPa) in branches collected at the coastal site and –4.5 MPa in branches collected from the estuarine site. Branches from both sites exhibited a baseline loss of k_h of *c*. 15% at the highest values of Ψ_L attained in the

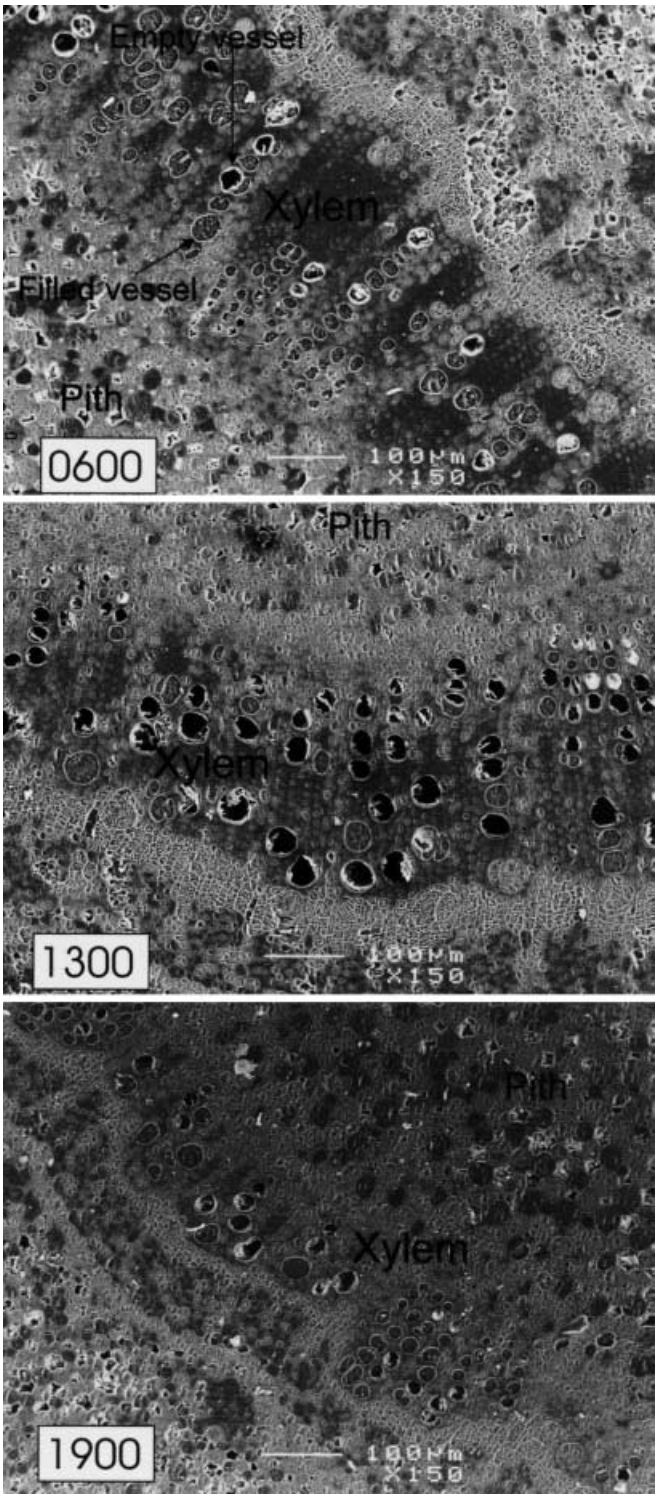

Fig. 6 Representative cryo-SEM images of *R. mangle* stems collected from the coastal population at different times during the same day: 0600, 1300, and 1900 hours. The ice-filled xylem vessels are uniform in appearance and generally lighter in color than the embolized vessels

