

Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status

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ABSTRACT

Vulnerability to water-stress-induced embolism and variation in the degree of native embolism were measured in lateral roots of four co-occurring neotropical savanna tree species. Root embolism varied diurnally and seasonally. Late in the dry season, loss of root xylem conductivity reached 80% in the afternoon when root water potential (Ψ_{root}) was about -2.6 MPa, and recovered to 25–40% loss of conductivity in the morning when Ψ_{root} was about -1.0 MPa. Daily variation in Ψ_{root} decreased, and root xylem vulnerability and capacitance increased with rooting depth. However, all species experienced seasonal minimum Ψ_{root} close to complete hydraulic failure independent of their rooting depth or resistance to embolism. Predawn Ψ_{root} was lower than Ψ_{soil} when Ψ_{soil} was relatively high (>-0.7 MPa) but became less negative than Ψ_{soil} later in the dry season, consistent with a transition from a disequilibrium between plant and soil Ψ induced by nocturnal transpiration to one induced by hydraulic redistribution of water from deeper soil layers. Shallow longitudinal root incisions external to the xylem prevented reversal of embolism overnight, suggesting that root mechanical integrity was necessary for recovery, consistent with the hypothesis that if embolism is a function of tension, refilling may be a function of internal pressure imbalances. All species shared a common relationship in which maximum daily stomatal conductance declined linearly with increasing afternoon loss of root conductivity over the course of the dry season. Daily embolism and refilling in roots is a common occurrence and thus may be an inherent component of a hydraulic signaling mechanism enabling stomata to maintain the integrity of the hydraulic pipeline in long-lived structures such as stems.

Key-words: cavitation; capacitance; hydraulic conductivity; water relations; xylem vulnerability.

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INTRODUCTION

The root system often represents the primary limitation on water movement along the soil-to-leaf continuum (Nobel & Cui 1992). Typically, 50% or more of the total resistance to water flow occurs below-ground despite the tendency for xylem conduit diameters to be largest in roots (Passioura 1984). Transpirational water loss generates tension, which is transmitted through continuous water columns running from the evaporative surfaces to the roots, making the entire xylem water transport pathway susceptible to cavitation and embolism. In roots, loss of functional xylem due to embolism prevents water uptake and reduces whole-plant hydraulic conductance (Linton & Nobel 1999) until the conduits are refilled with water overnight, presumably as a result of root pressure (Ewers, Cochard & Tyree 1997). Although the mechanism is unknown, there is also empirical evidence that even without root pressure embolized conduits are refilled on a daily basis while the surrounding xylem is still under tension (Salleo *et al.* 1996; McCully, Huang & Ling 1998; Melcher *et al.* 2001). In the root xylem of woody plants, field studies have shown significant embolism during drought with recovery occurring following rain (Jaquish & Ewers 2001; Domec *et al.* 2004). Surprisingly, daily cycles of embolism and refilling of root xylem have never been reported in woody plants, despite the potential physiological and ecological importance of this phenomenon.

The mechanisms involved in xylem refilling following embolism are a subject of intense discussion and investigation (Canny 1998; Tyree *et al.* 1999; Hacke & Sperry 2003; Trifilo *et al.* 2003). It has been proposed that refilling involves the loading of osmotically active solutes in living cells surrounding the xylem (Canny 1998; Pickard 2003a, b). The resulting water uptake, swelling and additional tissue pressure has been hypothesized to cause a transient extrusion of water into the vessel lumens resulting in refilling (Canny 1995, 1998). More recently, Bucci *et al.* (2003) confirmed that refilling of xylem in petioles was associated with an increase in osmotically active solutes and also showed that simple longitudinal incisions in the cortex prevented refilling of embolized vessels. Their explanation was

that the increase in tissue volume was partially constrained by the cortex, resulting in internal pressure imbalances that may drive radial water movement in the direction of the embolized vessels. This result shown in petioles suggests that further investigations of this phenomenon would be worthwhile in roots and stems.

Previous studies have demonstrated that roots are not only more vulnerable to embolism than stems, but also operate closer to critical tensions at which vessels embolize (Hacke & Sauter 1995). It is possible that this narrow safety margin for disruption of water movement might serve to buffer leaf water status by inducing early stomatal closure (Jones & Sutherland 1991). A recent study suggests that in conifers, embolism in roots may be involved in stomatal regulation of seasonal minimum water potentials (Domec *et al.* 2004). Stomatal conductance has been shown to decline even in the absence of reduction in bulk leaf water potential (Davies, Mansfield & Hetherington 1990), suggesting that stomata are able to sense loss of root conductivity (Fuchs & Livingston 1996) through chemical signals transported from roots to leaves in the xylem sap (Zhang & Davies 1987). Although common negative relationships between stomatal behaviour and the degree of embolism have been found within species (Sperry, Alder & Eastlack 1993; Cochard 2002) and across different ages of the same species (Domec *et al.* 2004), no trends have yet been established across species.

