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Seasonal changes in hydraulic conductance, xylem embolism and leaf area in *Eucalyptus tetrodonta* and *Eucalyptus miniata* saplings in a north Australian savanna

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ABSTRACT

Eucalypt saplings in north Australian savannas commonly die back, sometimes to ground level, during the 5 months of the long dry season. Water potentials are lower in saplings than large trees during the dry season, and we hypothesized that low water potentials may lead to high levels of xylem embolism and consequent death of branches and whole shoots. As the dry season progressed, hydraulic conductance of terminal branches decreased by 50% in *Eucalyptus tetrodonta* but not in *Eucalyptus miniata* saplings. Hydraulic conductance per leaf area decreased seasonally by 34% in *E. tetrodonta* branches. These decreases may be associated with the loss of leaves recorded from *E. tetrodonta* but not *E. miniata* branches. We modelled the effect of sequential loss of parallel resistors, representing petioles on a branch. This showed there is a non-linear decrease in flow as basal resistors are lost, which can lead to a decrease in mean flow per resistor due to increased mean path-length. Thus the observed loss of basal leaves, together with xylem embolism, probably contributed to the seasonal loss of hydraulic conductance in *E. tetrodonta* saplings. Loss of hydraulic conductance due to xylem embolism was generally low (< 15%) in both species, and the seasonal increase in embolism could not fully account for the decline in hydraulic conductance of *E. tetrodonta* branches. There was little evidence that branch and shoot death was caused by these levels of embolism. Developing an embolism vulnerability curve for species with long vessels is problematic and this issue is discussed.

Key-words: *Eucalyptus*; drought stress; evergreen; path-length; tropics; vulnerability curve; water potential.

INTRODUCTION

The evergreen tree *Eucalyptus tetrodonta* F. Muell is dominant over large areas of north Australian savannas (Brock 1993). Mature trees maintain high leaf water potentials and high rates of assimilation, transpiration and leaf flushing during the dry season (Myers *et al*. 1997; Williams *et al*. 1997b; Myers *et al*. 1998; O'Grady, Eamus & Hutley 1999; Eamus *et al*. 1999). However, in saplings, leaf water potentials, stomatal conductance, transpiration and assimilation rates all decline (Prior, Eamus & Duff 1997; Prior & Eamus 1999). Saplings may lose more than 80% of their leaves by the end of the dry season, and often die back to ground level (Prior *et al*. 1997). Nine of 25 tagged saplings died back to ground level during 2 years of study, although all subsequently resprouted from the base of the main stem or from the lignotuber (Prior, unpublished).

Eucalyptus miniata Cunn. ex Schauer is an evergreen tree often codominant with *E. tetrodonta* (Brock 1993). Mature trees of the two species have similar leaf water potentials, stomatal conductance and rates of assimilation and transpiration and patterns of growth throughout the year (Myers *et al*. 1997; O'Grady *et al*. 1999; Eamus *et al*. 1999). However, *E. miniata* saplings may grow faster and more consistently than *E. tetrodonta* saplings. In an unburnt savanna, average increase in height over 3 years for saplings 1–3 m high was 0·81 m for *E. miniata*, compared with –0·47 m for *E. tetrodonta* (R. J. Williams, unpublished).

We hypothesized that the widespread death of branches and shoots of *E. tetrodonta* saplings may be due to xylem embolism induced by seasonal drought stress. High mortality rates of *Adenostoma fasciculatum* seedlings during severe drought were associated with very high levels of xylem embolism and extremely low leaf water potentials (Williams, Davis & Portwood 1997a). Seasonal increases in xylem embolism have been demonstrated in many woody species (see Eamus & Prior 2000) including deciduous trees of Venezuelan dry tropical forests (Sobrado 1997), but we are unaware of any studies in Australian savannas.

Vulnerability curves (which present the relationship between percentage loss of hydraulic conductance due to xylem embolism, L_x, and water potential) can be used together with information about xylem water potentials to predict the loss of conductance in terminal branches in field-grown plants. However, it is difficult to derive

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vulnerability curves for plants with long xylem vessels (Cochard & Tyree 1990). Maximum vessel length in eucalypts may exceed 1 m (Skene & Balodis 1968; Franks, Gibson & Bachelard 1995; Pammenter & Vander Willigen 1998). Pammenter & Vander Willigen (1998) were able to construct vulnerability curves for several *Eucalyptus* clones only by using 5 m long branches. This is not possible for small saplings, and we were forced to develop an alternative method, described below.

For this study we monitored leaf water potentials, hydraulic conductivity, L_x and leaf area of terminal branches of *E. tetrodonta* saplings during two dry seasons. In the second season, *E. miniata* saplings were included for comparison, and survival of tagged leaves and branches was also recorded. Relationships between L_x and water potential were compared for intact field-dried and excised laboratory-dried *E. tetrodonta* branches.

MATERIALS AND METHODS

Experimental sites

The experimental site for the 1996 study was an area of native savanna at the Tropical Ecosystems Research Centre, Berrimah, 10 km east of Darwin, in northern Australia (see Prior *et al*. 1997 and Myers *et al*. 1998). In order to include enough *E. miniata* saplings, the 1998 study used both this site and native savanna at the Palmerston campus of the Northern Territory University, 14 km east of Darwin. Both sites had been unburnt for many years.

Temperatures are high year round, with monthly means for Darwin ranging from 24·9 °C in July to 29·2 °C in November. Rainfall is highly seasonal. Ninety-seven per cent of Darwin's annual average 1672 mm rain falls between October and April inclusive; June, July and August are virtually rainless (Commonwealth Bureau of Meteorology, Australia 1998). Rainfall records were obtained for Darwin Airport and Palmerston from the Bureau of Meteorology.

Growth and survival of shoots, branches and leaves

In 1998, shoot growth and survival of shoots, terminal branches and leaves was compared in *E. tetrodonta* and *E. miniata* saplings between 1 and 3 m high. We use the term 'shoot' to refer to the above-ground part of a sapling. Forty saplings (32 at Palmerston and eight at Berrimah) of each species were tagged. Sapling height and diameter of the main stem at 1·0 m height were measured in May (start of dry season) and October (end of dry season). Numbers of original and new leaves on three tagged terminal branches per sapling were recorded monthly between May and October. In July 1998, wildfire passed through a section of the Palmerston campus and destroyed 11 saplings each of both species. These saplings were excluded from the analysis, as were several branches that suffered obvious mechanical damage.

