

Xylem Hydraulic Characteristics, Water Relations and Wood Anatomy of the Resurrection Plant *Myrothamnus flabellifolius* Welw.

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Myrothamnus flabellifolius Welw. is a desiccation-tolerant ('resurrection') plant with a woody stem. Xylem vessels are narrow (14 µm mean diameter) and perforation plates are reticulate. This leads to specific and leaf specific hydraulic conductivities that are amongst the lowest recorded for angiosperms (k_s 0.87 kg m⁻¹ MPa⁻¹ s⁻¹; k_l 3.28 × 10⁻⁵ kg m⁻¹ MPa⁻¹ s⁻¹, stem diameter 3 mm). Hydraulic conductivities decrease with increasing pressure gradient. Transpiration rates in well watered plants were moderate to low, generating xylem water potentials of -1 to -2 MPa. Acoustic emissions indicated extensive cavitation events that were initiated at xylem water potentials of -2 to -3 MPa. The desiccation-tolerant nature of the tissue permits this species to survive this interruption of the water supply. On rewatering the roots pressures that were developed were low (2.4 kPa). However capillary forces were demonstrated to be adequate to account for the refilling of xylem vessels and re-establishment of hydraulic continuity even when water was under a tension of -8 kPa. During dehydration and rehydration cycles stems showed considerable shrinking and swelling. Unusual knob-like structures of unknown chemical composition were observed on the outer surface of xylem vessels. These may be related to the ability of the stem to withstand the mechanical stresses associated with this shrinkage and swelling.

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INTRODUCTION

The vegetative tissue of few angiosperm species can survive severe water loss. Of those that are tolerant of the air-dry state (termed 'resurrection' plants; Gaff, 1971), only *Myrothamnus flabellifolius** Welw. (Myrothamnaceae) has a woody stem. *M. flabellifolius* is a multi-stemmed shrub which grows to a maximum height of approx. 1.5 m, and occurs on shallow soils (often less than 15 cm deep) on rocky outcrops. The soils become fully saturated after rain but dry rapidly thereafter. On drying the leaves fold against the stem and in the dry state the plant appears brown and dead. Within 24 h after receiving water, the leaves unfold and display their green adaxial surfaces (Sherwin and Farrant, 1996).

There are two descriptions of the wood anatomy of *M. flabellifolius* in the literature: the first by Tippe (1938) included no micrographs and was very brief; the second, by Carlquist (1976), is a detailed description of the wood anatomy with brief reference to the ecology of this plant. However, neither author seemed aware of the unique resurrection ability of this plant, with Carlquist (1976) only mentioning that the leaves contracted and expanded

depending on water availability and that it had both xeric and mesic aspects in its niche preferences.

A woody stem poses some unique challenges to a resurrection plant. The roots and stems also dehydrate and thus the xylem vessels will be completely embolized when the plant is in the air-dry state. The xylem must be able to refill during rehydration in order to provide a supply of water to the leaves and restore the water soil-plant-atmosphere continuum. Leaf rehydration occurs via the soil-root-stem-leaf xylem continuum as aerial parts do not take up water directly (Sherwin and Farrant, 1996). After watering there is a 12 h delay in water uptake by leaves; this is suggested by Sherwin and Farrant (1996) to be the time taken for the xylem to again become functional.

The tension in xylem water depends on the resistance to flow which is determined in part by the physical structure of the xylem. If the tension becomes too great the water column will cavitate, resulting in an air embolism, leading to a reduction in xylem conductance (Tyree and Sperry, 1988). Vulnerability depends on wood anatomy, particularly the sizes of pores in intervessel pits, with larger pores being more susceptible to air entry than smaller pores (Sperry and Tyree, 1988). The vulnerability of xylem to embolism has been measured in a number of species (Sandford and Grace, 1985; Salleo and Lo Gullo, 1986; Sperry, Donnelly and Tyree, 1988; Cochard, Ewers and Tyree, 1994; Raschi *et al.*, 1995) using either loss of hydraulic conductivity or accumulation of acoustic emissions. Acoustic emissions

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* Note: this species is often referred to as *M. flabellifolia*. However, *M. flabellifolius* is the correct name.

from stems of woody plants have become an accepted way of measuring cavitation (reviewed by Jackson and Grace, 1996).

For an embolized xylem conduit to become functional again the embolism must be displaced or dissolved in water. The exact mechanism of xylem refilling is not yet fully understood (Grace, 1993). Positive root pressure has been shown to contribute to refilling in some species (Milburn, 1979; Sperry *et al.*, 1988; Cochard *et al.*, 1994). Sperry *et al.* (1987) demonstrated that in spring the root pressure developed in wild grape vines is adequate to force air out of leaf scars. Borghetti *et al.* (1991) showed that when ray parenchyma tissue is killed the xylem still refills and concluded that refilling is a purely physical or physico-chemical process. Capillarity has also been suggested to enable water uptake into embolized xylem (Borghetti *et al.*, 1991; Grace, 1993). The capillary effect of traces of water left in the end of tracheids is thought to reduce the water potential below that of adjacent cells thus creating a pressure differential between adjacent cells. The increased pressure as a consequence of menisci at the air-water interface will also contribute to dissolving the air in the xylem (Tyree and Yang, 1992; Yang and Tyree, 1992; Edwards *et al.*, 1994).