Previously, we have shown that vessels in petioles of savanna trees experience daily cycles of embolism and refilling (Bucci *et al.* 2003). In the present study, we measured root conductivity and leaf and soil water potential in four savanna tree species to test for the occurrence of daily cycles of embolism and refilling in the presence of tension-driven water flow. Three hypotheses were tested: (a) as previously shown for petioles, daily embolism and refilling in roots is a normal occurrence in neotropical savanna trees; (b) as in petioles, disrupting the mechanical integrity of roots prevents afternoon refilling of embolized vessels; and (c) root embolism may constitute a hydraulic bottleneck that governs stomatal conductance in a similar manner across species.

MATERIALS AND METHODS

Field site

This investigation was conducted in the IBGE Ecological reserve, an experimental field station located in central

Brazil approximately 33 km south of Brazilia (15°56' S, 47°53' W, 1100 m above sea level). Mean annual rainfall in the reserve is 1600 mm (1980–2004), more than 90% of which falls between October and April, resulting in a pronounced dry season from June to September. Mean monthly temperature ranges between 19 and 23 °C. Measurements were taken through the 2003 dry season from May to August. The dry season is accompanied by low relative humidity and high irradiance with a typical diurnal temperature range of 21 °C (<http://www.recor.org.br/servicos/estacao>). Vegetation in the reserve comprises an assortment of various stages of regeneration and subtypes of Cerrado vegetation (Eiten 1972). The study site was located in Cerrado *sensu stricto* (dense savanna woodlands with a well-drained deep oxisols rich in clay) with a mean tree and shrub density of approximately 1400 individuals ha⁻¹.

Plant material

We chose four dominant woody species: the evergreen *Blepharocalyx salicifolius* Berg. (Myrtaceae), the deciduous *Kielmeyera coriacea* Mart. (Gluciaceae), and the brevideciduous *Qualea parviflora* Mart. (Vochysiaceae) and *Byrsonima crassa* Nied. (Malpighiaceae). In the brevideciduous species, all leaves are shed annually and a flush of new leaves follows within 1–2 weeks.

Roots of 8–10 representative individuals of the four species were excavated to determine their spatial distribution (Table 1). The root system of *Q. parviflora* resembled the fibrous system of grasses, with a large number of different size roots located 20–30 cm below soil surface and no tap root. Root systems of *B. crassa* and *B. salicifolius* were characterized by a tap-root with six to 10 small prominent lateral roots with thin bark located 5–25 cm below the soil surface. *Kielmeyera coriacea* had a few thick primary roots with deep small sparse and inconspicuous lateral roots with thick, grey corky bark and located 35–50 cm below the soil surface.

Diurnal variation in hydraulic conductivity and embolism

Specific hydraulic conductivity (k_s , kg m⁻¹ s⁻¹ MPa⁻¹) and embolism were measured in roots collected twice a day: in the afternoon between 1400 and 1530 h and the following morning between 0530 and 0700 h on the 14th and 15th of the month between May and August 2003.

Table 1. Mean (\pm SE) tree height and diameter at breast height ($n = 15$ – 20), maximum rooting depth of representative individuals ($n = 8$ – 10), and total diameter and xylem diameter ($n = 15$ – 20) of the lateral roots sampled from four savanna tree species

	<i>B. salicifolius</i>	<i>B. crassa</i>	<i>Q. parviflora</i>	<i>K. coriacea</i>
Mean tree height (m)	2.8 \pm 0.2	2.3 \pm 0.2	2.2 \pm 0.3	2.3 \pm 0.1
Mean diameter at breast height (cm)	11.6 \pm 1.2	16.3 \pm 1.9	13.0 \pm 1.2	13.1 \pm 0.5
Mean rooting depth (cm)	5–15	10–20	30	35–50
Mean total root diameter (mm)	4.4 \pm 0.2	7.1–0.4	4.5 \pm 0.3	8.6 \pm 0.8
Mean root xylem diameter (mm)	3.1 \pm 0.1	4.6 \pm 0.3	3.6 \pm 0.3	4.3 \pm 0.4

