Vulnerability of xylem to embolism in a mangrove vs an inland species of Rhizophoraceae

John S. Sperry, Melvin T. Tyree and John R. Donnelly

Sperry, J. S., Tyree, M. T. and Donnelly, J. R. 1988. Vulnerability of xylem to embolism in a mangrove vs an inland species of Rhizophoraceae. – Physiol. Plant. 74: 276–283.

Vulnerability of xylem conduits to cavitation and embolism was compared in two species of Rhizophoraceae, the mangrove *Rhizophora mangle* L. and the tropical moist-forest *Cassipourea elliptica* (Sw.) Poir. Cavitation (water column breakage preceeding embolism) was monitored by ultrasonic detection; embolism was quantified by its reduction of xylem hydraulic conductivity. Acoustic data were not predictive of loss in hydraulic conductivity, probably because signals from cavitating vessels were swamped by more numerous ones from cavitating fibers. *Rhizophora mangle* was the less vulnerable to embolism of the two species, losing 80% of its hydraulic conductivity between −6.0 and −7.0 MPa. *Cassipourea elliptica* lost conductivity in linear proportion to decreasing xylem pressure from −0.5 to −7.0 MPa. Species' vulnerability correlated closely with physiological demands of habitat; the mangrove *Rhizophora mangle* had field xylem pressures between −2.5 and −4.0 MPa, whereas the minimum for *Cassipourea elliptica* was −1.6 MPa. Differences in vulnerability between species could be accounted for by differences in the measured air permeability of intervessel pit membranes. According to this explanation, embolism occurs when air enters a water-filled vessel from a neighboring air-filled one via pores in shared pit membranes.

Key words – *Cassipourea elliptica,* cavitation, ecological wood anatomy, embolism, hydraulic conductance, mangroves, pit membrane, *Rhizophora mangle.*

J. S. Sperry (corresponding author) and M. T. Tyree, Dept of Botany, Univ. of Vermont, Burlington, VT 05405, USA; J. R. Donnelly, School of Natural Resources, Univ. of Vermont, Burlington, VT 05405, USA.

Introduction

The Rhizophoraceae is a family of subtropical and tropical trees including both mangrove and inland species; many of the latter occur in tropical moist- and wet-forest (Holdridge Life Zone system; Holdridge et al. 1971). The mangrove and forest habitats place very different demands on the xylem: whereas a moist-forest tree may seldom have xylem pressures (Ψ_{px}) more negative than −2.0 MPa, a mangrove tree growing in pure seawater never has Ψ_{px} less negative than −2.5 MPa. This is because sea water has an osmotic potential near −2.5 MPa, and roots of most mangrove species exclude salt (Scholander et al. 1962). Mid-day Ψ_{px} in species of the mangrove genus *Rhizophora* drop as low as −5.5 MPa (Scholander et al. 1965).

Ecologically-minded wood anatomists have compared mangrove and inland genera of Rhizophoraceae in order to determine what might constitute an adaptation to the mangrove habitat. The most recent study by van Vliet (1976) is in agreement with previous ones (Panshin 1932, Jansonnius 1950) in concluding that mangrove genera have a greater density of vessels per transverse sectional area, and smaller diameter vessels than do inland genera. In addition, van Vliet (1976) reports that mangrove genera tend to have thicker vessel walls than do inland genera, and can be further distinguished by qualitative features including the type of vascular pitting, perforation plate, fibers, rays and parenchyma. The functional significance of these features, however, is ambiguous. In general, these studies

do not make it clear why the xylem in these habitats should be different.

The lower Ψ_{px} in mangrove species means that they are potentially more susceptible than inland species to drought-induced embolism. Embolism is the presence of air-filled (embolized) tracheids and vessels, and it can cause a substantial impairment of xylem transport. Low Ψ_{px} leads to embolism via the process of 'cavitation', the breaking of water columns under negative pressure. The immediate result of a cavitation is a lumen filled with water vapor and some air. Eventually, the lumen becomes fully embolized when more air comes out of solution to fill the void left by the cavitation. Cavitation occurs at pressures much less negative than predicted from the tensile strength of water (Pickard 1981) and, therefore, is probably caused by some property of the xylem itself. One reasonable explanation is that it occurs when air is aspirated into xylem conduits via pores in the conduit wall (Oertli 1971). This has recently been dubbed the 'air seeding' hypothesis (Zimmermann 1983).

In many plants, positive Ψ_{px} has been shown to refill embolized vessels (Tyree et al. 1986, Sperry et al. 1987, 1988b). In a mangrove tree, however, embolized vessels cannot be refilled in this way, because Ψ_{px} must always be negative in order to balance the osmotic potential of sea water (ca -2.5 MPa). As previously mentioned, this negative Ψ_{px} is necessary because the *Rhizophora* roots exclude salt, and the osmotic potential of the xylem sap is always near that of pure water (Scholander et al. 1962).

The xylem of every species studied thus far has exhibited a characteristic vulnerability to embolism (e.g., see Tyree and Dixon 1986). Typically this is expressed as a 'vulnerability curve' showing how embolism or cavitation increases as Ψ_{px} decreases. Embolism can be quantified by how much it reduces the xylem hydraulic conductivity (e.g., Sperry et al. 1988a). Cavitation can be monitored acoustically, because it is accompanied by shock waves (e.g., Tyree et al. 1984). For arborescent species, vulnerability curves seem to reflect the range of xylem pressures the species experiences in nature; over the normal range embolism accounts for a 10–20% loss in hydraulic conductivity, but as soon as Ψ_{px} becomes more negative, embolism increases dramatically (Sperry 1986, Tyree and Dixon 1986).