Most of the recent research on desiccation tolerance in resurrection plants has been conducted on leaf tissue and has been focused at the biochemical and molecular level (see Bewley, 1995; Ingram and Bartels, 1996; Oliver and Bewley, 1997 for recent reviews). To date very little work has been done on the effect of desiccation on the roots and stems of resurrection plants and there have been no reports pertaining to the significance of hydraulic properties of the water conducting pathway in recovery from the dehydrated state. The aim of the current work is to briefly describe aspects of the wood anatomy of *M. flabellifolius* that may be pertinent to its habit as a 'resurrection' plant, and to present some related aspects of the water relations and hydraulic characteristics of the species during both dehydration and rehydration. Suggestions are made as to the mechanism whereby this plant refills its xylem.

MATERIALS AND METHODS

Plant material

Myrothamnus flabellifolius plants were collected in the Mpumalanga Province of South Africa (25° 40' S, 28° 32' E). They were repotted in a peat:river-sand:potting-soil mix and maintained in glasshouses at the University of Cape Town and the University of Natal. No supplemental lighting was provided, although during the summer plants were placed under 30% shade-cloth in order to reduce temperature. The plants used for the experiment were held under natural light for the duration of the experiment.

Wood anatomy

Eight twigs with diameters of between 3 and 7 mm were studied from both hydrated and dehydrated plants. Three transverse sections and one maceration per twig were prepared for analysis. For light microscopy studies, wood

samples were softened by boiling before being cut in transverse section (30 μm) using a Reichert Jung (Vienna, Austria) base sledge microtome. The sections were stained for 2 d in a mixture of alcohol, glycerol and safranin red before mounting in Kaiser's gelatine-glycerine on glass microscope slides. Macerations were prepared using Franklin's methods (Panshin and de Zeeuw, 1970) in which small blocks of wood were macerated in equal volumes of acetic acid and hydrogen peroxide (50%/50%) at 60 °C until the wood turned white (72–96 h for *M. flabellifolius*). The macerations were stained with Fast Green and safranin and mounted on glass microscope slides. Vessel diameters were measured from transverse sections at the widest part of the opening while excluding cell wall thickness. Fifty vessels were measured per section and three sections were viewed per twig. Vessel frequencies are the means of five areas per transverse section. Vessel element length was measured using the macerations and included the tails. Average values for lengths are based on 80 individual vessel elements. Measurements of vessel elements were made directly from glass microscope slides using a Leitz (Wetzlar, Germany) Laborlux K incident light microscope at a magnification of 40 \times and the image analysing program, Flexible Image Processing System (Council for Scientific and Industrial Research, Pretoria, South Africa). All measures were in accordance with the procedures recommended by the International Association of Wood Anatomists (IAWA Committee, 1989). Small, cleanly shaved blocks of wood and some macerated wood were sputter coated with gold-palladium and photographed with a Cambridge S-200 scanning electron microscope.

Water relations of whole plants

Water was withheld from three potted plants while they were allowed to dry naturally. During the drying process the water contents of the soil, leaves and stems (five replicates per plant) were determined gravimetrically by drying at 103 °C for 24 h. Soil samples were taken from the centre of the pot without damaging the roots. The relationship between soil water content and soil water potential (measured by the thermocouple psychrometry, C52 sample chambers and HR-33T Dew Point Microvoltmeter, dew point mode, Wescor Inc, Logan, Utah, USA) was determined for the soil used to pot the plants. Xylem water potential was determined on three twigs per plant using the Scholander pressure bomb technique. Transpiration rates were measured (at least ten measurements per plant) using a steady state porometer (Li-1600, Li-Cor, Nebraska, USA).

After reaching the air-dry state the plants were left for a further week and then rehydrated by wetting the soil. Care was taken not to wet the leaves. The procedures described above were repeated at regular intervals during rehydration.

Hydraulic characteristics

Conductivity. Maximum hydraulic conductivity was determined by measuring the flux of degassed, distilled water, which had been passed through a 0.2 μm filter, through excised twigs (Sperry *et al.*, 1988). The twigs were first flushed with the perfusion solution at a pressure of 200 kPa

for approx. 40 min to ensure that all the vessels were functional. Maximum hydraulic conductivity was measured using a pressure head of 2 kPa. Active xylem area was measured after passing 0.1% safranin through the twigs, and the data were expressed as specific conductivity (k_s —conductivity per unit active xylem area) and leaf specific conductivity (k_l —conductivity per unit leaf area supplied). The effect of the pressure gradient on conductivity was determined by moving the supply reservoir up and down flights of stairs. Measurements were made starting from a maximum pressure head of 11.5 m, reducing stepwise to a value of 0.5 m, and then increasing stepwise to the initial pressure head. To ensure that blockages did not occur, a fresh cut was made to the input end of the twig prior to each measurement. All twigs were approx. 20 cm long and 3 mm in diameter (fully hydrated). Maximum vessel lengths of approx. 15 cm were measured according to the methods of Ewers and Fisher (1989).