Two lateral medium roots 3–5 mm in diameter and greater than 25 cm in length were excised near the base of four to five trees of each species. Before excision, the roots were checked to ensure that they were attached to the target tree. After excision, the roots were immediately recut under water in the field and transported to the lab for measurement of k_s within 30 min of excision from the trees. In the laboratory, a 16–18 cm long section of each root was cut, and the bark and cambium were removed from the entire sample. The proximal end was then attached to a tubing system and perfused at a pressure of 1–2 kPa with filtered (0.22 μm) water. The hydraulic pressure head was adjusted to avoid any refilling of embolized vessels. The rate of efflux was measured in a 1-mL-graduated pipette. When the flow was steady, the time required for the meniscus to cross five consecutive graduation marks (0.5 mL) was recorded. Specific conductivity was calculated as the mass flow rate of the perfusion solution divided by the pressure gradient across the root segment, normalized by the xylem cross-sectional area. Native embolism was determined by comparing the initial (or native) conductivity ($k_{s(i)}$) of root segments to the maximum conductivity ($k_{s(max)}$) after removal of air emboli after a series of 130 kPa flushes of filtered water. Percentage loss of conductivity (PLC) was computed as $\text{PLC} = 100(1 - k_{s(i)}/k_{s(max)})$.

Root xylem vulnerability to embolism and water loss curves

Vulnerability to embolism and water loss curves describe the percentage loss of hydraulic conductivity (% embolism) and relative water content (RWC) as functions of xylem tension. The air injection method was used to measure vulnerability of root xylem to water-stress-induced embolism (Sperry & Saliendra 1994). Root segments were collected in the field as described above, and air emboli were removed by flushing the samples at 130 kPa with filtered water. Even though vessel length was not measured, a preliminary experiment showed that there was no significant difference in vulnerability to embolism between root segments of 15, 25 and 40 cm long, suggesting that segment length was not critical. The segments were inserted into a locally built (11 cm in length) double-ended pressure chamber (Salleo *et al.* 1992) with both ends protruding and attached to the tubing system for measuring k_s as described above. A vulnerability curve was generated by first pressurizing the air chamber to 0.05 MPa to avoid water extrusion from lateral fine root scars when axial flow was induced (Sperry & Saliendra 1994), and allowing the system to equilibrate for 3 min. Water flow through the root was initiated and $k_{s(max)}$ was measured as described above. A pressure of 0.25 MPa was then applied and held constant for 2 min. After equilibration, the air chamber pressure was reduced to 0.05 MPa, and $k_{s(0.25)}$ measured. This process was repeated for pressures ranging from 0.25 to 3.00 MPa, or until the conductivity of the segment was negligible. Root xylem water potentials (Ψ_{root}) were estimated using the vul-

nerability curves determined in the laboratory and the PLC measured in the field.

On a subsample of roots, we followed the same pressurization steps and we measured the fresh mass (M_f) of the root segment after each pressure to determine the decrease in RWC associated with embolism. After determining the root mass at full saturation (M_{sat}) and the root dry mass (M_d), RWC was computed as $\text{RWC} = (M_f - M_d)/(M_{\text{sat}} - M_d)$. Water storage capacity, or capacitance, can be defined as the amount of water withdrawn from a tissue for a given decline in water potential. The volumetric RWC-based capacitance (in RWC MPa⁻¹), defined as $C = d_{\text{RWC}}/d\psi$ (Running 1980) was computed over the natural range of Ψ_{root} encountered by each species. Because wood density varies between species, it was more accurate to express the capacitance as the ratio of change in RWC, which represents the proportion of non-cell wall space that is occupied by water, to change in Ψ_{root} (Domec & Gartner 2001). However, for comparative reasons, capacitance (in g cm⁻³ MPa⁻¹) was also expressed as the change in water mass relative to the sample volume per unit change in Ψ_{root} .

Field manipulation

A manipulation experiment was conducted to examine factors influencing diurnal patterns of embolism and refilling of embolized vessels. In June 2003, 10 *B. salicifolius* trees were chosen and three of their lateral roots were excavated. We chose this species for its large number of easily assessable roots with uniform size. Three shallow longitudinal incisions were made in the bark and phloem tissues of one of the lateral roots and served as treated root. We took extra precaution in cutting through all the phloem without damaging the xylem. Each incision represented a very small fraction of the whole phloem volume that prevented any wound response. Immediately after the incisions were made, the treated roots were covered with silicone grease (100% silicone sealant; Dow Corning Corp, Midland, MI, USA) and wrapped in aluminium foil to limit air penetration and prevent water loss (Bucci *et al.* 2003). The other two lateral roots of each tree served as control roots and were also protected from sunlight by reflective insulation. Hydraulic conductivity and PLC of control roots were measured the morning prior to the incision on one of the three sampled roots on the 10 trees as described above. Hydraulic conductivity and PLC of the second control root and on the treated root were measured on five of the 10 trees in the afternoon following the incision and on the other five trees the next morning.

