

# The role of freezing in setting the latitudinal limits of mangrove forests

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## Summary

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- Mangrove trees dominate coastal vegetation in tropical regions, but are completely replaced by herbaceous salt marshes at latitudes above 32°N and 40°S. Because water deficit can increase damage caused by freezing, we hypothesized that mangroves, which experience large deficits as a result of saline substrates, would suffer freeze-induced xylem failure.
- Vulnerability to freeze-induced xylem embolism was examined in the most poleward mangrove species in North America, in an area where freezing is rare but severe, and in Australia, in an area where freezing is frequent but mild. Percentage loss in hydraulic conductivity was measured following manipulations of xylem tension; xylem sap ion concentration was determined using X-ray microanalysis.
- Species with wider vessels suffered 60–100% loss of hydraulic conductivity after freezing and thawing under tension, while species with narrower vessels lost as little as 13–40% of conductivity.
- These results indicate that freeze-induced embolism may play a role in setting the latitudinal limits of distribution in mangroves, either through massive embolism following freezing, or through constraints on water transport as a result of vessel size.

**Key words:** *Aegiceras*, *Avicennia*, freeze-induced embolism, mangrove, *Rhizophora*, vessel diameter, xylem ion content.

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## Introduction

Mangroves occur widely in the tropics but species diversity and stand complexity fall rapidly in temperate regions (Tomlinson, 1994; Spalding *et al.*, 1997; Duke *et al.*, 1998). Coastal ecosystems at latitudes above 32°N and 40°S lack woody vegetation, in contrast with the tropics, where an estimated 75% of coast can be classified as mangal (Spalding *et al.*, 1997). This worldwide transition from woody to herbaceous vegetation has long been attributed to temperature (McMillan, 1971; Lugo & Zucca, 1977; McMillan & Sherrod, 1986; Sherrod *et al.*, 1986; Tomlinson, 1994; Duke *et al.*,

1998), but the fundamental mechanisms excluding woody plants from tidal habitats at high latitudes remain unknown. Because mangroves are often considered tropical by definition (Tomlinson, 1994), few have questioned why they do not spread to temperate areas.

Mangroves are a large and diverse ecological assemblage. Trees of widely disparate families are found in the mangrove habitat, representing at least five major and many additional minor independent evolutions of salt-tolerant, anoxia-tolerant, woody plants (Tomlinson, 1994). Vivipary, breathing roots and salt glands have evolved multiple times within this ecological classification (Ball, 1988; Tomlinson, 1994; Duke *et al.*, 1998),

suggesting strong, convergent selective pressures. Given the wide range of species filling this niche, and the opportunities for adaptation present in different lineages, it is surprising that natural selection has not produced woody plants that can tolerate the combined stresses of salinity and freezing.

Vulnerability to the disruption of water transport after freezing corresponds with range limits of many species (Sperry *et al.*, 1994; Langan *et al.*, 1997; Pockman & Sperry, 1997; Cavender-Bares & Holbrook, 2001). Significant losses in the ability to supply water to stems and leaves can occur when air bubbles form in xylem sap during freezing. If the sap thaws under tension, these pockets of air can expand to block water transport (Tyree & Zimmerman, 2002).

Whether a bubble will shrink or grow depends on the hydrostatic pressures within both the bubble and the surrounding fluid, as well as the force resulting from the gas : liquid interface. The bubble will expand when

$$P_B - P_x > 2\gamma/r \quad \text{Eqn 1}$$

where  $2\gamma/r$ , the inward pressure resulting from surface tension  $\gamma$  divided by the radius of the bubble  $r$ , is less than the difference between the hydrostatic pressure of the xylem sap ( $P_x$ ; typically  $< 0$ ) and the pressure of the gas within the bubble itself ( $P_B$ ,  $> 0$ ). Thus, as pressure in the sap drops, smaller bubbles will cause xylem embolism (Davis *et al.*, 1999; Tyree & Zimmerman, 2002). Mangroves experience comparatively large xylem tensions because of the high osmotic concentration in their substrates (Scholander *et al.*, 1962; Scholander, 1968). The xylem water potential of a tree growing in seawater will be  $\leq -2.5$  MPa even when stomata are closed. The magnitude of this tension increases during the day, as transpiration drives the water potential in the leaves below that of the soil. These substantial xylem tensions cannot be eliminated by shedding leaves, and appear to preclude the generation of positive root pressures as a means of refilling embolized conduits (Sperry *et al.*, 1988).