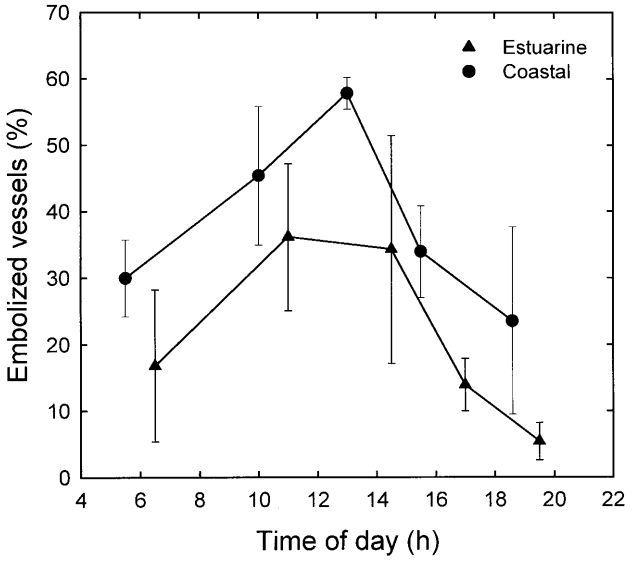

Fig. 7 Diurnal variation in the percentage of embolized vessels in coastal and estuarine *R. mangle* plants. The percent embolism was determined by counting gas- and ice-filled (liquid-filled) vessels in cryo-scanning electron micrograph images taken of stem segments that were frozen in liquid nitrogen (LN_2) in the field while they were still attached to the plant. *Values* are means±1 SE (n=3)

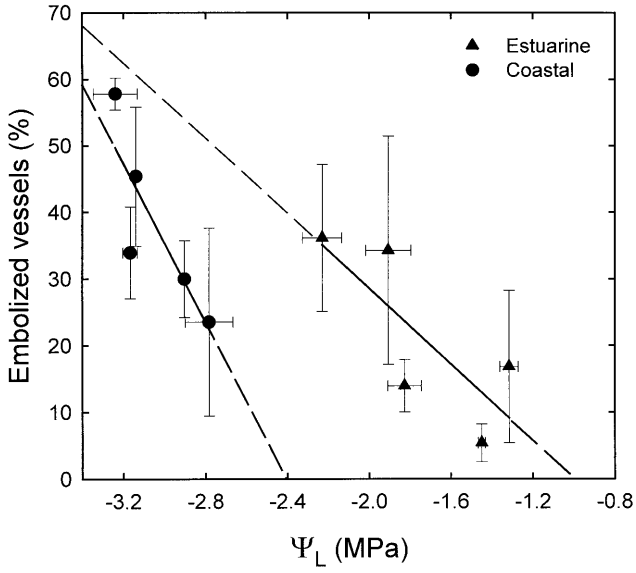

Fig. 8 The percentage of embolized vessels in cryo-SEM images of stem segments in relation to covered (non-transpiring) leaf water potential (Ψ_L) for intact *R. mangle* plants growing in the field. Stem segments for cryo-SEM analysis were frozen in LN_2 in the field while they were still attached to the plant. *Values* are means±1 SE (n=3). *Lines* are linear regressions fitted to the data: coastal site y=-143.9-59.8x, r^2=0.73; estuarine site y=-28.4-28.5x, r^2=0.61

field. The percentage of embolized vessels determined through inspection of cryo-SEM images increased in a nearly linear fashion as Ψ_L became more negative in excised coastal *R. mangle* branches dehydrated in the laboratory (Fig. 5). About 90% of the vessels were embolized at –7.0 MPa, a value of Ψ_L never observed in the field during the study period. At –4.0 MPa, a value of Ψ_L frequently observed in coastal plants at midday, about 30% of the vessels were embolized. At equivalent values of Ψ_L, the percentage of embolized vessels determined with the cryo-SEM technique and the percent loss of k_h were positively correlated (y=1.63x-0.73, r^2=0.82) in excised stems of coastal *R. mangle* plants dehydrated in the laboratory.