Vulnerability to cavitation. The vulnerability of the xylem to water-stress induced cavitation was measured using ultrasonic acoustic emissions on five excised, hydrated leafy twigs (approx. 12 cm in length and 5 mm in diameter) using a commercial sensor (Type 8314, Bruel & Kjaer, Naerum, Denmark) and preamplifier (Type 2637, Bruel & Kjaer, Naerum, Denmark) in a set-up similar to that described in Sandford and Grace (1985). The NR mode was used and a threshold of 290 mV was set to give a background of less than one emission per min (EPM). The sensor used was particularly responsive to frequencies at around 800 kHz; this was found to give the best results with *M. flabellifolius*. The bark was stripped off a portion of the twig which was immediately smeared with a gel which prevented local dehydration and increased the acoustic contact between the stem and the sensor. Cumulative acoustic emissions were measured as the twig dehydrated, with measurements being taken every 10 min during periods where the rate was high or every 30 min when emission rate was lower. To determine water content during drying the twig (with the sensor attached) was weighed periodically and the weight of the sensor and dry weight of the twig (103 °C, 24 h) were determined at the end of the experiment. Acoustic emissions were not recorded during weighing as the handling resulted in false emissions. The relationship between twig water content and water potential was determined by measuring the water potential of excised twigs as they dried in a manner similar to the acoustic emission experiments.

Root pressure. Root pressure generated on rewatering of dehydrated plants was measured by attaching a mercury manometer by water filled tubing to stems cut close to the soil surface. Root pressure was measured on two stems of each of three plants.

Capillary rise. This was measured by tracing tritium through detopped excised dry twigs with no leaves with their bases placed in tritiated water (specific activity 1.7 $\mu\text{Ci ml}^{-1}$). Twig segments (1 cm long) were analysed for radioactivity using a Packard 1900TR liquid scintillation analyser (Meriden, CT, USA) after the twigs had been left standing in the labelled water for 24 h.

Recovery of conductance. To characterize the refilling process in more detail, dehydrated twig segments (5%

RWC) were attached to a hydraulic system similar to that described by Borghetti *et al.* (1991). The basal end of the twig segment was connected to an upper reservoir and the distal end to a reservoir 20 cm lower. Both reservoirs were placed on balances connected to a computer to automatically record weight changes every 4 min. The twig segment was sealed in a transparent plastic bag to reduce transpirational water loss and was held horizontal in a fixed position relative to the reservoirs for the duration of each experiment. The position of the twig relative to the upper reservoir was varied between experiments, ranging from 20 cm below (equivalent to a positive pressure of 2 kPa), to 80 cm above (equivalent to a tension of -8 kPa). It was not possible to measure hydraulic refilling at pressure heads greater than 80 cm because of air entry from the twigs into the hydraulic system. A perfusion solution of filtered (0.2 μm pore size) degassed, distilled water was used and room temperature was kept at 20 °C (± 1 °C). Conductivity was determined from the rate of water flow into the lower reservoir. Measurements were taken until conductivity reached a constant value. The twig was then flushed with the perfusion solution for 30 min at 200 kPa, and the maximum hydraulic conductivity for that arrangement of pressure gradients was subsequently measured.

RESULTS

Wood anatomy

The description of wood anatomy is limited to features thought to be important for water conduction. For a more detailed description see Carlquist (1976).

In transverse section the wood of *M. flabellifolius* was diffuse porous with indistinct growth rings. Fibre tracheids were numerous and thick walled (Fig. 1A). Axial parenchyma was absent and the rays were uniseriate (Fig. 1A, arrow). Vessels were generally solitary and concentrically arranged. Diameters ranged from 10.1 to 22.5 μm with no distinct size classes. Mean tangential vessel diameters were 14.4 μm (± 2.1) in dehydrated and 14.0 μm (± 1.3) in hydrated wood with no significant difference ($P > 0.05$) between the wood taken from hydrated and dehydrated twigs (Table 1). Mean vessel density was 128 vessels mm^{-2} . The percentage of conducting surface occupied by vessels was 14%. Vessel element length was 556 μm and the mean tracheid length was 787 μm . Intervessel pit sizes were 'minute', 1–2 μm . Perforation plates were reticulate (Fig. 1B). The combination of narrow vessels, small intervessel pits and reticulate perforation plates probably offers a considerable resistance to water flow.

The outer surface of the vessels and tracheids had unusual knob-like structures (Fig. 1C). These were not reported by Carlquist (1976) and neither the nature nor function of these structures is known. They could not be clearly distinguished by light microscopy and so differential staining could not be used to identify their chemical nature. They appeared to form links between conducting elements and it is possible that they act to 'clip' or bind the various elements together. It was difficult to completely macerate the wood of this species, despite prolonged digestions (72–96 h *vs.* the standard 48 h). If these knob-like structures do hold the