Plant life in saline habitats can be compatible with cold temperatures; well-developed salt marsh communities dominate tidal plains and estuaries where winter ocean isotherms fall below  $10^\circ\text{C}$  (Duke *et al.*, 1998), and extend well into the Arctic (Mitsch & Gosselink, 1986). The feature that distinguishes the salt marsh from the mangal is the lack of woody, arborescent plants. We hypothesize that the unique combination of freezing and tension caused by salinity results in the exclusion of woody plants from coastal wetlands at latitudes where freezing is a regular occurrence.

We tested this hypothesis in three mangrove communities, sampling the five most poleward mangrove species on two continents at or near their absolute latitudinal limits, using excised-branch techniques to examine how the interaction of freezing and tension influences hydraulic conductivity. Experimental manipulations of xylem tension were performed to separate the effects of tension and freezing.

## Materials and Methods

### Plant material

Five species of mangroves were collected from natural populations growing near their latitudinal limits. In Florida, USA, *Avicennia germinans* (L.) Stearn (Acanthaceae) and *Rhizophora mangle* L. (Rhizophoraceae) were collected at Marineland ( $29^\circ40'\text{N}$ ,  $81^\circ12'\text{W}$ ) and at Ponce Inlet ( $29^\circ4'\text{N}$ ,  $80^\circ55'\text{W}$ ), respectively. Both species were collected during the first 2 wk of January 2003. In Australia, *Avicennia marina* (Forsk.) Vierh. var. *australasica* (Walp.) Moldenke (Acanthaceae) and *Aegiceras corniculatum* (L.) Blanco (Myrsinaceae) were collected in New South Wales, at Batemans Bay ( $35^\circ42'\text{S}$ ,  $150^\circ12'\text{E}$ ), during July and August 2003. *Rhizophora stylosa* Griff. (Rhizophoraceae) was collected in Queensland, near Jacobs Well ( $27^\circ46'\text{S}$ ,  $153^\circ22'\text{E}$ ) during September 2003. Cold-hardening can affect measurements of freeze-induced cavitation (Sakai *et al.*, 1981). Collections were therefore made in the coldest month at three of the four sites, and during very early spring at the Jacobs Well site. It can be difficult to determine the age of mangrove wood in all species (Tomlinson, 1994) because of their generally aseasonal habitats (but see Verheyden *et al.*, 2005); however, all measurements were made on mature branches at least 1 yr old and 2.5–7.7 mm in diameter; most of the variation in diameter was the result of differences between species.

### Occurrence of freezing at collection sites

In Florida, average winter minimums were high,  $8.2^\circ\text{C}$  in Jacksonville, near the Marineland site, and  $9.5^\circ\text{C}$  at Daytona Beach, near the Ponce Inlet site, but minimum recorded temperatures were  $-10^\circ\text{C}$  and  $-9.4^\circ\text{C}$  (21- and 30-yr records, respectively, NOAA-CIRES, 2002). During the past century, severe freezes ( $< 8^\circ\text{C}$ ) occurred on average once every 8 yr (Henry *et al.*, 1994). In southern Australia, mild frosts are common: minimums of  $0^\circ\text{C}$ ,  $-0.61^\circ\text{C}$  and  $-3^\circ\text{C}$  were recorded in mangrove communities during the study (data not shown) and 13-yr records report minimums of  $-2.9^\circ\text{C}$  (Commonwealth Bureau of Meteorology, 2001).

### Ion contents of xylem sap

Ion contents of xylem vessels were measured using energy-dispersive X-ray microanalysis (EDX) (McCully *et al.*, 2000) to determine an appropriate perfusing solution for use in hydraulic measurements. Physiologically relevant measurements of hydraulic conductivity are best made with a perfusing solution matched to the ion content of the xylem sap *in vivo*, as ion content can markedly affect measured conductivity (Zwieniecki *et al.*, 2001; López-Portillo *et al.*, 2005).

One stem cross-section was taken from each of three individuals growing at an estuarine site in Nelligen, NSW,

Australia, and three individuals from a marine site in Batemans Bay, NSW, Australia ( $n = 3$ ). Replicates were rapidly frozen by submersion of intact, attached branches in liquid nitrogen ( $-196^{\circ}\text{C}$ ). After removal from the tree, segments were cut from each branch and transported to the laboratory in a cryo-shipper at  $-170^{\circ}\text{C}$ .