Sampling procedure for hydraulic conductance and studies of L_x

One terminal branch was sampled from each of eight *E. tetrodonta* saplings in May, July and September 1996 for determination of hydraulic conductance/conductivity and loss of conductance due to xylem embolism. (Each sapling can have multiple secondary, tertiary and quaternary branches, and thus more than one terminal branch). A different set of saplings was used each month. In 1998, terminal branches were sampled from *E. tetrodonta* and *E. miniata* saplings in May, June, July, August and September. Two saplings of each species were chosen from the Berrimah site, and six from the Palmerston site. Branches were collected before 0900 h, to avoid the time of day when water stress was most severe.

When measuring hydraulic conductance, most investigators initially cut the branch in air. They then recut under water from each cut end a segment longer than the maximum vessel length to excise artificially embolized xylem (e.g. Cochard & Tyree 1990; Magnani & Borghetti 1995; Kolb, Sperry & Lamont 1996). Maximum vessel length in *E. tetrodonta* saplings was measured by passing low-pressure air through freshly cut shoots and branches. Material was progressively shortened until bubbles emerged from one end which was held under water (Franks *et al*. 1995). Maximum vessel length was 0·74 to 1·04 m in shoots that were 1·07 to 1·36 m long, and 0·36 to 0·44 m long in terminal branches 0·41 to 0·62 m long. It was therefore necessary to make the initial cut under water, and this was done by placing the branch in a water-filled tray before cutting it with secateurs. Four branches were sampled each day. They were immediately placed upright in a bucket containing about 4 cm water, and covered with a dark plastic bag to minimize transpiration during the 30 min drive to the laboratory. Measurements of hydraulic conductance commenced immediately on two branches, and about 3 h later on the remaining branches, which were kept upright with their cut ends in water. There were no differences in mean L_x between branches measured immediately and those measured 3 h later.

Measurements of hydraulic conductance and L_x

The method for measuring hydraulic conductance of terminal branches was based on that described by Kolb *et al*. (1996) and Williams *et al*. (1997a). Branches were placed under water, and leaves were cut from petioles and the basal end of the branch was recut using a sharp razor blade. The basal end was then quickly inserted through the seal in the vacuum chamber lid and attached to graduated 5 mL pipettes via flexible tubing filled with degassed, filtered (0·2 μm), acidified (pH 2, HCl; Sperry *et al*. 1994) water. Unlike Williams *et al*. (1997a), we did not cut the distal end, since the normal pathway for sap flow is through petioles. The diameter of the basal end of the branch was measured with callipers, and the length from cut end to tip measured with a ruler. Leaf area distal to the cut end was measured with a leaf area meter (Delta-T Devices Ltd, Cam-

bridge, UK). The vacuum chamber was constructed from PVC pipe and was 1·05 m long, 160 mm in diameter and could accommodate two branches. The solution was pulled through the branch in the same direction as the normal transpiration stream by applying a vacuum to the chamber with a vacuum pump. Flow rate was measured at four levels of vacuum (pressure difference, ΔP, = 20, 30, 40 and 50 kPa), measured with a vacuum gauge (Leybold, Germany). Generally 10–30 mins were required to reliably establish flow rates at each pressure difference. Hydraulic conductance (g s^{-1} MPa^{-1}) was calculated as the slope of the relationship between flow rate and pressure difference using linear regression (SigmaPlot 4, Jandel Scientific, California, USA). Hydraulic conductance was also expressed on the basis of leaf area or transverse area of the branch at the proximal end of the cut (g s^{-1} MPa^{-1} m^{-2}). We avoid the terms 'leaf specific conductance' and 'specific conductance' since, within the SI system, 'specific' is exclusively reserved to describe a value per unit mass (Edwards, Becker & Čermák 1996). Huber values were calculated as the branch transverse area per leaf area, with leaf area being determined at the time of sampling. This will overestimate the Huber value if significant leaf loss had occurred prior to sampling, but the data we present are from May 1998, when minimal leaf loss had occurred.

Hydraulic conductivity (g m s^{-1} MPa^{-1}) was calculated by multiplying hydraulic conductance by branch length, and was also expressed on a leaf area and a branch transverse area basis. Trends were generally similar to those in hydraulic conductance, but variability was higher. We consider it more appropriate to present trends in hydraulic conductance rather than conductivity. When a branch is enclosed within a partially evacuated chamber, pressure gradients within the branch will vary. Average pressure gradients will be steeper between the cut end of the branch and the cut ends of basal petioles than between the cut end of the branch and the cut ends of distal petioles, because the path length is shorter in the former case. This may be why there was no correlation between hydraulic conductance and branch length.

The initial measurement of hydraulic conductance was followed by a 30–60 minute perfusion of acidified, filtered, degassed water at a positive pressure of 175 kPa to remove air emboli, where present (Williams *et al*. 1997a). Compressed nitrogen was used to pressurize a reservoir (2 L) of the perfusing water to 175 kPa. This reservoir was connected to the branches via tubing. Pressure was released for 10 min before readings were resumed, to allow tubing and xylem vessels to relax. The measurement of hydraulic conductance was then repeated. The percentage difference between the initial and final values was considered to represent the percentage loss in conductance due to embolism (Williams *et al*. 1997a).

Modelling loss of resistors

A simple electrical model of a series/parallel resistor network was used to represent the branch and attached petioles as a sequence of series (stem xylem) and parallel (petiole) resistors (Fig. 1). The effect of sequentially removing petioles from either the basal or the apical end was then investigated by calculating total flow for the branch, and as mean flow per remaining petiole. (At any given ΔP, flow is directly proportional to hydraulic conductance.) Flow was expressed as a dimensionless relative value. We examined two cases (i) where resistance of petiole pairs was 10 times that of stem segments, and (ii) where resistance of stem segments was 10 times that of petiole pairs. We assumed complete sealing of the branch where the petiole was lost, as happens with natural abscission of leaves and petioles.