The mangrove and moist-forest representatives of the Rhizophoraceae are the ideal natural experiment for evaluating how well vulnerability of the xylem to embolism in closely related species correlates with habitat. In the present paper, we compare the vulnerability to embolism of two Rhizophoraceae species, *Cassipourea elliptica*, which occurs in tropical moist-forest, and *Rhizophora mangle* which grows as a mangrove. Both acoustic and hydraulic methods were used to generate vulnerability curves. The comparison is discussed in the context of a traditional anatomical analysis of the xylem of the two species. If the xylem is highly adaptive to

habitat, we should see considerable differences in the vulnerabilities of the two species, and the structural comparison could give us a clue as to why one species is less vulnerable than the other.

Another set of experiments was designed to tell how well the air seeding hypothesis explained the measured vulnerabilities. The intervessel pit membrane is the site most vulnerable to air seeding. Once any vessel in a tree becomes air-filled, e.g., by physical damage, the spread of air into neighboring water-filled vessels is limited by capillary forces at the air-water interface at pores in the cellulosic mesh of these pit membranes. The pressure difference required to displace an air-water meniscus from a pore can be estimated using the capillary equation. This equation relates the rise of liquid in a capillary tube to tube diameter; the hydrostatic pressure of the liquid column is equal to the air pressure required to displace the liquid from the tube. Assuming the liquid is water, and that pit membrane pores are ideal capillaries, the pressure difference (ΔP, in MPa) required to force air through the pore is given by:

$$\Delta P = 4 \, (T/D) \qquad \text{(Eq. 1)}$$

where T is the surface tension of water (in N m^{-1}) and D is the diameter of the pore (in μm). Lewis (1988) has provided evidence that this equation accurately predicts the pressure at which air enters the pores in *Sphagnum* water storage cells. Our 'air permeability' experiments measured this pressure difference for pores in intervessel pit membranes by determining the conductivity of air through hydrated stems as a function of applied air pressure. These 'permeability curves' were compared with embolism vulnerability curves to see if air penetration of pit membranes could be a cause of embolism.

Abbreviations AE(s), acoustic emission(s); Ψ, water potential; Ψ_{px}, xylem pressure potential.

Materials and methods

Plant material

We studied *Cassipourea elliptica* (Sw.) Poir. at the Barro Colorado Island reserve of the Smithsonian Tropical Research Inst. in Panama. During February 1987 (the middle of the dry season), branches 3–4 m in length were cut from trees growing along the shore of the freshwater Gatun lake. Although this species grew in the forest, our main concern was to make repeated collections from the same general site, and the lakeside site was the most convenient. *Rhizophora mangle* L. was studied at the Fairchild Tropical Garden in Miami, FL. Branches 2–3 m in length were collected from mangrove swamp along Biscayne Bay during April and May 1987.

Embolism vulnerability-hydraulic method

Harvested branches were used to determine the relationship between Ψ_{px} and the percentage loss in hydraulic conductivity due to embolism. A branch was brought back to the laboratory, where it was partially dehydrated. After dehydration, we bagged the branch in a large plastic bag (or bags), inserted this in a damp cotton bag, and wrapped the whole with more plastic bags; the branch was left overnight to let Ψ_{px} equilibrate. Dehydration times varied from zero for branches bagged in the field, to approximately 5 days. *Rhizophora* dried out much more slowly than *Cassipourea*. After overnight bagging, mean Ψ_{px} was measured with the pressure bomb on 5–10 shoots clipped from the branch. If the branch was so dry that foliage was drying, we measured water potential (Ψ) with 1 or 2 temperature-corrected stem pyschrometers (Dixon Instruments, Guelp Canada; see Dixon and Tyree 1984) attached near the branch base. For branches with living foliage, pressure bomb measurements of leafy shoots averaged 0.17 MPa more negative than psychrometer measurements at the branch base. Reasons for this include transpiration within the bag or during transfer of shoots to the pressure bomb, and erroneous pressure bomb measurements caused by refilling of embolised vessels (West and Gaff 1976).

The branch, minus shoots clipped for water potential measurements, was placed in a large sink or tub filled with water. Ten segments 10–20 cm in length were cut from it, taking care that all cuts were made underwater to prevent air from entering severed vessels. Segments ranged from 0.31–0.56 cm in diameter for *Cassipourea*, and 0.52–1.22 cm in diameter (all diameter measurements including bark) for *Rhizophora*. Segments were fitted with gaskets cut from rubber tubing, and each end was trimmed with a sharp razor blade. All 10 were attached in parallel to a tubing manifold designed for the measurement of hydraulic conductivity and percentage loss in conductivity.