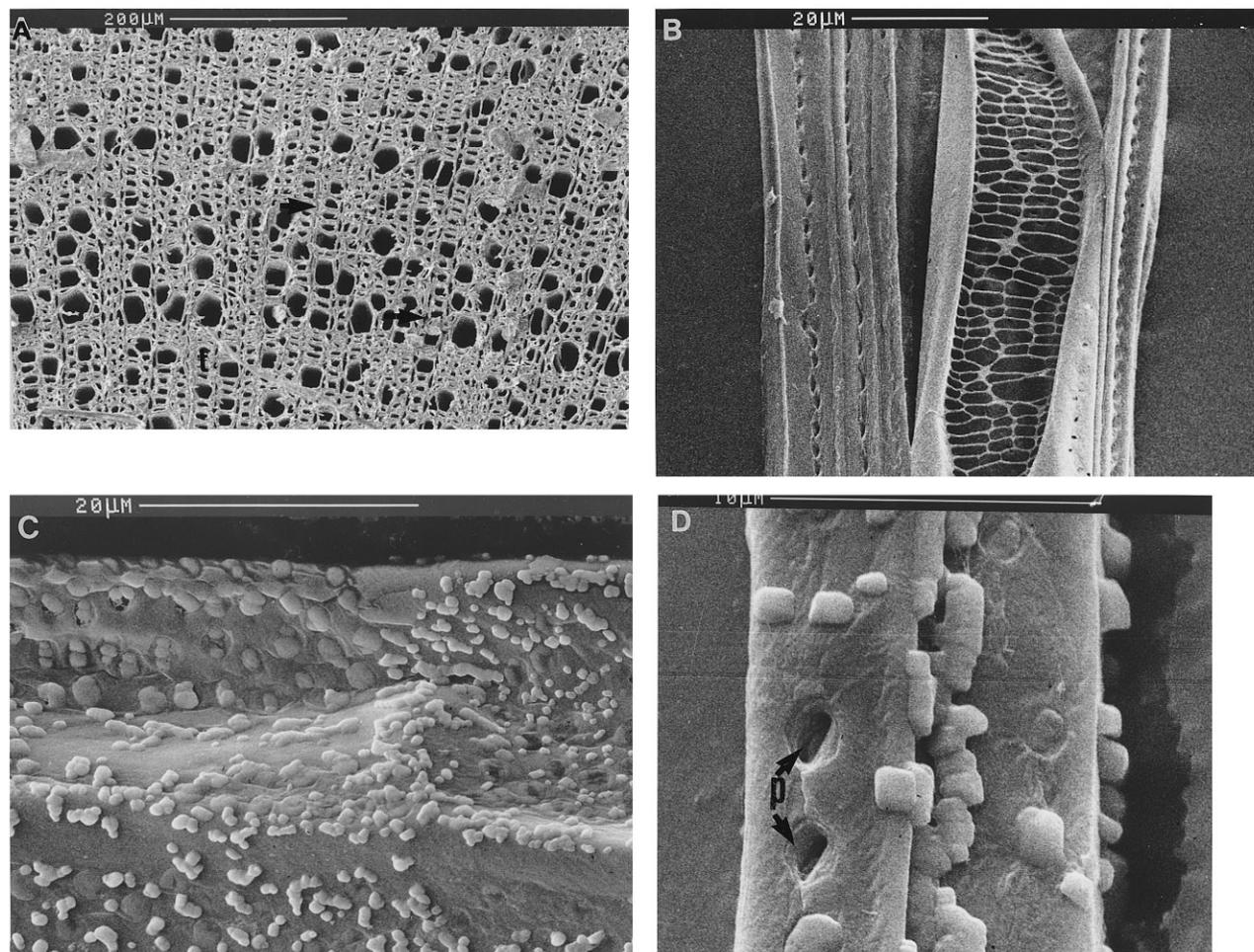


FIG. 1. SEM micrographs of the xylem anatomy of *Myrothamnus flabellifolius*. A, Transverse section of wood. t, Thick walled tracheids; arrows indicate some of the numerous uniseriate rays. B, Modified scalariform (reticulate) perforation plate with no distinct bars just interconnected wall material. C, Knobbed outer surface of a xylem element. D, These knobs appear to connect to xylem elements together. p, pits.

TABLE 1. Quantitation of wood anatomy features and stem diameter changes of twigs *M. flabellifolius*

Feature	Measurement (mean \pm s.d.)
Vessel diameter	
Hydrated (μm)	14.1 (\pm 2.1)
Dry (μm)	14.5 (\pm 1.3)
Vessel density (mm^{-2})	128 (\pm 28)
Percentage of conducting area covered by vessels (%)	14 (\pm 3.4)
Pit diameters (μm)	1 (\pm 0.2)
Vessel elements lengths (μm)	556 (\pm 35)
Tracheid lengths (μm)	787 (\pm 136)
Dehydration stem diameter (% of hydrated control)	75 (\pm 3)
Rehydrated stem diameter (% of hydrated control)	97 (\pm 7)

Vessel diameters and densities were measured on transverse sections while vessel and tracheid lengths were measured on macerated tissue. The shrinkage and recovery in stem diameters with dehydration and rehydration were measured on two twigs each of three different plants.

conducting elements together, these difficulties might be explained. During natural drying, the stems of *M. flabellifolius* shrunk to 75% of their original diameter. On rehydration they regained 97% of their original diameter within 48 h of rewatering. This regular shrinkage and expansion would place considerable stress on the elements of the wood.

Water relations of whole plants

Dehydration. The dehydration time course of a single *M. flabellifolius* plant is shown in Fig. 2. Soil water content declined over the first 6 d and only slightly thereafter. Leaf and stem water contents showed an initial slow decline over the first 7 d, followed by a sharp drop, with little further water loss thereafter. The decline in soil water content preceded the drop in leaf and stem water content. Leaf water potentials varied between -1.1 and -1.9 MPa until leaf water content dropped sharply. At this point leaf water potential showed a similar drop: one value of -2.4 MPa and another of -2.9 MPa were measured before values

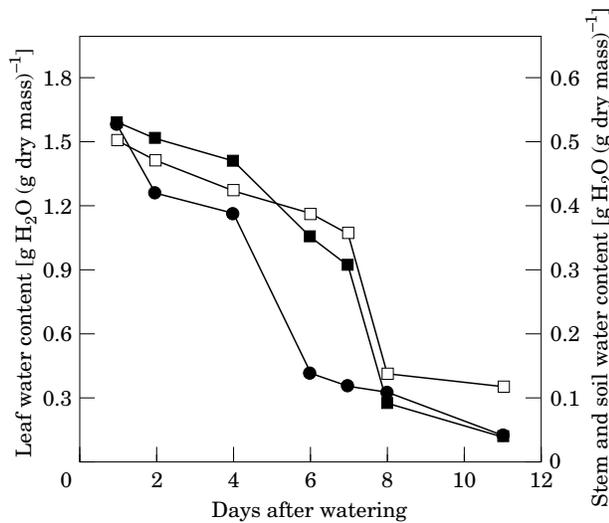


Fig. 2. Dehydration time course for an individual plant of *Myrothamnus flabellifolius*. Soil (●), stem (□) and leaf (■) water contents are measured against time in days.