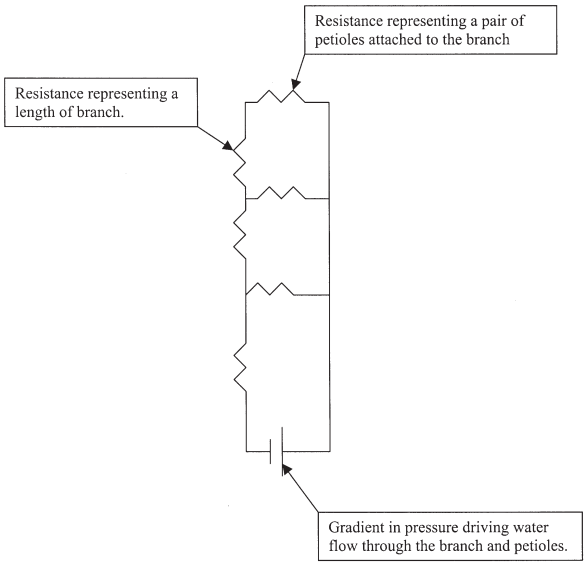


Figure 1. Schematic diagram of the model representing the branch and attached petiole pairs as a sequence of series (branch xylem) and parallel (petiole xylem) resistors. Resistance was assumed to be equal for all petioles, and resistance of all stem segments was also assumed to be equal. Two ratios of stem segment resistance to petiole pair resistance were used in the model, namely 10 : 1 and 1 : 10. For simplicity we show three resistors in parallel representing three pairs of petioles, but in the model analysis we also used five, seven and nine pairs.

Measurement of water potential

For the 1996 measurements, predawn leaf water potentials were used as a measure of plant water status. One leaf was sampled from another branch of each sapling on the morning following measurement of embolism, and its water potential was determined using a pressure chamber (Soil Moisture Equipment Corporation, California, USA). In 1998, midday water potentials were determined as an indication of the seasonal minimum water potential experienced by the sapling. Between May and September, most days are sunny with little day-to-day variability in temperature, and significant rainfall is highly unusual, so there is a consistent, progressive decrease in leaf water potentials (Prior *et al*. 1997; Prior & Eamus 1999). At the end of the week of the hydraulic conductance measurements, one leaf

per sapling was wrapped in a plastic bag, then enclosed in foil. This was done before 0900 h, when plant water potentials are still high (Prior *et al*. 1997). Leaf water potential is minimal and stable between 1130 hours and 1330 hours (Prior *et al*. 1997). During this period, the wrapped leaves, and a similar non-wrapped leaf from each sapling, were cut for determination of xylem water potential and leaf water potential, respectively.

Vulnerability curves

The long vessels in *E. tetrodonta* saplings presented difficulties in developing vulnerability curves using excised artificially dehydrated material. Even when precautions were taken, we suspect that many more vessels were embolized than in intact naturally dehydrated material at similar water potentials, as described below. However, it appeared possible to easily refill these vessels, leaving residual embolism similar to that expected in naturally dehydrated material. We adopted the following procedure to develop a vulnerability curve for excised, laboratory-dried, *E. tetrodonta* saplings that appeared to coincide with that for intact field-dried material, although variability was high for both data sets. A total of seven *E. tetrodonta* saplings between 1 and 1·5 m high were used for the excised laboratory-dried curve.

Water potential was determined on one leaf immediately before collecting each sapling. We found that branches could be embolized by cutting leaves for determination of leaf water potentials, so we always avoided sampling leaves from branches on which hydraulic conductance was to be measured.

The sapling was cut off under water near ground level by placing a split bucket, lined with a plastic bag, around the base. The bucket was filled with water, and the sapling was cut with secateurs and immediately placed in an intact bucket of water and brought into the laboratory. The cut end was wrapped in parafilm, moist paper towelling and a plastic bag, and the sapling was placed in a large plastic bag, partially open to allow gradual drying. This was to avoid possible problems associated with rapid drying and development of unnaturally large water potential gradients. Saplings were allowed to dehydrate for 1–6 d to water potentials between –2·1 and –5·4 MPa. The value of water potential to which the excised sapling declined was always lower than the water potential observed when measured on leaves sampled from another branch (a branch not used for measurements of conductance) at the time of removal of the whole sapling. The bag was then sealed overnight to allow water potential to equilibrate throughout the sapling, and the water potential of two to six leaves was measured next morning. Two terminal branches were then cut under water and placed in the vacuum chamber for determination of hydraulic conductance. We selected terminal branches that were further than the maximum vessel length distant from the cut end of the shoot. If there were additional suitable branches remaining on the shoot, the shoot was allowed to dry further and these branches were sampled on a later day.

In these excised laboratory-dried branches, flow rates increased at constant vacuum, even for ΔP = 10 kPa. This did not occur in branches that dried naturally on intact plants through the dry season unless they were severely embolized. Flow rates continued to increase for 1 to 4 h and then stabilized. Once stable flow rates were achieved at ΔP of 10–20 kPa, flow rates were constant at constant ΔP of 30, 40 and 50 kPa. Hydraulic conductance could thus be reproducibly measured once this refilling had occurred. We developed a vulnerability curve by applying a constant vacuum (usually 50 kPa, since this was faster than using a ΔP of 20 kPa) until constant flow was achieved, then measuring hydraulic conductance before and after pressure perfusion. Branches were then perfused with solution at 175 kPa pressure, which led to an increase in hydraulic conductance. These findings suggest that there were two distinct classes of air-filled vessels. One class could be very easily refilled by modest vacuum (ΔP = 10 kPa), and appeared to be generated when an excised shoot was dehydrated (by contrast with the field situation where the xylem and root system are intact). Refilling of these vessels presumably involved simple displacement of air by water (Yang & Tyree 1992). The second class was only refilled by pressures in the order of 175 kPa, and appeared to correspond to embolized vessels in field-dehydrated branches. Refilling of these vessels probably required dissolution of bubbles (Yang & Tyree 1992).

Statistical analyses

Significance of differences between species in survival of shoots and branches was determined using a χ^2 test. Differences in growth between species were tested using a *t*-test.