Embolism in branch segments was quantified as the percentage by which the initial hydraulic conductivity of the segments was below its maximum measured after removal of emboli (Sperry et al. 1988a). The tubing manifold linking the 10 segments in parallel was attached to a graduated pipette at one end and a reservoir of pre-filtered (0.1 or 0.22 µm) solution at the other. We used 10–20 mM oxalic acid in tap water as our solution, because its low pH (<2) prevented microbial growth associated with long-term declines in conductivity (Sperry et al. 1988a). Stopcocks allowed us to direct the flow of solution through one segment at a time. Hydraulic conductivity (L, kg m s^{-1} MPa^{-1}) was calculated from the mass flow rate ($\dot{m}$, in kg s^{-1}) of solution through a segment and the pressure gradient (dP/dl, in MPa m^{-1}):

$$L = \dot{m} \ (dl/dP) \qquad \text{(Eq. 2)}$$

Volume flow rate in the pipette was measured with a stopwatch and converted to mass flow rate assuming a solution density of 1 kg l^{-1} (we use mass flow rate, because in most of our related work we measure flow rate gravimetrically). The pressure gradient was determined by segment length (l) and the height of the reservoir above the pipette. Once all 10 segments had been measured in succession, pre-filtered solution was forced through all at 175 kPa for 10–120 min by pressurizing a tank of solution with compressed air. Following this 'flush', conductivity was remeasured. Flushing was repeated until conductivity reached a maximum value.

Preliminary experiments were performed with both species to determine if hydraulic conductivity declined with repeated flushes after a maximum value was achieved. Declines of 3 ± 6% (n = 30) per 1 h flush were achieved with *Rhizophora* by using a 0.1 µm prefilter and stripping the bark off the end of the branch inserted in the tubing. Declines of 6 ± 10% (n = 38) per 10 min flush occurred with *Cassipourea* despite stripping the bark. Unfortunately, 0.1 µm filters were not available to us at the time we were measuring this species, or else we might have achieved lower rates of decline.

The large and aerated pith of *Rhizophora* presented a problem, especially when it developed large cracks in drought-stressed twigs. We prevented water flow through the pith in this species by hollowing it out at the influx end and filling it with plasticine. The surrounding xylem was then retrimmed with a razor blade. Safranin (0.1%) dye perfusions confirmed that no water was flowing through the pith when plugged in this manner.

Embolism vulnerability-acoustic method

This method is based on considerable preliminary research (e.g. Tyree et al. 1984) showing that cavitation is associated with shock waves, or acoustic emissions (AEs). These presumably originate from the rapid pressure change in a cavitating lumen as liquid water at negative pressure is rapidly replaced by vapor and air at close to vacuum pressure.

Harvested branches were brought into the laboratory and rehydrated. An ultrasonic transducer (model R15 or I15I, Physical Acoustics Corp., Princeton, NJ) was attached approximately in the center of the branch by first carefully removing the bark from one side of a short length (ca 4 cm) and coating it with vacuum grease to prevent local dehydration and to improve acoustic transfer. The transducer was clamped in place with a constant force of 30 N maintained by a spring-loaded clamp. Acoustic emissions picked up by the transducer were amplified 76–78 dB by a custom built amplifier based on LH0032 operational amplifiers, or by a model 2615 Drought Stress Monitor (Physical Acoustics Corp.). The AEs were counted with a dedicated microcomputer (custom built or as part of the model 2615 monitor); both cumulative number and current rate of

AEs were displayed. For more technical details the reader is referred to Tyree et al. (1984). Within 8 cm of the transducer and on the same branch, a temperature-corrected stem psychrometer was attached for measuring Ψ.

With the psychrometer and transducer attached, the branch was placed with the cut end in water and its foliage enclosed in plastic bags overnight. During this time Ψ was near 0 MPa, and a background AE rate was determined to insure that it was <0.1 emissions per min. The acoustic system was designed to monitor AEs in the 100–300 kHz range, thus screening out all audible laboratory noise. Dehydration was initiated by removing the water and plastic bags. Periodic measurements of Ψ were made, and plotted vs the cumulative number of AEs until Ψ decreased to the value shown to cause complete loss of hydraulic conductivity from the experiments described above.

Anatomical measurements

Vessel diameters, wall thicknesses and densities were compared between the two species using a subsample of segments used in the embolism studies. Transverse sections, approximately 60 μm thick, were cut from the middle of these segments with a sliding microtome. Sections were mounted in glycerin, and anatomical parameters measured using a compound microscope-drawing tube assembly and a bit pad. Vessel diameter was calculated by bit pad software as the diameter of a circle having the same area as the vessel lumina. Both species had slightly elliptical vessel lumina with mean form factors (minor/major axis) of 0.92 ± 0.043 (*Rhizophora*, n = 56) and 0.90 ± 0.122 (*Cassipourea*, n = 64). Diameter statistics were based on measurements from 5–10% of the total number of vessels in cross section. All vessels within sectors (wedges) of xylem reaching from pith to cambium were measured to insure a representative sample. Vessel densities and wall thicknesses were measured in the same sectors. Wall thickness was measured only where two vessels contacted one another. The distance between the two luminae divided by two gave the wall thickness.