dropped to below -7 MPa, the lowest value that could be measured with the equipment available. Transpiration rates and leaf and stem water contents (Fig. 3) remained relatively high until the soil water content dropped below 0.15 to 0.1 g H₂O (g dry mass)⁻¹ (hereafter referred to as g g⁻¹). The relationship between soil water potential and water content was typical of that for a sandy soil, with water potentials remaining high until a water content of about 0.12 g g⁻¹, declining precipitously with further water loss (data not shown). The decline in plant water status coincided with the steep drop in soil water potential and was associated with the folding up of the leaves against the stem.

Rehydration. Rehydration of the desiccated plants took 24 to 48 h, depending on the size of the plant. Water appeared to move up the stem in a column rather than through a few xylem vessels at a time: when measuring water contents, values were either very low (above the hydration front) or at maximum values with a corresponding water potential of zero (below the hydration front). The leaves hydrated very rapidly, increasing from 5 to 65% RWC in 2 h, once the hydration front reached them. Once water was delivered to the leaves they started to uncurl and transpiration started almost immediately.

The distal portions of some stems on the whole plants did not rehydrate within the 48 h period. When these portions were excised and the bases placed in water they hydrated within 12 h, suggesting that failure to rehydrate was not due to permanent xylem dysfunction. This continued rehydration occurred irrespective of whether the twigs were excised above or below the hydration front, indicating that the phenomenon was not a consequence of localized blockages. There was no consistency in the length of a stem upon which the tips failed to rehydrate.

Hydraulic characteristics

Conductance. Maximum specific (k_s) and leaf specific conductivities (k_l) were found to be 0.87 kg m⁻¹ MPa⁻¹ s⁻¹

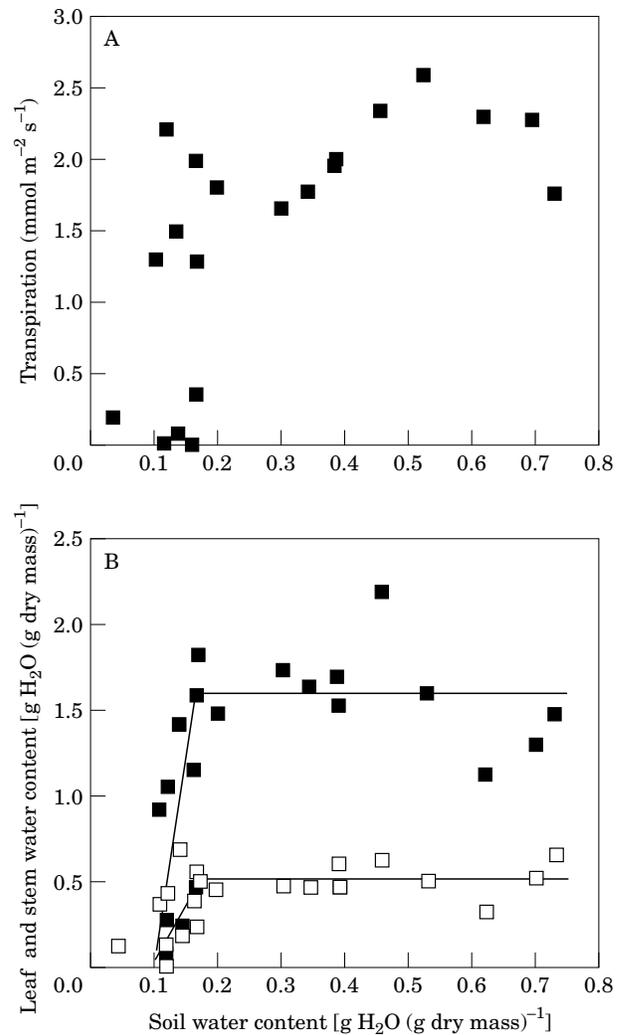


Fig. 3. Relationship between transpiration rate (mmol m⁻² s⁻¹) and soil water content [g H₂O (g dry mass)⁻¹]. Relationship amongst leaf (■), stem (□) and soil water content all measured in g H₂O (g dry mass)⁻¹. Trend lines were fitted by eye.