Analysis of variance was performed using Statistica version 5·0 (Statsoft, Tulsa, OK, USA) to test for effects of month, species and year on branch hydraulic parameters and leaf area per branch. Two sets of analyses were performed: one set used all the 1998 data, to establish effects of species and month for both species separately in 1998. The other set of analyses used the *E. tetrodonta* data for May, July and September of both years, since 1996 data were available only for these months. The effect of month for *E. tetrodonta* branches in both years combined and for 1996 alone, and the year effect were determined from the second set of analyses. Planned comparisons were used to test for linear trends over month in individual parameters for one species or year. For all data, means and standard errors were positively correlated, so square root transformations were performed prior to analysis.

The decrease in midday leaf and xylem water potentials between May and August 1998 was also analysed using analysis of variance and planned comparisons. Linear regressions were used to test for relationships between L_x and predawn leaf water potential in 1996, and between L_x and midday leaf and xylem water potentials for both *E. tetrodonta* and *E. miniata*.

Data points for the vulnerability curve were fitted to a linearized form of an exponential sigmoidal equation:

$$\ln(100/L_x - 1) = a\Psi - ab \quad (1)$$

where Ψ is the water potential, L_x is the corresponding percentage loss of hydraulic conductivity and a and b are constants, respectively, relating to the slope of the conductivity loss and the water potential corresponding to a 50% loss of conductivity (Pammenter & Vander Willigen 1998).

RESULTS

Rainfall

The 1995/96 wet season was slightly drier than average, with 1452 mm rain falling in Darwin between October and April, compared with the long-term mean of 1621 mm (Fig. 2). The dry season of 1996 was typical in that only 2 mm rain fell between June and September inclusive. By contrast, 2500 mm rain fell between October 1997 and April 1998, the highest rainfall on record. In addition, Darwin and Palmerston received 13 mm and 48 mm rain, respectively, in September 1998, so that the dry season ended earlier than usual.

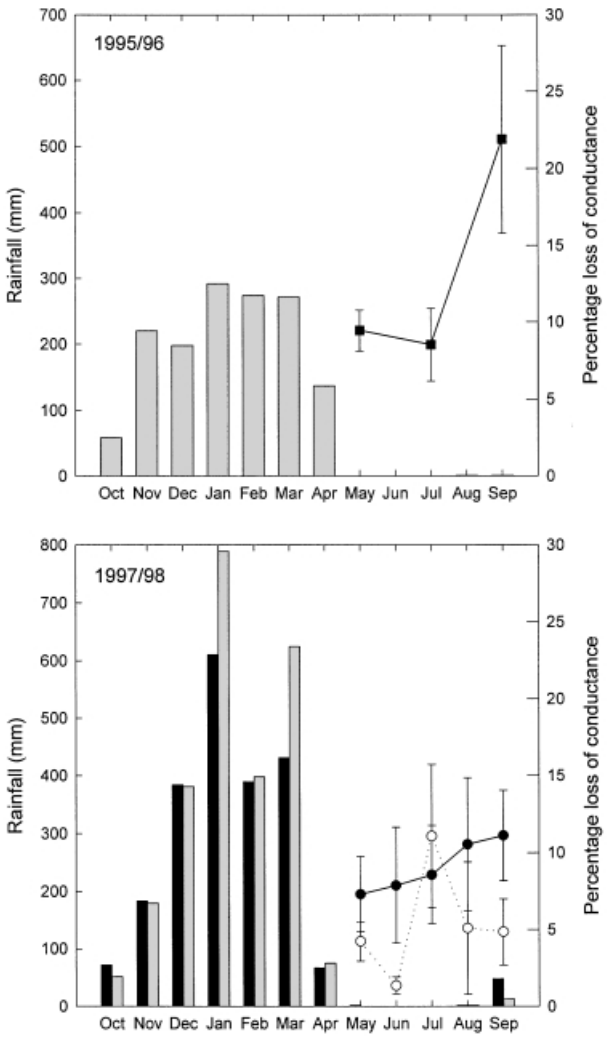


Figure 2. Rainfall for Darwin Airport (stippled bars) and Palmerston (solid bars 1997/98 only) during the study periods. Percentage loss of hydraulic conductance due to xylem embolism in terminal branches of *E. tetrodonta* (●) and *E. miniata* (○) saplings is also shown. Vertical bars indicate standard errors.

Table 1. Initial height and stem transverse area at 1 m height of tagged *E. tetrodonta* and *E. miniata* saplings. The change (Δ) in sapling height and stem transverse area between June and October is shown for all shoots, and for only those still surviving in October. Values are means ± standard errors

	E. tetrodonta	*E. miniata*
Initial height (m)	1·88 ± 0·13	1·74 ± 0·16
Δ height (m)	– 0·19 ± 0·09	– 0·48 ± 0·11
Δ height, survivors only (m)	– 0·05 ± 0·05	– 0·17 ± 0·07
Initial transverse area (cm^2)	1·59 ± 0·30	1·80 ± 0·40
Δ transverse area (cm^2)	0·02 ± 0·05	0·08 ± 0·09
Δ transverse area, survivors only (cm^2)	0·05 ± 0·06	0·17 ± 0·11

Growth and survival of shoots, branches and leaves

Average height of saplings declined between June and October 1998, even when only the surviving shoots were considered (Table 1). The mean decrease was larger for *E. miniata* than for *E. tetrodonta* saplings, but the difference was not significant. However, there was an increase in mean transverse area of the main stem. This was larger for *E. miniata* saplings than for *E. tetrodonta* saplings, but again the difference was not significant (Table 1).

The proportion of leaves lost between May and October was around 70% for both species (Fig. 3). In *E. miniata*, this was almost entirely due to death of whole branches (or shoots), whereas for *E. tetrodonta*, there was also a loss of leaves from live branches. Thirty-one per cent of *E. miniata* shoots died back to ground level, compared with only 10% of *E. tetrodonta* shoots. This difference was marginally significant ($P < 0{\cdot}10$) (Fig. 3).

Hydraulic conductance and L_x

There were no significant differences between the two species in hydraulic conductance or conductivity of terminal branches (Fig. 4). Branch diameter was similar for the two species (Table 2), so there was no difference in hydraulic conductance or conductivity per branch transverse area (Fig. 4). However, leaf area per branch was significantly lower for *E. miniata*, so that both conductance per leaf area and conductivity per leaf area were higher for this species (Fig. 4). The Huber Value, which represents the investment in stem tissue per unit leaf area, was higher in *E. miniata* saplings (Table 2). The only significant difference between years for *E. tetrodonta* was that hydraulic conductivity per unit leaf area was higher in 1998.