Specific hydraulic conductivity for 50 stem segments of each species was calculated as their maximum hydraulic conductivity divided by the transverse-sectional area of their xylem (measured at the middle of the segment). Theoretical hydraulic conductivity (L) was calculated for stem segments of each species from vessel diameter measurements using the Hagen-Poisieulle relationship between capillary radii and conductivity:

$$L = (\pi/8\eta) \Sigma r^4, \qquad \text{(Eq. 3)}$$

where r is the radius of a capillary (vessel), and η is the viscosity of water. In practice, theoretical conductivity was calculated for the vessels constituting the sector sample, and the total conductivity of the cross-section

estimated from the fraction of total area represented by the sector. Vessel lengths in both species were measured using paint infusion and air methods outlines by Zimmermann and Jeje (1981).

Air permeability measurements

In the wood technology literature, 'permeability' is the relationship between volume (or mass) flow rate of a fluid (gas or liquid) and pressure gradient (Siau 1984). For incompressible fluids such as water, permeability is the hydraulic conductivity as defined by Eq. 2. For a compressible fluid such as air, volume is a function of pressure and the equation is modified to the following general form (Siau 1984):

$$L_a = Q [P \, l/(\Delta P) \, \bar{P}]^x, \qquad \text{(Eq. 4)}$$

where L_a is the conductivity of air ($m^{3+x} \, s^{-1} \, MPa^{-x}$), Q is the volume flow rate of air ($m^3 \, s^{-1}$, l is the stem length (m), P is the pressure at which Q was measured, ΔP is the pressure difference across the stem, and $\bar{P}$ is the average pressure (all pressures in MPa) inside the stem (= sum of absolute pressures at the two ends of the stem divided by 2). The value of the exponent, x, is equal to one for laminar flow. If air flow is non-linear or turbulent, then x is 0.50 or 0.57, respectively (see Siau 1984). As air flow-rate increases there is a transition from laminar to non-linear to turbulent flow.

We determined x experimentally by measuring the volume flow rate of air vs the pressure term (the term raised to the x power in Eq. 4) in air-dried stems of the same dimensions used in the experiments described below. The value of x was given by the slope of a log-log plot of volume flow rate of air vs the inverse pressure term. For *Cassipourea*, x was 0.78 ± 0.058 (n=6). Similar values were obtained for sugar maple (*Acer saccharum*), and we used the same number for *Rhizophora*. Air flow in our experiments was apparently transitional between laminar and turbulent.

On hydrated stems we generated 'permeability curves' by measuring the conductivity of air through stem segments as pressure was increased. The stems were longer than most of the vessels, and so an increase in conductivity signalled the displacement of the air-water interface at intervessel pits. In this way we could measure the range of pressures over which the pit membranes became 'unsealed'. These experiments were done at the Univ. of Vermont (Burlington, VT) in September 1987, using material collected at the same sites used for the embolism vulnerability experiments. Material was 2 to 5-day-old when it arrived in Vermont. Stems were re-cut under water to a length of 55–72 cm in *Cassipourea* and 23–40 cm in *Rhizophora*, and allowed to hydrate for several hours. A stem was inserted a few cm into a pressure bomb, and the exserted end attached to an inverted side arm flask filled with water. The side arm was linked to a reservoir of water on an

electronic balance via water-filled tubing. The volume flow rate of air moving through the stem was measured by the volume flow rate of water displaced from the inverted flask into the balance reservoir. High volume flow rates were sometimes measured by diverting air from the stem into a water-filled inverted graduated cylinder and timing its accumulation.

The volume flow rate of air was measured for each 0.34 MPa increase in bomb pressure up to 4.5 MPa. This was as high as we felt it was safe to pressurize the bomb in this application, because some stems tended to split open and blow out of the bomb at higher pressures. Conductivity of air at each pressure was calculated according to Eq. 4. If the stem was longer than the longest vessel, as was the case for most *Rhizophora* stems, no air passed through the segment until the pressure became high enough to displace the menisci at the intervessel pit membranes. When no air penetrated, the pressure was held for 5 min before being raised another 0.34 MPa. In *Cassipourea* stems, <2% of the vessels extended through the segment and therefore air flow was present from the lowest bomb pressure (0.34 MPa). The conductivity of these continuous vessels (as measured at 0.34 MPa) was subtracted from subsequent values measured at higher pressures, because we were only interested in conductivity due to air penetrating intervessel pits. For each stem we expressed conductivities relative to the maximum value obtained for that stem over the pressure range from 0 to 4.5 MPa.

Air flowing through the large and well-aerated pith of *Rhizophora* was diverted from the collection flask by drilling a small hole into the pith through the xylem at one side of the stem near its middle. This interruption of the xylem did not affect the results, because they were expressed relative to maximum conductivity of the drilled segment.

Some stems of both species were pre-perfused at 0.34 MPa with 5–10 ml of 80% (v/v) ethanol immediately prior to being used for a permeability curve. Relative surface tensions of distilled water and ethanol were estimated by measuring the pressure required to force air through 0.22 μm membrane filters soaked in water vs those soaked in 80% ethanol.