Representative cryo-SEM images of *R. mangle* stems collected at different times of the day are shown in Fig. 6. These transverse sections reveal the sizes and positions of the vascular strands as well as the arrangement of the vessels in rows separated by parenchyma tissue. The lumens of gas-filled, cavitated, and embolized vessels appear markedly darker than those filled with liquid. An increase in the relative number of gas-filled vessels was observed from predawn to midday, followed by a decrease in the evening. Diurnal courses of increasing embolism followed by vessel refilling were evident in cryo-SEM data obtained from both the coastal and estuarine populations (Fig. 7). The proportions of embolized vessels were 5 and 30% at dawn for estuarine and coastal populations, respectively, increasing to 35 and 60% at midday. By dusk, the percentage of embolized vessels had fallen below its initial predawn value at each site. The proportion of embolized vessels was significantly higher (P<0.05) at midday than in the evening in both populations.

The percentage of embolized vessels appeared to increase linearly with decreasing Ψ_L of covered leaves at both sites (Fig. 8). Although there was no overlap in values of covered Ψ_L between the two sites, the fraction of embolized vessels appeared to be greater in the estuarine plants at a given value of Ψ_L. When the linear relationships in Fig. 8 were extrapolated to their x-intercepts (0% embolized vessels), the resulting values of Ψ_L were very close to the osmotic potential of the interstitial soil water at each site. Extrapolation of the relationships to 100% embolized vessels yielded Ψ_L values of –4.1 and –4.5 MPa for the coastal and estuarine sites, respectively. The predicted values of Ψ_L corresponding to 100% embolized vessels based on balance pressure and cryo-SEM data taken from intact plants in the field were substantially less negative than values of Ψ_L corresponding to 100% loss of k_h during determinations of vulnerability curves on excised branches in the laboratory (cf. Figs. 4, 8).

Discussion

A one-to-one relationship between P_x, measured with the xylem pressure probe, and Ψ_L, measured with the pressure chamber on covered maize (*Zea mays*) and sugarcane (*Saccharum* spp.) leaves, was observed in the 0 to –0.4 MPa range (Melcher et al. 1998a). A similar relationship was also obtained with *Zea mays* plants growing under lower light conditions (Wei et al. 1999). If cov-

ered-leaf balancing pressures also provide reliable estimates of P_x for *R. mangle*, then pressure-chamber measurements on previously transpiring leaves of *R. mangle* may have underestimated P_x by at least 0.8 MPa during periods of rapid transpiration. When the values of P_x using non-transpiring leaves, in this study, are further corrected by the osmotic potential of the xylem sap, then the estimated values of P_x could be about 1.0 MPa more positive than estimates based on Ψ_L measurements with transpiring leaves. The estimates of maximum (most positive) P_x reported here (–0.72 MPa, Table 2) are still substantially lower than the most negative value of *c.* –0.2 MPa recorded with the xylem pressure probe in *R. mangle* in a previous study (Zimmermann et al. 1994). The basis for the disparity between pressure-chamber and xylem-pressure-probe estimates of P_x in *R. mangle*, despite the good agreement reported for maize and sugarcane (Melcher et al. 1998a; Wei et al. 1999) remains to be elucidated. The discrepancies could be the result of the limited measurement range of the xylem pressure probe, as well as factors related to the point of insertion of the xylem pressure probe in the plant. The glass microcapillary of the probe is not capable of penetrating woody tissues and therefore measurements are mostly confined to leaf veins and petioles. The vasculature in leaves, where the xylem is in intimate contact with the phloem, and the short distance to the sites of carbohydrate production in the photosynthetic tissue, may provide an opportunity for osmotic regulation of P_x that does not exist in woody stems. However, if the discrepancies in P_x estimates with both techniques are real, they could lead to errors in characterizing the magnitude and nature of the driving forces involved in long-distance water transport. Apparently, covered leaf Ψ measurements obtained with the pressure chamber in *R. mangle* provide adequate estimates of P_x, at least in the upper range, under the assumption of overnight equilibration of plant and soil Ψ, because predawn estimates of P_x were similar to the osmotic potential of the interstitial soil water (π_{SW}) measured concurrently with a water vapor pressure osmometer in both study sites.