Soil, root and leaf water potentials and stomatal conductance

Soil water potential (Ψ_{soil}) was measured between May and August 2003 using individually calibrated screen-cage thermocouple psychrometers (PST-55; Wescor, Logan, UT, USA) installed at multiple depths in four locations. Measurements were recorded every 30 min with a data logger

(CR7X; Campbell Scientific, Logan UT, USA). Means of measurements made at 10, 10–20, 30 and 30–50 cm for *B. crassa*, *B. salicifolius*, *Q. parviflora* and *K. coriacea*, respectively, were reported here because these depths encompassed the mean depth at which root samples were collected (Table 1). Predawn and midday water potentials of upper canopy leaves (Ψ_{leaf} , MPa) from four different trees were determined on covered and exposed leaves with a Scholander-type pressure chamber (PMS, Corvallis, OR, USA). To compare with the measured embolism, predawn and midday covered Ψ_{leaf} was used in conjunction with the vulnerability curves to estimate morning and afternoon root embolism, respectively. Covered leaves were enclosed in plastic bags and aluminium foil to stop transpiration. The covered Ψ_{leaf} reflected stem Ψ , which was assumed to be close to Ψ_{root} because the tension gradient in the stem was not expected to be large. In addition, daily values of stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) of four sun exposed, upper to mid-canopy leaves per tree were determined with a null balance porometer (LI-1600; Li-Cor, Inc., Lincoln, NE, USA). In July and August, leaves were in different stages of development, from expanding to senescent and g_s was made on the most recently expanded leaves. For *K. coriacea*, the deciduous species, the trees had completely shed their leaves at the end of July. Water potentials and g_s were measured at different times of the day between 0600 and 1800 h on the same days that roots were collected for determination of native embolism on four trees from each species (on the 14th and 15th of the month). The trees sampled for Ψ_{leaf} and g_s measurements were not the same trees for which the root hydraulic tests were performed.

Statistical analyses

Vulnerability curves were fitted by the least squares method based on a sigmoidal function: $\text{PLC} = 100/(1 + e^{a(\Psi-b)})$, where Ψ is the negative of the applied air pressure, the parameter a is an indicator of the slope of the linear part

of the vulnerability curve and b is the pressure at which 50 PLC occurred (P_{50} , MPa). Differences in hydraulic parameters and field measurements between control and treatment were determined using a one-way analysis of variance (ANOVA). All stated equations in the figures had a significant regression coefficient at $P < 0.05$. All statistical procedures were conducted with Statistical Analysis Systems software (1999; SAS Inc., Cary, NC, USA).

RESULTS

Diurnal changes root k_s and xylem vulnerability

Values of $k_{s(\text{max})}$ (conductivity with embolism removed) for a given species did not vary significantly between morning and afternoon. The largest differences in $k_{s(\text{max})}$ between species occurred between *B. salicifolius* and *B. crassa*, with values of 21 ± 3 and $49 \pm 11 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$, respectively (Table 2). In all four species, roots were consistently more embolized in the afternoon than the next morning, except for *Q. parviflora*, which exhibited a higher degree of embolism in the morning than in the afternoon in August (Fig. 1). The maximum PLC observed in roots of the four species ranged from 50 to 85% in the afternoon, decreasing to 25–45% the next morning (Fig. 1) in May. Morning PLC was greater in August than in May, reaching 82% in August in *Q. parviflora*. As a consequence, values of native k_s varied significantly between morning and afternoon, fluctuating around overall seasonal means ranging from 18 ± 2 to $28 \pm 3 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ in the morning and from 5 ± 1 to $18 \pm 3 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ in the afternoon. The two species with shallow roots (10–20 cm), *B. salicifolius* and *B. crassa*, experienced higher daily fluctuation in root embolism than the two species with deeper roots (30–50 cm), *Q. parviflora* and *K. coriacea*. At the beginning of the study period, in May, Ψ_{soil} was close to zero for all rooting depths, but severe drought in the upper layers with Ψ_{soil} dropping to around -2.0 MPa was experienced by *B. salicifolius* and *B. crassa* in August before the rain resumed (Fig. 1).