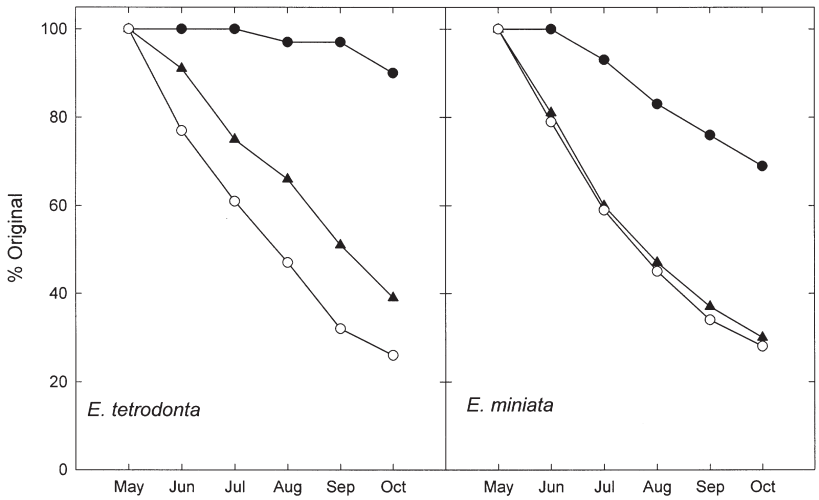


Figure 3. Percentage survival of shoots (●), terminal branches (▲) and leaves (○) that were tagged in May 1998.

Hydraulic conductance and conductivity *per se*, and normalized for leaf area or branch transverse area, all declined as the dry season progressed for *E. tetrodonta* branches, as did leaf area per branch (Fig. 4). The decrease in leaf area per branch was due to loss of basal leaves; average leaf number per branch used for measurements declined from 7·1 in June to 4·6 in September. When averaged over the 2 years, between May and September hydraulic conductance, conductance per branch transverse area, conductance per leaf area and leaf area per branch declined by 50, 60, 34 and 30%, respectively, in *E. tetrodonta* branches. Linear monthly trends were significant ($P < 0{\cdot}05$) or marginally significant ($P < 0{\cdot}10$) for all parameters in *E. tetrodonta* branches (both years combined), but were not significant for any parameters in *E. miniata* branches. Monthly effects were most significant for conductance per branch transverse area and conductivity per branch transverse area, but were also significant for conductance and conductivity. Effects were generally larger and more significant in 1996 than in 1998, but there were no significant interactions between species and month or between year and month.

Mean L_x was generally low (< 13%), except for *E. tetrodonta* branches in September 1996, when it was 22% (Fig. 2). The monthly increase in L_x was significant only for *E. tetrodonta* branches in 1996.

Modelling loss of resistors

The model showed that removal of basal petioles (parallel resistors) caused a decline in flow through the system (Fig. 5), because the total resistance of the system increased. We were able to replicate this experimentally by removing pairs of leaves and sealing the petiole with glue (to represent leaf abscission and subsequent sealing of the leaf scar). The largest decline in flow occurred for the first two pairs of petioles lost from the basal end of the stem. This was true whether the stem or the petiole constituted the principle source of resistance to flow, and irrespective of how many petiole pairs were initially present. By contrast, losing petioles from the apical end had much less effect on total flow, demonstrating the importance of average path-length.

Effects on flow per petiole (and therefore on hydraulic conductance per remaining leaf area for a *E. tetrodonta* branch) were more complex, and varied according to

Table 2. Size and hydraulic characteristics of branches of *Eucalyptus tetrodonta* and *E. miniata* saplings sampled at the beginning of the dry season, in May 1996 or May 1998. Values of hydraulic conductance and conductivity are those obtained before pressure perfusion. All values are means ± standard errors for eight branches

	E. tetrodonta 1996	*E. tetrodonta* 1998	*E. miniata* 1998
Branch length (cm)	27·8 ± 0·8	31·9 ± 2·6	38·2 ± 3·9
Diameter at base (mm)	2·8 ± 0·1	3·1 ± 0·2	3·2 ± 0·1
Leaf area (cm^2)	369 ± 59	432 ± 94	233 ± 34
Huber value ($\times 10^{-4}$)	1·62 ± 0·16	1·69 ± 0·16	2·94 ± 0·36
Hydraulic conductance (mg s^{-1} MPa^{-1})	59 ± 11	69 ± 14	45 ± 12
Hydraulic conductance per leaf area (g s^{-1} MPa^{-1} m^{-2})	1·69 ± 0·25	1·75 ± 0·20	1·74 ± 0·27
Hydraulic conductance per branch transverse area (g s^{-1} MPa^{-1} m^{-2})	9057 ± 1094	9454 ± 2036	5901 ± 1476
Hydraulic conductivity (mg m s^{-1} MPa^{-1})	16 ± 3	22 ± 5	16 ± 4
Hydraulic conductivity per leaf area (g s^{-1} m MPa^{-1} m^{-2})	0·47 ± 0·07	0·59 ± 0·12	0·64 ± 0·10
Hydraulic conductivity per branch transverse area (g m s^{-1} MPa^{-1} m^{-2})	2505 ± 302	3131 ± 777	2101 ± 568