TABLE 2. Various hydraulic and water relations parameters of *M. flabellifolius*

Feature	Measurement (mean ± s.d.)
Maximum specific conductivity (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	0.87 (±0.21)
Maximum leaf specific conductivity (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	3.28×10^{-5}
Maximum transpiration (mmol m ⁻² s ⁻¹)	2.55 (±0.2)
Water content at 50% cumulative acoustic emissions (MPa)	-2.5
Root pressure (kPa)	2.4 (±0.2)
Capillary rise	
Measured (cm)	> 27
Theoretical (cm)	212

and 3.28×10^{-5} kg m⁻¹ MPa⁻¹ s⁻¹, respectively (Table 2). These low values are probably a consequence of the narrow vessel diameters and reticulate perforation plates between

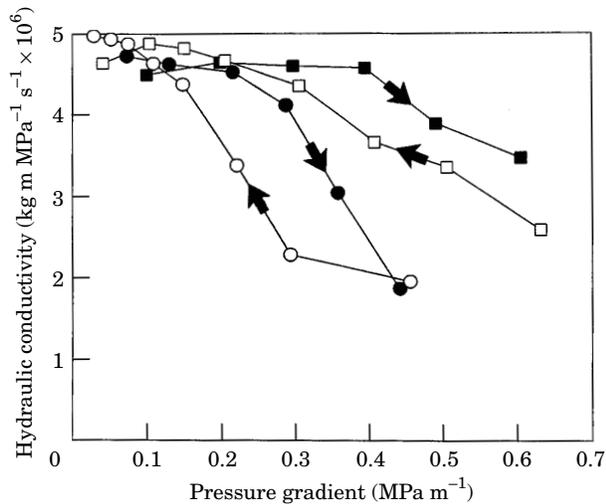


FIG. 4. The influence of pressure gradient on the hydraulic conductivity of two twigs of *M. flabellifolius*. Measurements were made at decreasing and then increasing pressure gradients. Arrows along the curves indicate the direction in which the pressure gradient was changed. ■, Twig 1 increasing pressure gradient; □, twig 1 decreasing pressure gradient; ●, twig 2 increasing pressure gradient; ○, twig 2 decreasing pressure gradient.

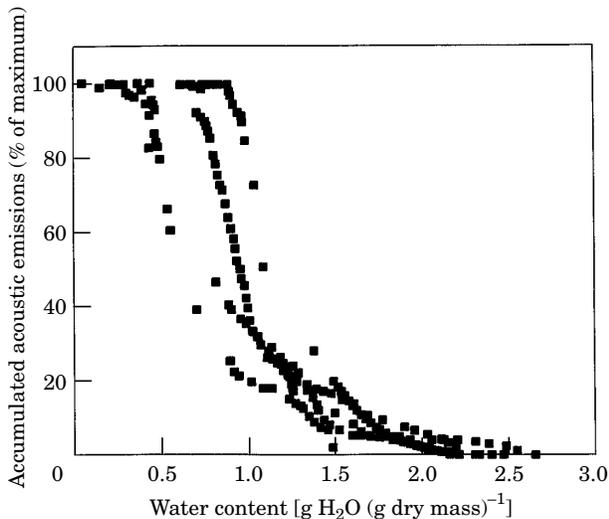


FIG. 5. Cumulative acoustic emissions of five twigs of *Myrothamnus flabellifolius* during dehydration. Data for each twig were expressed as a percentage of total emissions.

vessel elements. Hydraulic conductivity decreased as the applied pressure gradient increased (Fig. 4). An actively transpiring twig of *M. flabellifolius* will have a water potential in the region of -1 to -2 MPa, giving rise to pressure gradients (depending on the length of stem) of around 1 to 2 MPa m^{-1} . Thus it is likely that a transpiring plant will have a conductivity lower than the measured maximum.

Vulnerability to cavitation. Acoustic emission data (Fig. 5) showed that marked increases in emissions occurred when twig water content declined below 1.8 to 1.4 g g^{-1} . The twig water potentials corresponding to this range of water

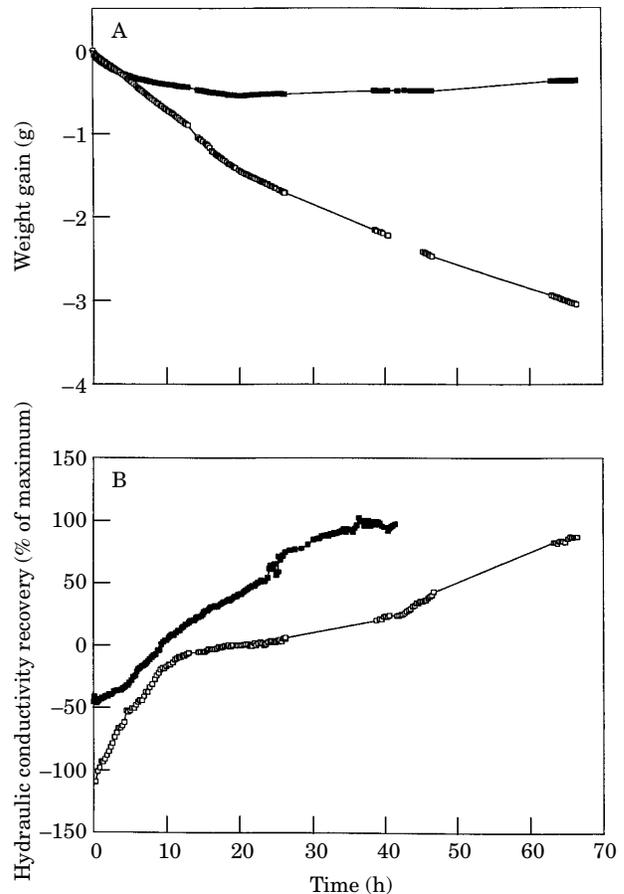


FIG. 6. Characterization of the refilling of twigs of *Myrothamnus flabellifolius*. A, Recovery of water flow as a function of time during the refilling of previously embolized twigs, illustrated as accumulated weight gain of water from the source (■) and sink (□) reservoirs. B, Time course for conductivity recovery for a twig under positive pressure (■) and a negative tension of 8 kPa (□). The pressure gradient between reservoirs was 2 kPa in both cases.

contents were -2 to -3 MPa, similar to the water potential at which the sharp decrease in leaf and stem water content was initiated. This suggests that at water potentials in this range a runaway embolism cycle occurred, which resulted in virtually all of the vessels becoming embolized below these water contents. This would cause an interruption of water supply to the leaves leading to the sharp drop in leaf water content observed.