Results

Figure 1 shows vulnerability curves for *Cassipourea elliptica* and *Rhizophora mangle* obtained by measuring percentage loss in hydraulic conductivity as a function of Ψ_{px}. The range of field Ψ_{px}, determined from pre-dawn (maximum Ψ_{px}) and mid-day (minimum Ψ_{px}) pressure bomb measurements, is also indicated. Despite considerable variability in the *Rhizophora* data, the vulnerabilities of the two species are very different and are correlated with the demands of their respective habitats. Within the normal range of field Ψ_{px}, each species showed only 10–15% loss in hydraulic conductivity. However, at −4.0 MPa, which is within *Rhizophora*'s
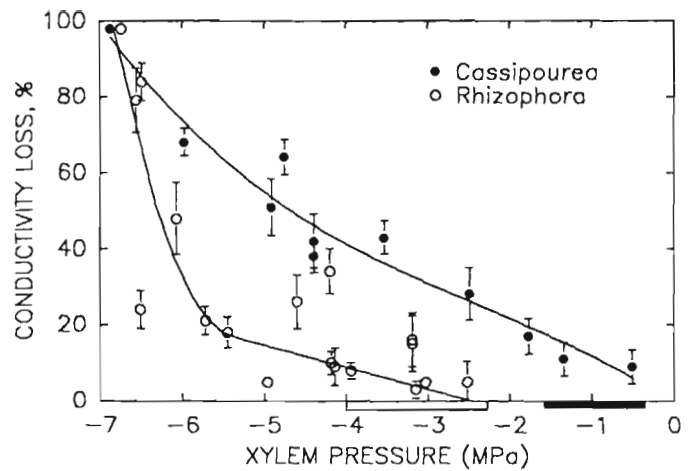

Fig. 1. Embolism vulnerability curves for *Rhizophora mangle* and *Cassipourea elliptica* showing how hydraulic conductivity of the xylem is lost as Ψ_{px} becomes increasingly negative. Each data point represents the mean of 10 segments from a single branch, error bars are 95% confidence limits. Bars on X-axis represent measured range of Ψ_{px} in the field for each species, *Rhizophora* on the left (open bar), *Cassipourea* on the right (solid bar).

normal range, *Cassipourea* xylem is 40% embolized. Although both species lose all hydraulic conductivity at around −6.9 MPa, *Cassipourea* shows an almost linear loss beginning at −0.5 MPa, whereas *Rhizophora* loses most of its conductivity in a narrow Ψ_{px} range between −6.0 and −6.9 MPa.

Figure 2 shows vulnerability curves obtained by the acoustic method. The data are combined from 5 experiments for each species. Cumulative AEs from each replicate were normalized to relative numbers by dividing each data set by the number of AEs at stem Ψs of −6.3 and −4.7 MPa, respectively, for *Rhizophora* and
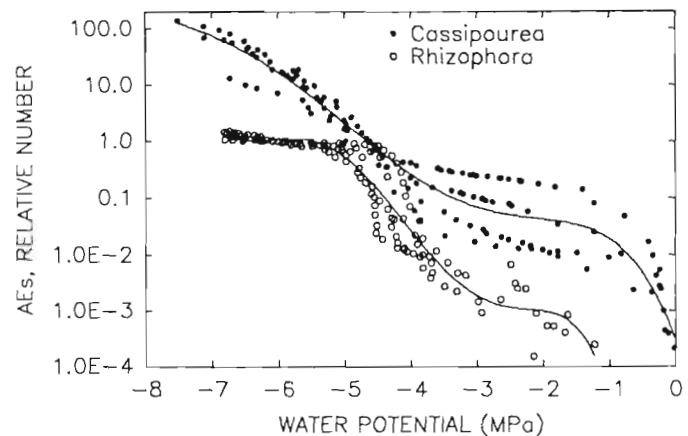

Fig. 2. Embolism vulnerability curves for *Rhizophora mangle* and *Cassipourea elliptica* showing how the relative number of AEs increase as Ψ_{px} becomes increasingly negative. Data are for 5 replicates of each species. A relative value of 1.0 corresponds to the number of AEs observed when Ψ of the stem equalled the value causing 50% loss of hydraulic conductivity (Fig. 1). The trend lines are from fourth order polynomial fits to all data sets for each species. See Results for more details.

280

Tab. 1. Comparative xylem anatomy of *Rhizophora mangle* and *Cassipourea elliptica*. Asterisk means significant species differences ($P <0.01$); n, sample size. For the first three parameters the mean is a grand mean from n stems, and SD is the standard error of the mean. Specific conductivity was calculated as maximum (no embolism) hydraulic conductivity divided by transverse xylem area. Theoretical conductivity was calculated from vessel diameters using Eq. 3; percentage theoretical conductivity is based on measured value of maximum conductivity (no embolism) for the same stem segment.

Parameter	*Rhizophora mangle*			*Cassipourea elliptica*		
	mean	SD	n	mean	SD	n
Vessel density (vessels m^{-2})	1.4×10^{8}*	0.46×10^{8}	10	2.5×10^{8}*	0.64×10^{8}	10
Vessel diameter (μm)	28.9	2.71	10	29.5	2.63	10
Vessel wall thickness (μm)	3.3*	0.48	5	1.70*	0.094	5
Specific conductivity (Kg MPa^{-1} s^{-1} m^{-1})	1.9*	0.92	49	1.3*	0.51	53
Theoretical conductivity (%)	53*	22	9	21*	7	10

Cassipourea; these Ψs caused 50% loss of hydraulic conductance (Fig. 1). There is no simple correlation between the pattern of AEs and the percentage loss in hydraulic conductivity in Fig. 1. This lack of correspondence is probably due to AEs being produced by cavitation in fibers, ray cells, etc. These cavitations would not alter hydraulic conductivity, but could easily obscure any pattern of vessel-related AEs, because the number of fibers per unit wood volume far exceeds the number of vessels.