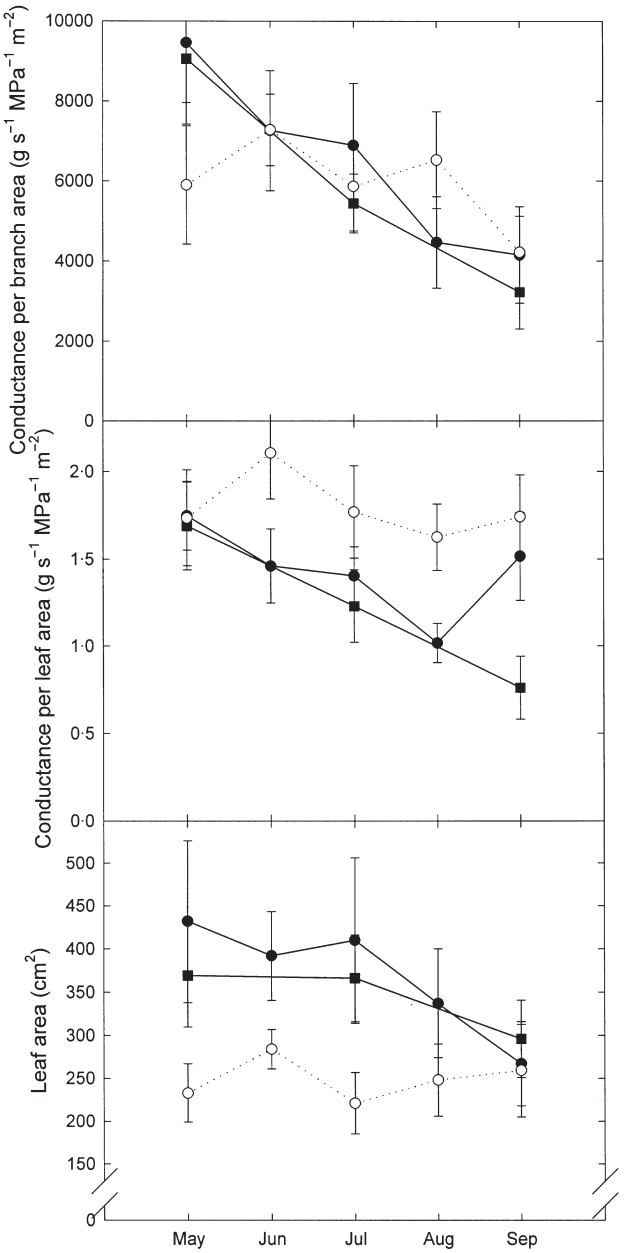


Figure 4. Monthly trends in hydraulic conductance per branch transverse area, hydraulic conductance per leaf area and in leaf area per branch for *E. tetrodonta* saplings in 1996 (■) and 1998 (●) and for *E. miniata* saplings in 1998 (○). Vertical bars indicate standard errors (n = 8).

whether basal or apical petioles were lost, whether the petiole or the stem constituted the larger resistance, and according to how many petiole pairs were initially present. Loss of petioles tended to increase the flow per remaining petiole, especially if apical petioles were lost first. However, if basal petioles were preferentially lost, this effect was sometimes outweighed by the increase in average path-length for water flow decreasing the average flow per petiole, especially if resistance of the stem was greater than that of the petiole. For a branch that initially had seven pairs of petioles, the loss of three basal pairs of petioles caused a 53% decrease in average flow per petiole (Fig. 5*a*), and for a branch that initially had three pairs of petioles, loss of the first basal pair caused a 22% decrease in average flow per petiole.

Relationship between L_x and plant water potential

In 1996, there was a good correlation (R^2 = 0·61) between pre-dawn leaf water potential and L_x in *E. tetrodonta* saplings (Fig. 6). Pre-dawn leaf water potentials were lower, and L_x higher, in September than in July or May 1996. In 1998, midday xylem water potential (i.e. covered leaves) decreased significantly between May and August for *E. tetrodonta* saplings (P = 0·0025) but not *E. miniata* saplings (data not shown). The decrease in midday leaf water potential (uncovered leaves) between May and August was significant for *E. tetrodonta* saplings (P = 0·0001), and marginally significant for *E. miniata* saplings (P = 0·075). Water potential data from September were excluded from analyses since 32 mm rain fell during the measurement week. Leaf and xylem water potentials increased substantially as a result of this unusual rainfall, and were not seasonal minima (data not presented), whereas L_x was still relatively high (Fig. 2). Seasonal minimum pre-dawn leaf water potentials were estimated, from relationships between pre-dawn leaf and midday leaf water potentials (Prior *et al*. 1997), to be similar in both years, but were below about –0·4 MPa for a longer period in 1996 than in 1998. Midday leaf and xylem water potentials (that is, field data) in 1998 were not good predictors of L_x for either species, with R-values ranging from 0·22 to 0·50. The relationship between L_x and midday xylem water potential for *E. tetrodonta* is shown as a vulnerability curve for field-dehydrated branches and excised, laboratory dried shoots (Fig. 7). When the latter were added (open symbols, Fig. 7) to the field data, the relationship was improved and extended.

Vulnerability curve

The relationship between L_x and xylem water potential in both intact material that was field-dried due to drought and in excised laboratory-dehydrated *E. tetrodonta* branches is shown in Fig. 7. Fitted values of the coefficients in Eqn 1 were a = 0·60 ± 0·15 and b = –6·27 ± 0·98. Variability was very large for both groups of branches, and there was only a narrow band of overlapping water potentials, between about –3·2 and –2·0 MPa. Within this band, mean L_x was not significantly different for the two groups: 8·7 ± 3·6% (n = 8) for intact field-dried branches and 5·7 ± 2·6% (n = 8) for excised laboratory-dried branches. Within the range of xylem water potentials experienced in the field in 1998, L_x was always less than 30%, and values predicted from Eqn 1 were less than 15%. Even at –5 MPa, predicted L_x was less than 40%. It was not possible to measure hydraulic conductance of branches at water potentials much below

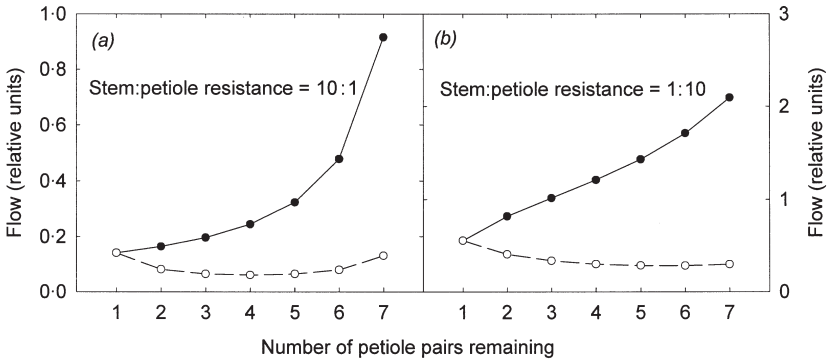


Figure 5. Modelled flow per branch (●), and per remaining petiole pair (○), as petiole pairs are sequentially removed. Petiole pairs were removed from the basal end, and total flow per branch decreased markedly, especially when the first pairs were removed. Flow per remaining petiole pair was lowest when some of the original petioles had been removed, especially when stem resistance was higher than petiole resistance. The ratio of stem resistance: petiole resistance was set at 10 : 1 in (a) and at 1 : 10 in (b).