Xylem refilling. Positive root pressures of only 2.4 kPa were measured on rehydrating plants (Table 2). This is only adequate to force water some 24 cm up an empty stem and alone is insufficient to refill the xylem of *M. flabellifolius*. Taking a mean vessel diameter of 14 μ m, the height water could attain by capillary rise in *M. flabellifolius* is 2.12 m (Table 2), and capillary rise to heights greater than this would be possible in narrower vessels. Experiments with twigs cut at both ends and the basal end placed in radioactively labelled water showed that water could rise to a height of 27 cm (the maximum twig length available when the experiments were conducted). This suggests that capillarity together with root pressure would provide the

potential necessary to refill the conduits in *M. flabellifolius*, a shrub which never exceeds 2 m in height.

A typical example of hydraulic refilling for *M. flabellifolius* is illustrated in Fig. 6. This pattern of hydraulic recovery was representative of all the experiments undertaken over a range from a positive pressure of 2 kPa to a tension of -8 kPa, differences being observed only in the time scale over which refilling occurred. Initially water was taken up at both ends of the twig with uptake from the lower balance being greater than that from the upper balance (Fig. 6A). This period of 'negative' conductivity ranged from 8 h when the twig was under positive pressure of 2 kPa, to 20 h when the twig was under a tension of -8 kPa. Even though attempts were made to keep evaporation from both the twig and the reservoirs at a minimum, the rate of inflow always exceeded the rate of out flow. The dry twigs reached maximum conductivity in 33 h when the water was under positive pressure (2 kPa) and after 70 h when the water was under a tension of -8 kPa (Fig. 6B). During these refilling experiments the leaves were observed to unfold and appear turgid shortly after conductivity became positive, some time before the establishment of maximum conductivity. Although the stem water contents measured during the rehydration experiments indicated that water moved up the stem in an 'hydration front', it appears that this front did not refill all the xylem vessels since there was some delay before the re-establishment of maximum conductivity.

DISCUSSION

Plants of *M. flabellifolius* occur in shallow soils and when soil water is depleted the plant dries and comes into equilibrium with ambient relative humidity. This dehydration implies total xylem embolism.

The tension developed in the water column in xylem conduits depends upon the transpiration rate. It has been suggested that a function of stomata is to prevent transpiration rates from becoming so high as to lead to tensions that can initiate catastrophic embolism cycles (Sperry and Pockman, 1993). This does not appear to be the case with *M. flabellifolius*: transpiration rates and leaf and stem water contents remained close to those in a fully saturated soil until the soil water had been virtually depleted. Although it is difficult to measure quantitatively, leaves appeared to start folding slightly before the initiation of massive acoustic emissions, continued drying during the peak of emissions, and became totally folded and appressed to the stem only after the emission rate had declined. The increase in acoustic emissions from stems occurred at a water potential similar to that at which leaf and stem drying occurred. Because of the desiccation tolerance of the tissues of *M. flabellifolius*, there is no advantage to the plant in either conserving water or preventing extensive embolisms. Rather, the plants utilize all available water, undergo embolisms and survive the consequent dehydration stress until water is again available.

Vulnerability to cavitation was measured on excised stem segments and the above interpretation assumes that it is embolisms in the stems that interrupt the water supply to

leaves. It has been pointed out recently that roots may be more vulnerable to cavitation than stems (Alder, Sperry and Pockman, 1996; Kolb, Sperry and Lamont, 1996) although if this is the case, the likelihood of cavitation in roots as opposed to stems will depend upon the water potential of roots relative to stems. Similarly, the soil-root interface in drying (particularly sandy) soils could become a significant resistance in the water flow pathway (discussed by Weatherly, 1976) and may be as important as xylem embolisms. Nothing is known of the vulnerability to cavitation of roots of *M. flabellifolius*, or of the resistances to water flow that might occur at the root-soil interface. However, the observation that in whole plants it is the distal portion of the twigs that dry first suggest that if embolisms do lead to leaf drying, these embolisms occur in the stem, and are initiated in the distal parts. This is in keeping with the hydraulic segmentation hypothesis of Zimmermann (1983) as well as the vulnerability segmentation shown to occur in *Juglans regia* (Tyree et al., 1993).

Stem tissues of *M. flabellifolius* survive repeated desiccation and rehydration and although there is no change in the diameter of the vessels there is some shrinkage and swelling of the stem. These cycles of alternate shrinking and swelling may place considerable physical stresses on the wood, which could cause it to split. Unusual knob-like protuberances were found on the external walls of vessels and tracheids. It was difficult to completely macerate the wood of this species, taking 72–96 h, rather than the usual 48 h. It is not known whether these protuberances were responsible for this unusual toughness, but if so, their role may be to provide the wood with the physical characteristics to enable it to withstand repeated shrinking and swelling stresses.