Table 2. Mean (\pm SE) xylem density, maximum specific conductivity ($k_{s(\text{max})}$), the negative of applied pressure at which 50% loss of $k_{s(\text{max})}$ is reached (P_{50}), the calculated minimum root water potential (Ψ_{root}), the daily fluctuation in root water potential in July–August ($\Delta\Psi_{\text{root}}$), the xylem capacitance at Ψ_{root} experienced in May (C_{May}) and in July–August ($C_{\text{July August}}$), and the amount of water released in July–August over the range of $\Delta\Psi_{\text{root}}$ in four savanna tree species

	<i>B. salicifolius</i>	<i>B. crassa</i>	<i>Q. parviflora</i>	<i>K. coriacea</i>
Xylem density (g cm^{-3})	0.55 ± 0.01 a	0.52 ± 0.03 a	0.66 ± 0.02 b	0.28 ± 0.02 c
$k_{s(\text{max})}$ ($\text{kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$)	21 ± 3 a	49 ± 11 bc	32 ± 6 b	47 ± 8 c
P_{50} (MPa)	-1.4 ± 0.3 a	-0.9 ± 0.3 ab	-1.0 ± 0.1 ab	-0.8 ± 0.3 b
Minimum Ψ_{root} (MPa)	-2.6 ± 0.4 a	-2.0 ± 0.1 a	-2.4 ± 0.2 a	-1.1 ± 0.1 b
Maximum $\Delta\Psi_{\text{root}}$ (MPa)	1.8 ± 0.1 a	1.1 ± 0.1 b	1.0 ± 0.2 b	0.5 ± 0.1 c
C_{May} (%RWC MPa^{-1})	8.6 ± 1.3 a	10.0 ± 1.7 a	17 ± 5 b	20.9 ± 1.9 b
$C_{\text{July August}}$ (%RWC MPa^{-1})	6.9 ± 1.2 a	8.9 ± 1.3 a	11.9 ± 2.5 b	19.4 ± 1.4 c
$C_{\text{July August}}$ (10^{-2} , $\text{g cm}^{-3} \text{ MPa}^{-1}$)	4.7 ± 0.6 a	6.0 ± 0.9 a	6.6 ± 1.2 b	17.1 ± 1.0 c
Water released (10^{-2} , g cm^{-3})	8.4 ± 1.1 a	6.5 ± 1.0 a	6.6 ± 1.2 a	8.3 ± 0.5 a

For comparison, the volumetric capacitance experienced in July–August is also given. Values with different letters within a row are significantly different ($P < 0.05$, one-way ANOVA).

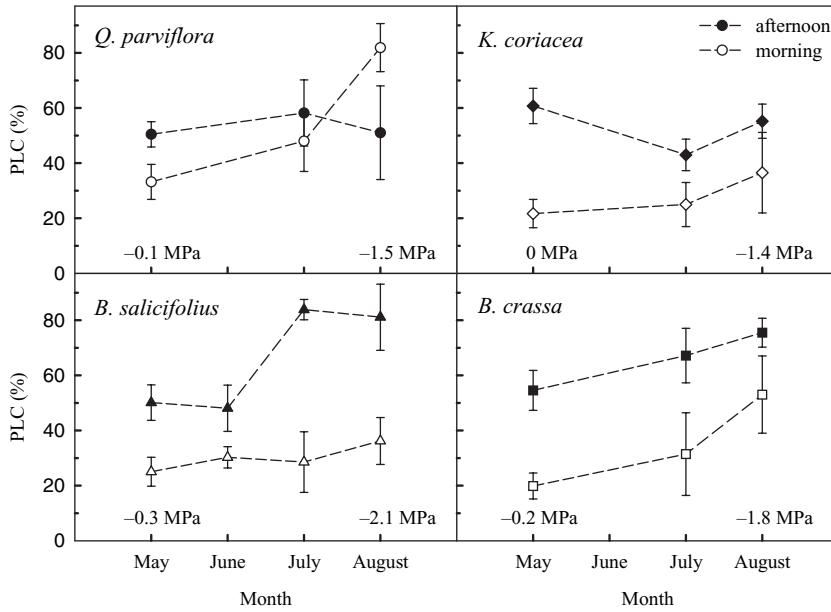


Figure 1. Diurnal changes in percentage loss of conductivity (PLC) for root xylem of four Cerrado woody species ($n = 5-6$). Closed and open symbols indicate midday and morning predawn measurements, respectively. Values above the x-axis correspond to the maximum (May) and the minimum (August) soil water potentials ($n = 4$) at the depth where roots were sampled.