–5 MPa since there was obvious shrinkage of petioles, and once a vacuum was applied, flow rates gradually decreased to near zero. Neither was it possible to determine embolism in laboratory-dried branches at water potentials less negative than –2 MPa, since they had already naturally been exposed to these xylem water potentials, and we would have measured native embolism.

DISCUSSION

Hydraulic conductance and conductivity *per se*, and conductance and conductivity normalized for leaf area or branch cross-sectional area, all declined as the dry season progressed, for branches of *E. tetrodonta*. There are several possible causes of this and these are now discussed in relation to experimental and modelled results.

Modelled and experimentally observed changes in total resistance/conductance to flow

Seasonal declines in hydraulic conductance could arise through: (1) an increase in xylem embolism; (2) loss of leaves, and consequent sealing of vessels that previously passed through petioles; (3) deterioration of xylem due to ageing (Sperry, Perry & Sullivan 1991); and (4) seasonal selection pressure for 'safer' branches, since within an individual plant, hydraulic conductance and 'safety' of xylem appears to be inversely related (Sperry & Saliendra 1994). We did not look for, or observe, any ageing of xylem, nor can we rule out seasonal selection pressure giving a hydraulically biased population of branches surviving the dry season. However, the first two factors both appear to contribute, as described below.

Between May and September there was an increase in L_x in *E. tetrodonta* branches from 9 to 22% in 1996, and from 7 to 11% in 1998. These increases can only account for a small proportion of the 60% observed loss in hydraulic conductance per branch transverse area in *E. tetrodonta* saplings (Fig. 4). During this time, average number of leaves per tagged branch decreased from 7·1 to 4·5 in *E. tetrodonta* saplings, and 8·5 to 7·7 in *E. miniata* saplings. Abscission occurs at the junction of the petiole and the branch, and sealing occurs, so that no flow was ever observed through the leaf scar of a naturally abscised leaf. It was predominantly basal leaves (and petioles) that abscised. The serial/parallel resistor network model showed that loss of petioles (parallel resistors) leads to loss of conductance of a branch, and that preferential loss of basal petioles can lead to loss of conductance per petiole because of increased average path-length for water flow. The predictions of the model are consistent with our results, in that declines in both hydraulic conductance and in numbers of leaves per branch were greater in *E. tetrodonta* than in *E. miniata* saplings. We conclude that the seasonal changes in hydraulic conductance are the result of increased embolism, an increase in the average path-length for water flow and a decrease in the number of parallel pathways (i.e. attached leaves) for water flow.

It is noteworthy that the model representing loss of

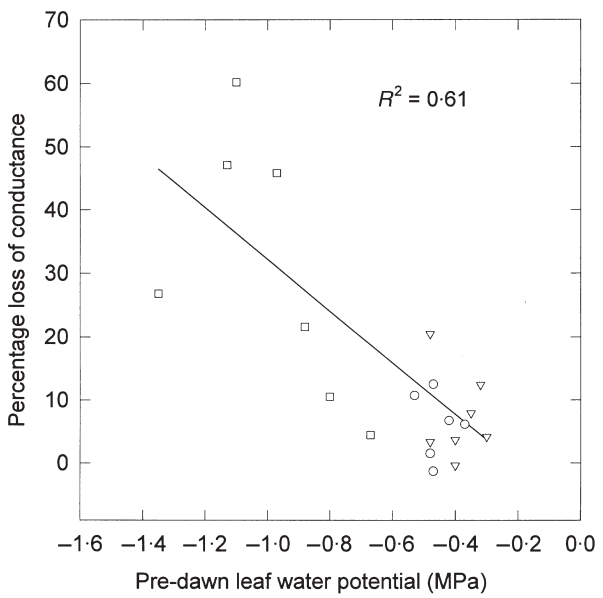


Figure 6. The relationship between percentage loss of hydraulic conductance due to xylem embolism in terminal branches of *E. tetrodonta,* and pre-dawn leaf water potential in *E. tetrodonta* saplings in May (▽), July (○) and September (□) 1996. The straight line indicates the fitted linear regression.

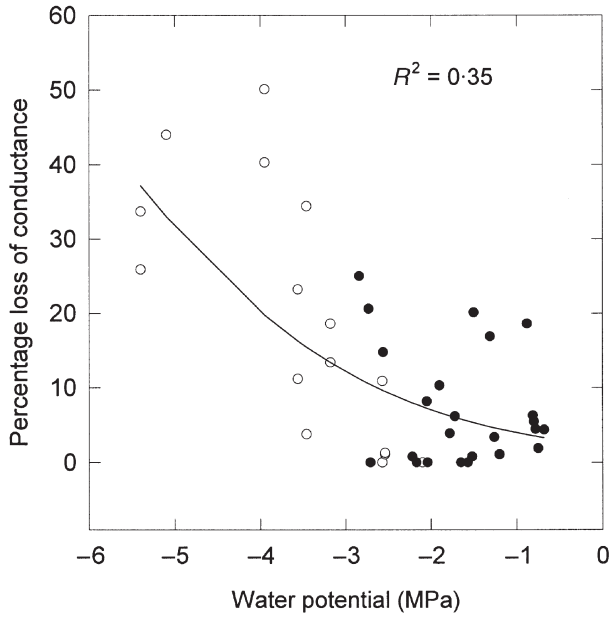


Figure 7. The relationship between percentage loss of hydraulic conductance due to xylem embolism in terminal branches of *E. tetrodonta,* sampled in 1998 and water potential of excised, laboratory-dried shoots (○) measured after shoots were enclosed in a plastic bag overnight; or intact, field-dried shoots (●), measured at midday. The line indicates the fitted version of Eqn 1, where a = 0·60 and b = –6·27.

parallel resistors is also applicable to whole trees. As lower branches are lost in a maturing tree, whole tree conductance to water will decline in a way similar to that seen here at branch level.