The difference in Ψ between covered and transpiring leaves increased linearly with increasing transpiration in both study sites (Fig. 2). The slope of the relationship between $\Delta\Psi_L$ and transpiration is, according to the Ohm's law analogy for water transport in plants, a measure of the resistance to water movement between the stem xylem and the evaporating surfaces in the leaves. The linearity of the relationship between $\Delta\Psi_L$ and transpiration suggests that leaf hydraulic resistance remained relatively constant and independent of flow rate throughout the day. The steeper slope of the relationship between $\Delta\Psi_L$ and transpiration in coastal *R. mangle* plants indicates that their leaf hydraulic resistance was greater. Leaf hydraulic resistance was also estimated in this study with a high-pressure flow meter by forcing water through leaves attached to excised stems. The general pattern observed was in agreement with that observed for intact plants in the field in that hydraulic resistance was consistently greater in leaves of coastal *R. mangle* plants. However, leaf hydraulic resistance increased continuously with the duration of water flow into leaves so that steady-state values were not obtained even after 40 min (Fig. 3). This may reflect infiltration of water into leaf compartments with progressively higher resistance or an elastic response of the leaf to a continued increase in volume, and it raises questions concerning interpretation of absolute leaf hydraulic resistance values obtained with the HPFM.

High-molecular-weight polysaccharides (mucilage or pectin-like substances) observed within the vessel lumens of *R. mangle* at coastal sites (Zimmermann et al. 1994) could partially explain why coastal plants had lower k_h and higher leaf resistance than estuarine plants. It is possible that these polysaccharide complexes could retard pressure-driven water flow, and the abundance of these substances could be elevated in plants subjected to larger levels of substrate salinity. However, quantitative data on the content of mucopolysaccharides in vessels of coastal versus estuarine *R. mangle* plants are lacking. Components of leaf water potential (e.g., Ψ_L at full turgor and at the turgor loss point) differences determined by pressure-volume relationships, were consistent with differences in hydraulic resistance in coastal and estuarine populations obtained "in vivo" (with intact plants) and "in vitro" (with detached stem segments) studies. Leaf water potentials at full turgor and at the turgor loss point were about 1.1 MPa more negative in coastal plants, compared to estuarine plants. Leaves of *R. mangle* trees, therefore, were able to exercise partial osmotic adjustments according to the salinity level of the interstitial soil water. It has been previously suggested that leaf osmoregulation in mangroves is most likely due to adjustment of levels of low-molecular-weight-carbohydrates found in high concentrations within leaf tissues of many mangrove species including several species within Rhizophoraceae (Popp 1983b).

The diurnal change in the frequency of embolized vessels observed in this study is contrary to previous claims that embolism in *R. mangle* is largely irreversible (Sperry et al. 1988b). However, the observed patterns are consistent with several recent reports of embolism repair *in situ* (Canny 1997; Zwieniecki and Holbrook 1998; Tyree et al. 1999). At both sites in this study, about 35% of the vessels became embolized and were apparently refilled diurnally, which could help to restore axial water flow to levels similar to those before the onset of cavitation. The daily minimum and maximum proportions of embolized vessels were both higher at the coastal site, consistent with the more stressful growing conditions. Over short periods of time, during rapid transpiration, water released by cavitated vessels may help prevent desiccation of the neighboring cells (Lo Gullo and Salleo 1993). The tension transmitted from the xylem water columns to the living cells is released when the continuity of the column is broken, resulting in tensions near that of water vapor (approximately 0 MPa). When the negative pre-existing P_x is released, water can migrate

from cell to cell, i.e., from cells located further away from xylem vessels, permitting transient partial rehydration to occur. Under conditions of high evaporative demand, stomatal regulation could intervene to prevent excessive dehydration of the leaves before the continuity of the water-conducting pathway is restored. Even though the daily maximum percentage of embolism ranged from about 35% at the estuarine site to 60% at the coastal site, its impact on total resistance of the soil/leaf pathway, and therefore on Ψ_L, may have been negligible. The relative magnitudes of the serial resistances in the root/leaf pathway are typically such that stem resistance is a small fraction of the root and leaf resistance. In fact, transient release of water from internal storage via cavitation or other processes may serve to dampen diurnal fluctuations in leaf water status (Lo Gullo and Salleo 1993) and temporarily increase apparent hydraulic conductance (Andrade et al. 1998).