Apart from these knob-like structures, the wood of *M. flabellifolius* has no unique features. It does, however, have many characteristics which would normally be considered 'safety features' e.g. narrow xylem vessels with a high density of vessels, presence of thick-walled tracheids, reticulate perforation plates and small intervessel pits which are typical of woods in which severe tensions may develop as a consequence of low water availability (reviewed by Tyree, Davis and Cochard, 1994). Despite these features, massive cavitation events are initiated in *M. flabellifolius* at water potentials between -2 and -3 MPa. *M. flabellifolius* is thus not particularly resistant to cavitations (see the range of values cited in the review by Tyree and Ewers, 1996). The air-seeding mechanism of cavitation suggests that the size of pores in intervessel pit membranes is the critical anatomical feature determining vulnerability to cavitation (Zimmermann, 1983; Sperry and Tyree, 1988). The intervessel pits of *M. flabellifolius* are minute (1–2 µm), however, the vulnerability of the xylem to cavitation suggests that the pores are not particularly small. The presence of reticulate perforation plates has been suggested to contribute to the mechanical strength of xylem vessels required to withstand the physical stresses associated with high xylem tensions (Carlquist, 1976). Because of the vulnerability of the xylem of *M. flabellifolius*, excessive tensions do not occur in the xylem water column. The 'safety' features observed in the xylem anatomy of this species may have little functional

significance and may simply reflect its evolutionary history of which little is known.

The narrow vessels and perforation plates of the xylem of *M. flabellifolius* give rise to specific and leaf specific hydraulic conductivities which are lower than those reported for most angiosperms (reviewed by Patiño, Tyree and Herre, 1995 and Tyree and Ewers, 1996). In their review of hydraulic properties of a range of tree species, Patiño *et al.* (1995) normalized data to branch diameters of 15 mm because of variation in conductivities with branch size. The values presented here for *M. flabellifolius* are for stem diameters of 3–5 mm. Nothing is known of the relationship between conductivity and stem diameter in *M. flabellifolius*, but stems rarely exceed 7 mm in diameter. The only other angiosperm with similarly low conductivities is *Clusia uvitana* ($k_s = 1.1 \text{ kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$ for stem diameter 15 mm; Zott, Tyree and Cochard, 1994). Despite the moderate to low transpiration rates of well watered plants (compared with values of 12 to 15 $\text{mmol m}^{-2} \text{ s}^{-1}$ for co-occurring savanna trees; Scholes and Walker, 1993) and the comparatively short transport pathway, the low leaf specific conductivities of *M. flabellifolius* gave rise to relatively low leaf water potentials.

The hydraulic conductivity of fully hydrated wood was shown to decrease with increasing pressure gradient. This effect has previously been reported in some gymnosperms (Sperry and Tyree, 1990) and was ascribed to the increased pressure forcing the torus of the pit membrane closed. As far as we are aware this is the first report of the phenomenon in an angiosperm. The maximum pressure gradient developed in the experiments was less than would occur in a transpiring hydrated plant, so that the actual conductivity under these conditions may be less than the maximum measured. Although mean vessel diameters do not change on dehydration and subsequent rehydration, there is swelling and shrinkage of the stem. It is not known whether this is in anyway related to the influence of pressure head on conductivity. It would require only small changes in the diameters of the widest vessels to generate the effect on conductivity that was observed.

On rewatering, the xylem tissue of *M. flabellifolius* refills readily. Positive root pressures have been measured in wild grape vines (Sperry *et al.*, 1987), *Betula* and *Alnus* spp. (Sperry *et al.*, 1994) and Tyree and Ewers (1996) have reviewed the occurrence of positive root pressures in tropical species. Although this may play a role in refilling of some of those species, generally the root pressures developed in woody species are considered to be inadequate to bring about refilling. Certainly, the root pressure generated on rewatering of *M. flabellifolius* (2.4 kPa) is inadequate to provide the driving force for refilling in this species.

Capillary rise to a height of 2 m is possible in tubes with a diameter of 14 μm , the mean vessel diameter. The reticulate perforation plates could also reduce the meniscus radius and contribute to the capillary effect. Using tritiated water, capillary rise to the height of the longest twig available was demonstrated and refilling of an excised dry twig by capillary forces could be demonstrated when the water was under a tension of -8 kPa (the highest tension that could be developed). Refilling from the air-dry state has not been

demonstrated previously, although the model of Yang and Tyree (1992) does permit this. In *M. flabellifolius*, root pressure, although low, will provide water at the base of the stem on which capillary forces can act. It thus appears that capillary forces are adequate to account for the refilling of dehydrated stems of *M. flabellifolius*. During the refilling experiments water uptake by both ends of the twig occurred. This has been reported previously by Borghetti *et al.* (1991) and Edwards *et al.* (1994) for *Pinus sylvestris*, but it is not known how common this is. During these experiments, the weight loss from the upper balance always exceeded the weight gain by the lower balance. A similar phenomenon was noted by Tyree and Yang (1992), who ascribed it to transpirational loss of water from the twig. In the experiments with *M. flabellifolius*, the twigs were enclosed in plastic bags to reduce transpiration, but condensation was always noted on the inside of the bag, suggesting a similar process was occurring.

When dehydrated whole plants were rewatered the distal portions of some twigs failed to rehydrate. This could not be a consequence of localized blockages or damage to the xylem because if these twigs were excised and their bases placed in water, they completed rehydration irrespective of whether they were excised above or below the hydration front. The reason for this behaviour is unknown.

M. flabellifolius differs from other resurrection plants in that it has a woody stem. There appears to be little stomatal control of water loss as the soil dries, and extensive xylem cavitation results. The drought survival strategy of this species involves desiccation tolerance of cellular constituents and rapid recovery of the water conducting pathway after rewatering.

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