Table 1 summarizes the anatomical analysis of segments used in the vulnerability experiments. In contrast to previous anatomical surveys (Panshin 1932, Jansonnius 1950, van Vliet 1976), we found no significant difference in vessel diameters, and the inland species *Cassipourea* had significantly higher vessel densities than the mangrove *Rhizophora*. In agreement with van Vliet (1976) we found the mangrove *Rhizophora* to have significantly thicker vessel walls than the inland *Cassipourea*.

Despite greater vessel densities, the specific hydraulic conductivity of *Cassipourea* was slightly lower than for

Tab. 2. Vessel lengths in *Rhizophora mangle* and *Cassipourea elliptica*. Percentages are relative to number of vessels in transverse section. *Rhizophora* data are pooled from 5 stems 0.71 to 1.12 cm in diameter (including bark) using both air and paint methods. *Cassipourea* data are pooled from 3 stems 0.32 to 0.33 cm in diameter (including bark) using the air method (Zimmermann and Jeje 1981). The longest *Rhizophora* vessels were only 35 cm, whereas the longest *Cassipourea* vessels exceeded 220 cm.

Length class (cm)	Vessels in length class (% of total)	
	R. mangle	*C. elliptica*
0–5	82.0	94.4
5–10	12.0	1.5
10–15	3.0	0.7
15–20	1.8	0.0
20–	1.2	3.4

Rhizophora. This is explained by the fact that theoretical conductivities in *Cassipourea* were only 21% of measured values vs 53% for *Rhizophora* (Tab. 1). There is no readily apparent reason for this.

The most striking anatomical difference between the two species is in vessel length. The quantitative similarity apparent in the vessel distributions shown in Tab. 2 obscure a rather dramatic qualitative difference. Although in both species over 80% of the vessels are shorter than 5 cm, a small percentage (<2%) in *Cassipourea* are over 1.2 m, with some longer than 3 m. The longest vessels in *Rhizophora* stems are only 20–30 cm. The air and paint-perfusion methods of Zimmermann and Jeje (1981) gave similar results for *Rhizophora*, but in *Cassipourea* paint-perfusions did not fully penetrate the longer vessels.

Air permeability studies indicated that intervessel pit membranes in *Cassipourea* were much more permeable to air than in *Rhizophora*. Figure 3A shows permeability curves in native branch segments of both species that were hydrated in water but otherwise untreated. Conductivity of air in *Cassipourea* increased abruptly between bomb pressures of 0.34 and 2 MPa, despite the fact that air flow through vessels running straight through the stem was subtracted out. We interpret this to mean that over this range, air is penetrating at least some intervessel pits. There was a plateau in air conductivity between 2 and 3.75 MPa and a slight upward trend at higher pressures indicating that until the highest pressures there was little further penetration of pits. In contrast to *Cassipourea*, *Rhizophora* vessels were impermeable to air over the range of pressures applied.

Figure 3B shows the effect of pre-perfusing the stems with 80% ethanol, which has a surface tension 37% that of water at 25°C. Theoretically, this allows us to estimate what the permeability curve of native stems in Fig. 3A would look like if we could extend the X-axis by a factor of 2.7, i.e. from 4.5 to 12.2 MPa. Ethanol-perfused *Rhizophora* shows an abrupt increase in air conductivity at pressures above 2.5 MPa signalling the penetration of intervessel pits; if we extrapolate to native stems, this would correspond to air penetration at pres-
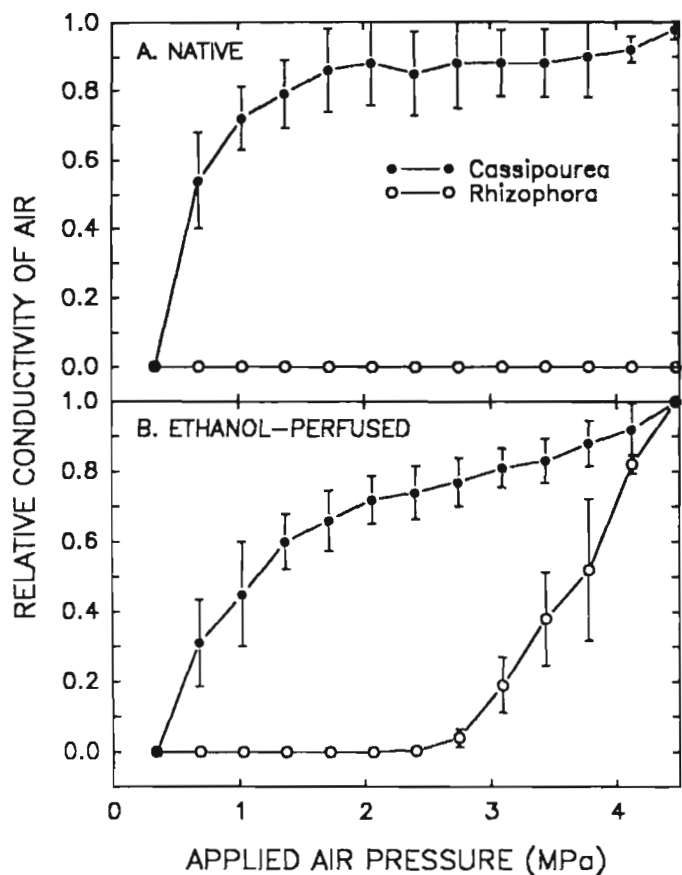
Fig. 3. Permeability curves for *Rhizophora mangle* and *Cassipourea elliptica* showing range of applied air pressure required to cause increase in conductivity of air through hydrated stem segments. Increasing conductivity indicates air penetration of intervessel pit membranes. For each stem, conductivity of air was calculated relative to the maximum value obtained for the stem. Each curve represents mean relative conductivities of 5 stem segments; error bars are 95% confidence limits. A, Native stems hydrated in water but otherwise untreated. B, Segments perfused immediately before the experiment with 80% ethanol, which has a surface tension 37% that of water at 25°C.