Leaf loss, shoot death and water status

The L_x of *E. tetrodonta* and *E. miniata* branches increased with declining plant water potentials. The strongest relationship was between L_x and predawn leaf water potential for *E. tetrodonta* in 1996, and there was also a significant correlation with midday xylem water potential for *E. tetrodonta* in 1998 (Figs 6 and 7). It may be that L_x is best correlated with soil water availability (as indicated by predawn leaf water potential), or alternatively, that the correlation was better in 1996 than 1998 because the dry season was more extreme and embolism levels generally higher in 1996.

Variability in the relationship between L_x and xylem water potential has been reported and is attributed to variation between individual plants (Sperry & Saliendra 1994; Sperry *et al*. 1994). In addition, when plants are severely dehydrated, xylem water potentials are more difficult to determine accurately (Sperry & Saliendra 1994), and may take longer to equilibrate throughout the plant as hydraulic connections are lost through embolism. Despite the variability, *Eucalyptus tetrodonta* was relatively resistant to xylem embolism. Its vulnerability was similar to that of the least vulnerable tropical trees (Tyree, Patiño & Becker 1998), but greater than that of the least vulnerable temperate trees (Tyree, Davis & Cochard 1994). *Eucalyptus tetrodonta* was less vulnerable than *E. grandis* or its hybrids (Pammenter & Vander Willigen 1998).

Leaf loss and shoot death in *E. tetrodonta* saplings between May and October were 64 and 10%, respectively, both slightly less than in a previous study when they averaged 86 and 18%, respectively, per year over 2 years (Prior *et al*. 1997). This was probably because in 1998 the wet season was wetter, and the dry season shorter, than average. However, shoot dieback (death of above-ground parts but followed by regeneration from below-ground) and death (no regeneration observed) was higher than expected for *E. miniata* saplings, so that they did not provide the planned comparison of a species whose growth and survival was relatively unaffected by drought stress.

Hydraulic conductivities per leaf area and per branch transverse area were significantly lower for *E. tetrodonta* and *E. miniata* than for *E. grandis* (Vander Willigen & Pammenter 1998). This could be because we used much smaller branches. Hydraulic conductivity on both a leaf-area basis and sapwood-area basis increases with stem diameter (Patiño, Tyree & Herre 1995). Values for all three *Eucalyptus* species are within the range reported for temperate angiosperm trees (Patiño *et al*. 1995), and higher than those reported for other evergreen trees from savannas or dry tropical forests (Goldstein, Rada & Catalan 1987; Sobrado 1993, 1996, 1997). Mature eucalypt trees in savanna near Darwin are able to access subsoil moisture throughout the dry season (Myers *et al*. 1997; O'Grady *et al*. 1999), and consequently maintain high rates of sap flow, which are facilitated by conductive xylem. Trees from drier habitats, such as Venezuelan dry tropical forest, tend to have smaller, less conductive vessels (Tyree *et al*. 1994).

Mean L_x was less than 13% for all but one sampling time, and generally lower than those reported for other species during seasonal drought. In several temperate Northern Hemisphere trees, L_x values of 20–50% has been measured during summer (Cochard & Tyree 1990; Tognetti & Borghetti 1994; Magnani & Borghetti 1995). Sobrado (1997) found L_x of Venezuelan evergreen trees was between 30 and 35% during the wet season, and between 40 and 48% during the dry season. Recent evidence suggests that conductivity decreases during the day and increases during the night (Zwieniecki & Holbrook 1998). Therefore by sampling branches before 0900 h we may have underestimated L_x that occurs in the middle of the day when evaporation is highest.

Methodological considerations

Our low values of L_x could be because we always cut branches under water, whereas most other workers cut branches in air, then recut under water. We suspect that in some cases at least, this has artificially induced emboli when xylem tension was suddenly released, and may explain very high apparent native levels of embolism at times of high soil

water availability. In addition, in some studies, pressure perfusion caused filling of unnatural conductive pathways, leading to an artificially high estimate of xylem embolism. In any case, we urge extreme care in, and detailed reporting of, sampling procedures.

There was a strong indication that there were many empty but easily refilled vessels in terminal branches when excised shoots were dried in air, even though these branches were further than the maximum vessel length from the cut end. This suggests that not only the cut vessels, but also vessels adjacent to cut vessels, may be artificially embolized when xylem is cut and exposed to air. Columns of water may be drawn from the xylem into the leaves, thus emptying vessels without air-seeding having to occur. It was not possible to obtain a reproducible estimate of hydraulic conductance until these vessels were refilled, since flow increased rapidly and non-linearly. It is possible that we underestimated the L_x in laboratory-dried material by refilling embolized vessels prior to 175 kPa pressure perfusion. It is also likely that the air-injection method overestimates vulnerability in branches with long vessels. In this method, air entry into the vascular system is facilitated by cut branch ends, side-branches and notches (Sperry & Saliendra 1994). The proportion of xylem volume directly air-filled by cutting (as opposed to that embolized by subsequent air-seeding) would be considerably larger in plants with very long, wide vessels than those with small vessels, and is probably not negligible. We stress that the increase in flow rate with time only ever occurred in the laboratory-dried samples, not in branches that had undergone natural drying in the field as soil water availability declined. It is recommended that the generation of vulnerability curves using laboratory-dried material be approached cautiously and that this effect is investigated experimentally in all such experiments.

In conclusion, both predicted and actual L_x in *E. tetrodonta* saplings were low compared with many other reports of L_x during drought. This reflects relatively low vulnerability combined with exposure to only moderate xylem water potentials. There was little evidence that death of *E. miniata* or *E. tetrodonta* shoots was related to L_x in terminal branches. It is possible that embolism increases suddenly and death of shoots or branches occurs very quickly after that. It is also possible that in these two species, only modest levels of embolism are required to induce catastrophic xylem dysfunction. A further possibility is that death of whole shoots may be related to embolism in the roots or lignotuber, rather than branches. Nonetheless, L_x in terminal branches was not a useful predictor of shoot death.

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