Samples were planed with a diamond knife at  $-80^{\circ}\text{C}$  (McCully *et al.*, 1998, 2000), coated with aluminum, and transferred to the cryo-stage of a scanning electron microscope (Oxford CT 1500, Oxford Instruments, Oxford, UK) where they were viewed through a beryllium window. Ion concentrations in six to 12 filled xylem conduits per sample were measured using EDX (Link eXL, Oxford Instruments). Detection limits with this equipment are 30 mM for  $\text{Na}^+$ , and 1 and 7 mM for  $\text{K}^+$  and  $\text{Cl}^-$  (C. Huang, pers. comm.). Assuming cation/anion balance allows interpretation of the less reliable  $\text{Na}^+$  measurements.

#### Interaction of freezing and tension in excised branches

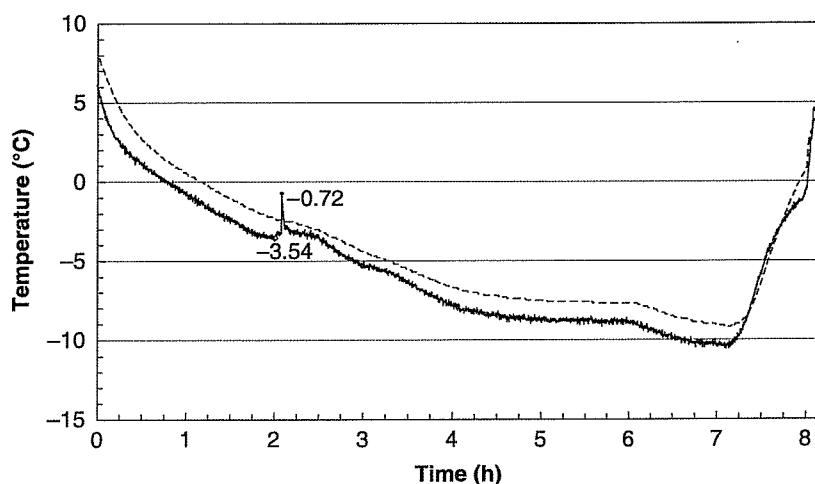
Terminal, leaf-bearing branches *c.* 1 m in length were collected during periods of minimal transpiration and xylem tension between 20:00 and 22:00 h for all species except *R. stylosa*, which was harvested between 05:00 and 07:00 h. For each species, 23–15 individuals were sampled; this was reduced to 10 in *R. mangle*, where only one experiment was conducted. Branches were cut in pairs, with one branch assigned to the freezing treatment and the other to the cold-storage control. 'Tension' and 'tension relieved' samples were gathered on successive days. In 'tension' treatments, branches were cut from each tree in air, and enclosed in plastic bags immediately following excision to prevent water loss and thus preserve xylem tensions at their native values. In 'tension relieved' treatments, branches from the same tree were cut under a sap solution containing 25 mM NaCl, placed in a bucket of sap solution, and allowed to hydrate until leaf water potentials

( $\Psi_l$ ) measured with a pressure chamber (Scholander *et al.*, 1964) were  $\geq -0.5$  MPa.

Before beginning the freezing treatments, the water potential of one leaf from each branch was measured using a pressure chamber. Branches were placed in a Styrofoam chamber approx. 0.6 m wide by 1.5 m long and cooled from  $0^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$  at a rate of  $2^{\circ}\text{C h}^{-1}$ , held at  $-10^{\circ}\text{C}$  for 1 h, and thawed at  $2^{\circ}\text{C min}^{-1}$ . Temperature of the freezing chamber was controlled using a programmable water bath and monitored with thermocouples throughout the experiment. Moist towels were placed in the freezing chamber to prevent further water loss. Freezing and thawing rates reflected rates of temperature change experienced during freezing in nature (Sperry & Sullivan, 1992; Sperry *et al.*, 1994; Pockman & Sperry, 1997; Cordero & Nilsen, 2002). Control branches were stored in sealed plastic bags at  $4^{\circ}\text{C}$  during the entire time the experimental branches were in the freezing chamber.

Thermocouples were attached to branches during freezing and branch temperatures were logged using a datalogger (Campbell Scientific, Logan, UT, USA). No external nucleation was used. Branches were frozen with leaves intact, as we presume this to be the site of nucleation *in vivo*. Freezing exotherms were observed and recorded for each branch. Because freezing is a stochastic process, freezing was observed to occur at a variety of temperatures between  $-2$  and  $-10^{\circ}\text{C}$ , although generally before  $-5^{\circ}\text{C}$ . A sample thermocouple trace from a branch undergoing the process of freezing appears in Fig. 1.