sures above 6.76 MPa (2.7×2.5 MPa). In ethanol-perfused *Cassipourea*, air conductivity increases with each pressure increment indicating continual unsealing of pits; extrapolating to native stems indicates they would show a continual increase in air conductivity with increasing pressure to 12.2 MPa (2.7×4.5 MPa). In *Cassipourea*, absolute conductivities (as opposed to relative values shown in Fig. 3) of ethanol-perfused stems should have been higher than native stems by a factor of 2.7 for the same pressure; in actuality they were on the average 1.8 times higher (data not shown). This suggests that the ethanol was diluted as it passed through the stem, and/or failed to fully penetrate all vessels and vessel walls.

Absolute conductivities of air in *Cassipourea* showed considerable variation for both native and ethanol-perfused stems; this is obscured in plots of relative conductivity because the shapes of permeability curves were quite similar within each treatment. This variation could

be explained neither by differences in cross-sectional xylem area nor by differences in hydraulic conductance.

Discussion

The poor correspondence between the acoustic (Fig. 2) and hydraulic (Fig. 1) vulnerability curves of *Rhizophora mangle* and *Cassipourea elliptica* suggests that in the Rhizophoraceae, at least, cumulative AEs are a poor predictor of loss in xylem conductivity from embolized vessels. This may be true for many vessel-bearing plants, because we strongly suspect that the lack of correspondence is due to AEs produced by cavitation of fibers and perhaps ray cells. Fibers vastly outnumber vessels on a per-volume basis in vessel-bearing trees. The cumulative AE count in *Rhizophora* levelled off at Ψ in the range of -5 to -6 MPa. This indicates that *Rhizophora* fibers cavitate at about the same range of Ψ_{px} as vessels. In *Cassipourea*, fibers apparently cavitate at much more negative Ψ_{px}. Previous work on conifers (Tyree and Dixon 1986), where the wood consist largely of tracheids, has shown a good correspondence between the two methods; the same was true for sugar maple (*Acer saccharum*; Tyree and Dixon 1986), a vessel-bearing dicotyledon whose fibers are mostly already embolized in their native state (Wiegand 1906).

On the basis of comparing the hydraulic vulnerability curves of *Rhizophora* and *Cassipourea* (Fig. 1), vulnerability of the xylem to embolism appears to be an adaptive trait that reflects the physiological demands of the environment. Some of the anatomical differences noted between the xylem of the two species could also reflect adaptation to their contrasting environments. The thick vessel walls in *Rhizophora* (Tab. 1) could substantially reduce the probability of air seeding where these walls contact air-filled extracellular spaces. Uniformly short vessels in *Rhizophora* (Tab. 2) promote greater compartmentalization of potential embolism in a stressful environment; this contrasts to the presence of a small number of very long vessels in *Cassipourea*, which may represent a minimal sacrifice of safety for greater xylem conductance in its less stressful habitat.

The air permeability experiments strongly suggest that air-seeding at intervessel pit membranes is causing embolism in these species, and that the greater permeability of the intervessel pit membranes in *Cassipourea* as compared to *Rhizophora* is responsible for the greater vulnerability of the former species to embolism. The increase in embolism beginning with Ψ_{px} of -0.5 MPa in *Cassipourea* (Fig. 1) corresponds to the increase in air conductivity beginning with applied air pressures above 0.34 MPa (Fig. 3A). The plateau in the permeability curve between 2 and 3.7 MPa is not reflected in the vulnerability curve, but there are only two data points in this region. The minimal embolism in *Rhizophora* at Ψ_{px} down to -4.5 MPa (Fig. 1) corresponds to zero air conductivity at applied pressures up to 4.5 MPa (Fig. 3A).

As indicated in the Results section, the air permeability curves from the ethanol-perfused stems (Fig. 3B) can be extrapolated to native stems by accounting for the difference in surface tension between ethanol and water. In this way, we estimated that native stems of *Rhizophora* would become permeable to air at 6.76 MPa, and native *Cassipourea* would show an increase in air conductivity with pressure up to 12.2 MPa. Both of these extrapolations overestimate the negative Ψ_{px} required to induce embolism if air-seeding at pit membranes is invoked, i.e. *Rhizophora* begins to embolize at ca -5.8 MPa (Fig. 1) rather than -6.76 MPa, and *Cassipourea* is completely embolized by -6.9 MPa (Fig. 1) rather than continuing to embolize at Ψ_{px} down to -12.2 MPa. This discrepancy could be explained by dilution and/or lack of complete penetration of the ethanol as it was perfused through the stem.