The mechanisms responsible for vessel refilling when P_x is negative in surrounding vessels are unknown. Although previous work has shown that living cells are not likely to be involved in embolism refilling in conifers (Borghetti et al. 1991; Edwards et al. 1994), more recent studies suggest that living xylem parenchyma surrounding cavitated vessels might play a role in vessel refilling (Zwieniecki and Holbrook 1998; Holbrook and Zwieniecki 1999). The ability of *R. mangle* to refill embolized vessels could also result from the high molecular weight mucopolysaccharides that have been previously observed within intact xylem vessel lumens (Zimmermann et al. 1994). These substances may remain within each vessel conduit, either by entrapment of the smaller diameter end-walls, or through attachment to the vessel walls themselves. Rapid replacement of liquid water with water vapor upon cavitation may lead to a rapid decrease in xylem osmotic potential associated with an increased concentration of mucopolysaccharides in the small amount of water remaining in the vessel. This could create a driving force large enough to refill the vessel. Further studies are needed to understand how the process of embolism repair occurs in *R. mangle*. Regardless of the mechanism(s) responsible for vessel refilling, the ability of *R. mangle* in particular, and of other species in general, to repair cavitated xylem conduits on a daily basis has important implications for understanding regulation of water use in plants.

Cryo-SEM determinations of percentages of embolized vessels in relation to covered leaf Ψ for intact plants in the field (Fig. 8) yielded information analogous to that obtained from xylem vulnerability curves determined on air-dried, excised branches in the laboratory (Fig. 4). Both methods gave similar results in that xylem of estuarine *R. mangle* was found to be considerably more vulnerable to cavitation than that of coastal *R. mangle*. Nevertheless, a comparison of cryo-SEM data obtained from coastal *R. mangle* plants in the field and laboratory suggested that cavitation occurred more readily in intact plants than in excised branches. About 22% of the vessels were embolized in excised, air-dried branches with corresponding values of Ψ_L near –3.2 MPa (Fig. 5) compared with 48% embolism in intact branches of plants growing in the field (Fig. 8). A similar discrepancy between xylem vulnerability of branches dehydrated *in situ* and air-dried after excision was observed in the woody species *Hibiscus rosa* (Melcher 1999). Based on measurements of k_h, branches excised from field-grown plants experienced a 50% loss of k_h at Ψ_L (covered leaf) of –1 MPa, whereas the loss of k_h in excised, air-dried branches was only 25% at the same Ψ_L. The basis for this disparity is uncertain, but factors such as the rate of dehydration and differences in gradients of tension and pathways of water movement in intact and excised branches may be involved.

In conclusion, a major finding of this study is that estimated values of P_x determined from covered leaf Ψ_L and further corrected by π_x in *R. mangle* was found to be about 1.0 MPa more positive compared to previous reports that rely on balancing pressures obtained with freely transpiring leaves. Large diurnal variations in the proportion of embolized vessels, using cryo-SEM techniques, were observed in plants growing at both study sites. In particular, it was found that the number of gas-filled vessels increased during the morning and then decreased sharply during the afternoon, suggesting that refilling of embolized vessels occurred when considerable xylem tension was present in neighboring vessels. Consequently, cavitation was prevented from being cumulative, and therefore catastrophic, by repair processes that apparently operated continuously during the day, particularly under high evaporative demand conditions. *Rhizophora mangle* plants growing at the coastal site were found to be less vulnerable to cavitation and exhibited more negative leaf water potentials at full turgor and at the turgor loss point, than the estuarine plants. This species, therefore, adjusts hydraulic properties of the water-transport system, as well as the cellular osmotic content in leaves, in concert with the environmental growing conditions.

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