The effect of freezing on xylem hydraulic conductivity was quantified by comparing percentage loss of hydraulic conductivity (PLC) in paired frozen and unfrozen (control) branches. A 3–7 cm segment, located  $> 1 \times$  maximum vessel length from the cut end, was excised from each branch. Maximum vessel lengths were determined using the air injection method of Zimmerman & Jeje (1981). All cuts were made under 25 mM NaCl solution to prevent entry of air into the test segment; this solution, passed through a  $0.2 \mu\text{m}$  syringe filter,



**Fig. 1** Graph of temperature vs time for one branch frozen on 7 January 2003. The dark trace shows branch temperature, and the dotted line shows the temperature of a reference thermocouple placed distal to the chilling unit. The temperature at which freezing occurred ( $-3.54^{\circ}\text{C}$ ) and the highest temperature of the freezing exotherm ( $-0.72^{\circ}\text{C}$ ) are labeled. Temperatures at which freezing occurred varied between  $-2$  and  $-10^{\circ}\text{C}$ , although nucleation generally occurred by at least  $-5^{\circ}\text{C}$ . The large variation in nucleation temperature was the result of the stochastic freezing process and the fact that nucleation was not artificially induced but allowed to occur *in situ* in attached leaves.

was also used for perfusion. The flow rate through each segment was then measured with a steady-state flow meter (Brodribb & Feild, 2000) at an initial hydraulic head of approx.  $2.3 \times 10^{-2}$  MPa (US) or  $1.9 \times 10^{-3}$  MPa (Australia). These pressures, which occurred before the first resistor, are insufficient to flush emboli from open vessels in all five species, based on measurements of maximum vessel diameter. The actual delivery pressures experienced by the branches were on the order of half that supplied by the initial pressure head.

Each segment was then flushed with perfusing solution using a syringe to remove air emboli from vessels. Segments were flushed using a 0.2  $\mu\text{m}$  filter and 25 mM NaCl solution at  $\sim 100$  kPa for 5 min, or until no more bubbles emerged from the distal end of the segment, and then re-measured to estimate maximum conductivity. Measurements are reported as PLC:

$$\text{PLC} = (1 - (K_i/K_m)) \times 100 \quad \text{Eqn 2}$$

( $K_i$ , initial conductivity after treatment;  $K_m$ , maximum conductivity after flushing). The short length of the segments ensured that a majority of vessels were cut open at both ends. This approach has been used by Cochard *et al.* (2002) and Brodribb *et al.* (2003) to determine PLC and was chosen because tests showed conductivity decreased in *A. marina* during the longer flushing times required to dissolve emboli, possibly resulting from wounding effects (data not shown). However, if the majority of vessels are open, emboli can be pushed out of the end of the segment using only the pressure necessary to move a meniscus along an open capillary. Because vessel endings often contribute the majority of hydraulic resistance in the stem (Wheeler *et al.*, 2005; Choat *et al.*, 2006), a measurement excluding them would be misleading in comparisons; therefore, we do not report sapwood-specific conductivity.

#### Vessel diameter measurements

Vessel diameters were measured on sections taken from segments used for hydraulic measurements in both the US and Australia. Three images were taken per cross-section in each stem, accounting for approx. three-quarters of the total area of the section. For each species, two stems each from five individuals were used. In the US, segments were shaved smooth with a razor blade, oven-dried and sputter-coated, and viewed on a calibrated Quanta 200 ESEM (FEI Co., Hillsboro, OR, USA). In Australia, segments preserved in ethanol were hand-sectioned, stained with toluidine blue, and photographed at 100 $\times$  magnification on an Axioskop light microscope (Carl Zeiss) with a Spot Camera (Diagnostic Instruments, Sterling Heights, MI, USA). All images were analyzed using thresholding and particle analysis utilities in analySIS 3.2 (Soft Imaging System, Gulfview Heights, South Australia, Australia).

## Results

### Ion contents of xylem sap

Energy-dispersive X-ray microanalysis analysis of both *A. marina* and *A. corniculatum* revealed relatively low concentrations of ions in the xylem sap (Table 1). In both species, concentrations of  $\text{K}^+$  were frequently below detectable limits, while  $\text{Mg}^{2+}$ ,  $\text{P}^+$ ,  $\text{S}^{2-}$ , and  $\text{Ca}^{2+}$  were only rarely above the limits of detection in xylem conduits. Nondetects were included as zeros; this accounts for some standard errors being larger than means. There was no significant difference between ion concentrations in xylem conduits of plants growing at the higher salinity coastal site and the estuarine site, allowing measurements to be pooled. *Avicennia marina* had higher average ion concentrations than *A. corniculatum*, but again there was no significant difference between species.