Assuming that air seeding at intervessel pit membranes does occur, the greater vulnerability of *Cassipourea* to embolism implies that this species has larger pit membrane pores that the more invulnerable *Rhizophera*. Using the capillary equation (Eq. 1), the predicted pore diameters for the two species can be calculated from the range of Ψ_{px} shown to cause embolism in Fig. 1. Diameters reflect the maximum per vessel, because it is at the largest pore that air-seeding will occur. For *Rhizophora* embolism occurred between -5.8 and -6.9 MPa; using a surface tension for water of 0.072 N m^{-1} (25°C), this corresponds to pore diameters between 0.050 and 0.042 μm. Embolism in *Cassipourea* occurred between -0.5 and -6.9 MPa corresponding to pore diameters between 0.576 and 0.042 μm. Unfortunately, there are no reports in the literature of measured pore sizes in these species, or in the family Rhizophoraceae.

The conclusion that embolism is caused by air leaking through pits is supported by independent work on *Rhododendron* (Crombie et al. 1986) demonstrating a correspondence between xylem cavitation and air permeability of intervessel pit membranes. The same correspondence has been shown for grapevines (Sperry et al. 1987), and in recent work on sugar maple (Sperry and Tyree 1988). As far as drought-induced embolism is concerned, the intervessel pit membrane appears to be the 'Achille's heel' of the xylem. Future studies on ecological wood anatomy and water stress physiology should take this possibility into account.

Edited by A. Kylin

References

Crombie, D. S., Hipkins, M. F. & Milburn, J. A. 1985. Gas penetration of pit membranes in the xylem of *Rhododendron* and other species. – Planta 163: 27–33.

Dixon, M. A. & Tyree, M. T. 1984. A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. – Plant Cell Environ. 7: 693–697.

Holdridge, L. R., Grenke, W. C., Hatheway, W. H., Liang, T. & Tosi, J. A., Jr. 1971. Forest Environments in Tropical Life Zones. – Pergamon press, New York. ISBN 0-080-16340-8.

Jansonnius, H. H. 1950. The vessels in the wood of Javan mangrove trees. – Blumea 6: 465–469.

Lewis, A. M. 1988. A test of the air-seeding hypothesis using *Sphagnum* hyalocysts. – Plant Physiol. (In press).

Oertli, J. J. 1971. The stability of water under tension in the xylem. – Z. Pflanzenphysiol. 65: 195–205.

Panshin, A. J. 1932. An anatomical study of the woods of the Philippine mangrove swamps. – Philipp. J. Sci. 48: 143–205.

Pickard, W. F. 1981. The ascent of sap in plants. – Progr. Biophys. Mol. Biol. 37: 1281–229.

Scholander, P. F., Hammel, H. T., Hemmingsen, E. A. & Garay, W. 1962. Salt balance in mangroves. – Plant Physiol. 37: 722–729.

– , Hammel, H. T., Bradstreet, E. D. & Hemmingsen, E. A. 1965. Sap pressure in vascular plants. – Science 148: 339–348.

Siau, J. F. 1984. Transport Processes in Wood. pp. 73–104. – Springer-Verlag, New York. ISBN 0-387-12574-4.

Sperry, J. S. 1986. Relationship of xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. – Plant Physiol. 80: 110–116.

– & Tyree, M. T. 1988. Mechanism of water-stress induced xylem embolism. – Plant Physiol. (In press).

– , Holbrook, N. M., Zimmermann, M. H. & Tyree, M. T. 1987. Spring filling of xylem vessels in wild grapevine. – Plant Physiol. 83: 414–417.

– , Donnelly, J. R. & Tyree, M. T. 1988a. A method for measuring hydraulic conductivity and embolism in xylem. – Plant Cell Environ. 11: 35–40.

– , Donnelly, J. R. & Tyree, M. T. 1988b. Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). – Amer. J. Bot. 75: 1212–1218.

Tyree, M. T. & Dixon, M. A. 1986. Water stress induced cavitation and embolism in some woody plants. – Physiol. Plant. 66: 397–405.

– , Dixon, M. A., Tyree, E. L. & Thompson, R. G. 1984. Ultrasonic acoustic emissions from the sapwood of cedar and hemlock: An examination of three hypotheses concerning cavitations. – Plant Physiol. 75: 988–992.

– , Fiscus, E. L., Wullschleger, S. D. & Dixon, M. A. 1986. Detection of xylem cavitation in corn under field conditions. – Plant Physiol. 82: 597–599.

van Vliet, G. J. C. M. 1976. Wood anatomy of Rhizophoraceae. – Leiden Bot. Ser. 3: 20–75.

West, D. W. & Gaff, D. F. 1976. Xylem cavitation in excised leaves of *Malus sylvestris* Mill. and measurement of leaf water status with the pressure chamber. – Planta 129: 15–18.

Wiegand, K. M. 1906. Pressure and flow of sap in the maple. – Am. Nat. 40: 409–453.

Zimmermann, M. H. 1983. Xylem Structure and the Ascent of Sap. pp. 44–47. – Springer-Verlag, New York. ISBN 0-378-12268-0.

– & Jeje, A. A. 1981. Vessel length distribution in stems of some American woody plants. – Can. J. Bot. 59: 1882–1892.