Based on these measurements, a perfusing solution containing 25 mM NaCl was selected for use in all five species. This agrees with previous reports of mangrove xylem sap content (Ball, 1988; Tomlinson, 1994) and represents 92–94% exclusion of substrate salt. Although both *A. marina* and *A. corniculatum* have previously been thought to have higher sap salinities because of the fact that they have salt excretion glands, our results do not support this. Our results are consistent with reports of 24.4–36.6 mM NaCl by Melcher *et al.* (2001) in *R. mangle* (a nonexcreting species); however, they are less than the 56–316 mM reported by López-Portillo *et al.* (2005) in *A. germinans* and *Conocarpus erectus*. Differences between studies in the ion concentrations reported in xylem sap of mangroves may reflect differences in the methods of measurement.

Our direct measurements of ion concentrations in the xylem sap of *A. marina* and *A. corniculatum* agree well with those estimated for the same species by a mass balance method (Ball, 1988). In contrast, much higher concentrations of ions have been reported in the xylem sap of mangroves when a pressure bomb was used to express the sap from a cut stem; these high concentrations may result from contamination. High concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  typically accumulate within living cells of halophytes (Ball, 1996); given the substantial numbers of living cells that occur within the hydraulic tissue, expressed xylem sap could become contaminated with ions if the sap came into contact with the cut surfaces of living cells.

Table 1 Xylem sap ion concentrations for Na, Cl and K ions

Species	Na ( $\mu\text{M}$ ) <sup>a</sup>	Cl ( $\mu\text{M}$ ) <sup>a</sup>	K ( $\mu\text{M}$ ) <sup>a</sup>
<i>Aegiceras corniculatum</i>	30.00 $\pm$ 19.97	31.80 $\pm$ 18.57	5.19 $\pm$ 5.43
<i>Avicennia marina</i>	27.26 $\pm$ 22.29	24.68 $\pm$ 17.02	2.93 $\pm$ 5.48

<sup>a</sup>Measurements were made using energy-dispersive X-ray microanalysis (EDX) on branch tissue visualized by cryoscanning electron microscopy. Data for each ion are given as means  $\pm$  SE ( $n = 3$ ).

## Interaction of freezing and tension in excised branches

There was severe xylem impairment in three of the five species after freezing at native tension; in two species, there was no significant effect of freezing at native xylem tensions (Fig. 2). A separate one-way ANOVA was performed for each species, showing that branches of *A. germinans*, *R. mangle* and *Rhizophora stylosa* frozen under tension showed significantly greater loss ( $P < 0.05$  for all tests, Tukey HSD) in conductivity than in control branches. Freezing under tension did not significantly impair conductivity in either *A. corniculatum* or *A. marina* as compared with unfrozen branches (Fig. 2).

These tests also showed that, among species susceptible to freeze-induced damage, *A. germinans* and *R. stylosa* showed significantly greater dysfunction after freezing under tension than after freezing with tension relieved. Measurements on tension-relieved stems of *R. mangle* were not made because of time limitations and inaccessibility of the sample site.

In *A. corniculatum*, there was no significant effect of freezing, with or without tension. In *A. marina*, branches frozen

after tension was relieved showed significantly greater impairment than hydrated, unfrozen branches or branches frozen under tension. However, the overall loss of conductivity in this species was low ( $< 40\%$ ) and comparable to, or less than, the measurements for control branches in other species. Although the treatments were statistically distinguishable in *A. marina*, the magnitude of impairment in each case was so low that it is unlikely to have a distinguishable effect on the survival of the plant. Losses in conductivity of this magnitude are not likely to lead to death of the plant; while this difference is statistically significant, it is small enough in scale that it is unlikely to be physiologically important.

## Vessel diameter measurements

Mean and hydraulically weighted average vessel diameters in all five species corresponded with both latitudinal limits and observed loss of conductivity after freezing at native xylem tensions (Table 2). The percentage loss in hydraulic conductivity of excised branches frozen under tension increased

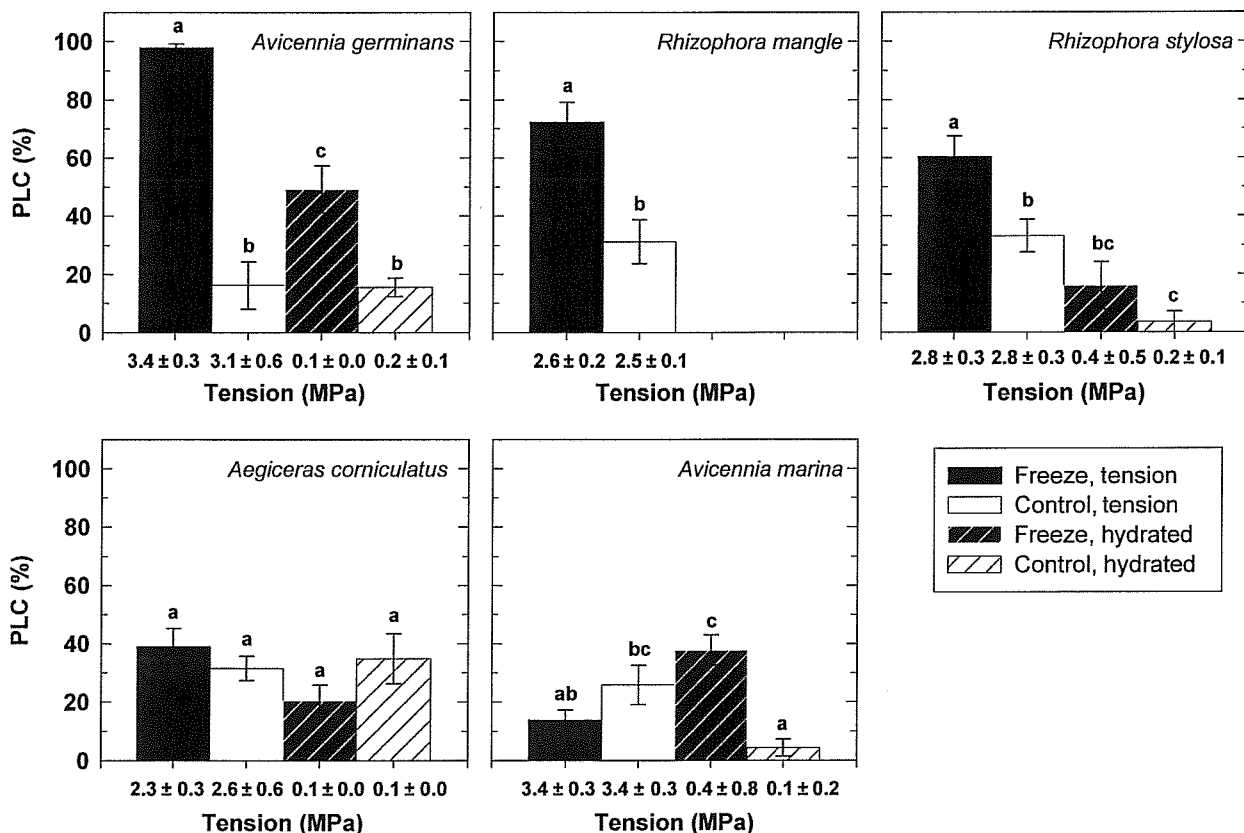


Fig. 2 Interactive effects of freezing and tension on percentage loss in stem hydraulic conductivity (PLC) in five mangrove species. The four treatments, indicated by pattern, were: branches frozen or held at 4°C at native xylem tension, and branches cut under sap solution and hydrated to relieve xylem tension. Mean water potentials for each treatment are reported below each group. Error bars represent standard error calculated for each mean,  $n = 5-13$ , with an average size of 9; unevenness of group sizes was the result of loss of samples because of high rates of clogging in some species. A separate ANOVA test was performed for each species; letters indicate groups which were significantly different at  $P = 0.05$  using Tukey HSD. For each tension treatment, 'frozen' and 'control' samples were paired branches from a single individual, for a total of 10–20 individuals per species.

with the hydraulically weighted average vessel diameter ( $r^2 = 0.72$ ) (Fig. 3). These data show that when the native tension was retained, interspecific differences in vessel diameter accounted for as much as 72% of the variation in freeze-induced embolism. Thus, for species inhabiting saline environments, vulnerability to freeze-induced embolism can be reduced by a decrease in the diameter of xylem vessels.

## Discussion

We tested the hypothesis that mangroves will be more vulnerable to freeze-induced cavitation when under tension generated by osmotic pressure. In three of five species, a combination of freezing at environmental temperatures and *in vivo* tensions caused severe embolism. Freezing under tension also

caused more severe loss of conductivity than did freezing with tension relieved in the two species for which this was examined. However, our results also indicate that *in vivo* tensions were not sufficient to cause significant xylem failure in *A. corniculatum* or *A. marina*, species with mean vessel diameters of 17.05 and 19.09  $\mu\text{m}$  (Table 2). We believe that these species were protected from embolism by their narrow vessels. Differences in hydraulically weighted average vessel diameter corresponded directly with loss of conductivity as a result of freezing (Fig. 3). In turn, loss of conductivity in response to freezing to  $-10^\circ\text{C}$  corresponded with the latitudinal limits of the species in this study.

Previous work has correlated conduit diameter with hydraulic impairment caused by freezing (Sperry *et al.*, 1994; Davis *et al.*, 1999), showing that, primarily for reasons of volume (Tyree & Zimmermann, 2002; Pittermann & Sperry, 2003), 30  $\mu\text{m}$  is the threshold at which severe (> 50%) loss of conductivity occurs (Davis *et al.*, 1999). By contrast, Feild & Brodribb (2001) found that 20–25  $\mu\text{m}$  was the threshold for 50% loss of conductivity. Our results were consistent with this finding. All species above 22  $\mu\text{m}$  showed significant loss in conductivity after freeze/thaw treatment; for species below this threshold, there was no major impairment after freezing.

**Table 2** Vessel diameters for five mangrove species from the northern and southern limits of the worldwide mangrove distribution

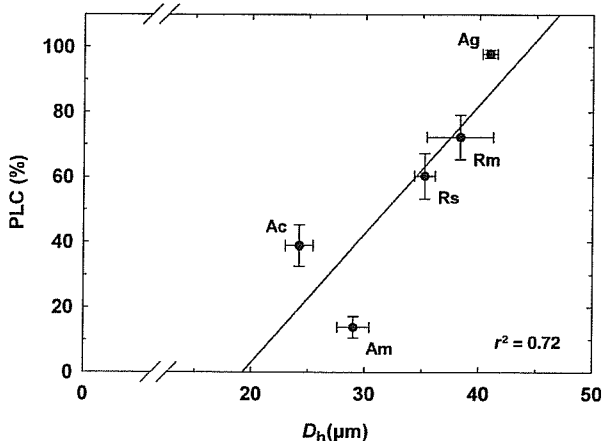
Species	$D$ ( $\mu\text{m}$ ) <sup>a</sup>	$D_h$ ( $\mu\text{m}$ ) <sup>b</sup>	Latitude <sup>c</sup>
<i>Aegiceras corniculatum</i>	17.1 $\pm$ 0.68	24.2 $\pm$ 1.22	35°42'30"S
<i>Avicennia marina</i>	19.1 $\pm$ 0.35	29.0 $\pm$ 0.66	35°42'30"S
<i>Avicennia germinans</i>	31.0 $\pm$ 1.01	40.9 $\pm$ 1.41	29°40'08"N
<i>Rhizophora mangle</i>	30.8 $\pm$ 2.52	38.3 $\pm$ 2.94	29°4'35"N
<i>Rhizophora stylosa</i>	22.2 $\pm$ 1.17	35.2 $\pm$ 0.90	27°46'41"S

<sup>a</sup>Mean vessel diameter ( $D$ )  $\pm$  SE.

<sup>b</sup>Hydraulically weighted mean vessel diameters, calculated as

$D_h = \Sigma D^5 / \Sigma D^4$ .

<sup>c</sup>Collection latitude.



**Fig. 3** Freeze-induced percentage loss in stem hydraulic conductivity (PLC) at native xylem tensions as a function of hydraulically weighted vessel diameter ( $D_h$ ) in five mangrove species. Hydraulically weighted vessel diameters ( $D_h = \Sigma D^5 / \Sigma D^4$ ) account for the disproportionate contribution of larger vessels to conductivity. Regression line is  $y = 3.961x - 76.08$ , with  $r^2 = 0.72$ . Bars denote standard error of the mean, with  $n = 5$ –13 stems for PLC and  $n = 5$  stems for vessel diameters. Two letter abbreviations indicate genus and species: Ac, *Aegiceras corniculatum*; Ag, *Avicennia germinans*; Am, *Avicennia marina*; Rm, *Rhizophora mangle*; Rs, *Rhizophora stylosa*.

## Vessel diameter and latitudinal range

The average vessel diameters for *A. germinans* and *R. mangle* collected from sites in Florida at 29°N were 54% greater than those of *A. marina* and *A. corniculatum* collected in Australia at 35°S. The latter two species have the most poleward range of all mangroves. The mean vessel diameters of 19 and 17  $\mu\text{m}$  in *A. marina* and *A. corniculatum*, respectively, are among the smallest observed in mangroves (Tomlinson, 1994) and are small among angiosperms in general (Carlquist, 2001). It is interesting to note that the vessel diameters for the genus *Avicennia* appear to vary more than those for *Rhizophora*. Whether the smaller diameters observed across latitudes and continents in *Avicennia* are the result of genetic variability or phenotypic plasticity remains to be established. A comparative study of vessel diameter in *A. marina* across latitude could help to address this question.

The climatic profiles of the collecting sites in Florida and New South Wales may contribute to the differences observed. In Florida, average winter minimums are warm, ranging from 8.2 to 9.5°C, but frosts as severe as  $-10^\circ\text{C}$  are regular events, occurring, on average, once every 8 yr over the past century (Henry *et al.*, 1994). For a mangrove growing under these conditions, there is no benefit to resisting freezing in an average year, and producing narrow vessels represents a significant fitness cost in years without frost. Because of the difficulty in aging mangroves (Tomlinson, 1994; Verheyden *et al.*, 2005), little information is available on the age at which mangroves reach reproductive maturity. Nonetheless, field observations indicate that it takes 3–5 yr for these trees to flower (M. C.

Ball, unpublished). Data from this study suggest that where mangroves are able to achieve reproductive success, either directly or through dispersal, they may face little or no pressure to adapt to intermittent frosts.

By contrast, the climate in southern New South Wales provides conditions that may select for frost resistance. Climate records indicate that frosts occur at least once a year in Batemans Bay, with an average of 5.6 d with minimums  $\leq 0^\circ\text{C}$  per year, but frosts are less severe, with no temperatures below  $-3^\circ\text{C}$  (Commonwealth Bureau of Meteorology, 2006). Our experiments showed that when leaves are attached, xylem freezing may occur at or near this temperature (Fig. 1). We suggest that freezing is a pressure mangrove species in this area face before reproductive age. Our results indicate that the smaller vessel diameters and higher vessel densities found at this site successfully limit freeze-induced embolism even under tension. Yet, these narrow vessels may contribute to the exclusion of *A. marina* and *A. corniculatum* from more seasonal habitats, as carbon gain is typically limited by high resistance in stems (Brodrigg & Feild, 2000; Santiago *et al.*, 2004). The extremely long periods of time between flowering and fruiting ( $> 1$  yr, Duke, 1990) observed in the southernmost populations of *A. marina* suggest that these populations have difficulty acquiring the photosynthate needed for reproduction. With shorter growing seasons at high latitudes, this could explain why these seemingly well-adapted species do not reach colder climates.

### Freezing as a disturbance

Cold temperatures alone do not exclude vegetation from saline habitats, and salt marshes dominated by low-growing grasses (e.g. *Spartina*), reeds (e.g. *Juncus*) and succulents (e.g. *Salicornia*) are widespread. Although these herbaceous plants also suffer from freezing, they do not have to invest in many years of woody growth before reproduction. The mangrove to salt marsh transition has not been extensively considered, but forest to grassland boundaries occur widely and are well studied (Schultz *et al.*, 1955; Longman & Jenik, 1992). Successional theory suggests that where nutrient availability permits, trees are the long-term climax vegetation (Clements, 1916). However, grass- or herb-dominated ecosystems persist where disturbances, such as fire, grazing or drought, occur regularly. Freezing has occasionally been considered in this context, although not as widely as fire or grazing (Longman & Jenik, 1992).

Our results suggest that at least two separate mechanisms are responsible for limiting the range of the mangroves species within the climates included in our study. For *A. germinans*, *R. mangle*, and *R. stylosa*, rare but severe freezes may act as disturbances which favor the herbaceous salt marsh vegetation. For *A. marina* and *A. corniculatum*, severe freezes are apparently not lethal, and mechanisms not tested here must be suggested to account for their distribution.

Many different factors can cause the death of a mangrove tree, and may be locally responsible for limiting the expansion of a particular stand. Cold ocean temperatures and drier land (Duke *et al.*, 1998), a lack of continuity in suitable habitat, and cold damage to living membranes (McMillan, 1975; Markley *et al.*, 1982) may all have helped establish the present geographic range of mangrove taxa. Our data add to these previous explanations, suggesting that freezing in a saline environment can severely limit the ability to supply water to leaves. This may be one more vital clue in understanding why temperate mangroves have never evolved in any group.

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