Refilling of embolized vessels in *B. salicifolius* roots was prevented by shallow longitudinal incisions (Fig. 2). PLC in roots with incisions did not decrease in the morning and was significantly higher than PLC the previous morning ($P = 0.02$), whereas the control roots recovered to lower PLC that was not significantly different ($P = 0.4$) from the previous morning.

Root xylem vulnerability curves were steep, with PLC increasing sharply above 0.25 MPa applied pressure in all species (Fig. 3a). The PLC reached 80 at -1.5 MPa in *K. coriacea*, at -2.0 MPa in *B. crassa* and *Q. parviflora*, and at -2.5 MPa in *B. salicifolius*. Values of P_{50} varied significantly ($P = 0.04$) from -0.8 MPa in roots of *K. coriacea* to -1.4 MPa in roots of *B. salicifolius* (Table 2). Values of P_{50} for roots of *B. crassa* ($P_{50} = -0.9$ MPa) and *Q. parviflora* ($P_{50} = -1.0$ MPa) did not differ significantly ($P = 0.6$). There

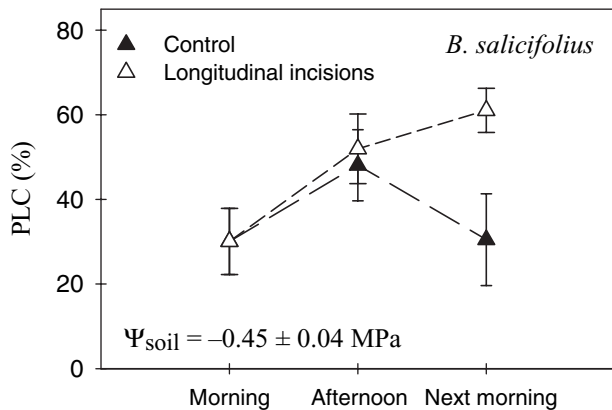


Figure 2. Diurnal changes in percentage loss of conductivity (PLC) in *Blepharocalyx salicifolius* in June for control roots (closed symbols) and for roots with longitudinal phloem incisions (open symbols) ($n = 5-10$). Mean soil water potential at the depth where roots were sampled is also given ($n = 4$).

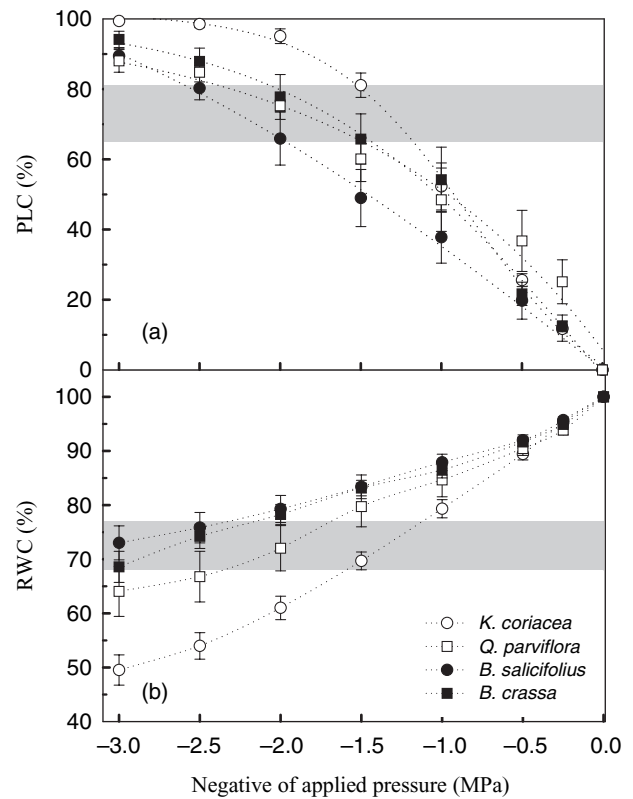


Figure 3. Vulnerability and water loss curves showing (a) the percentage loss of hydraulic conductivity (PLC) and (b) the decrease in relative water content (RWC) versus the negative of applied air pressure for root xylem ($n = 6$) of four savanna tree species. The grey shaded areas represent the range of PLC and RWC experienced among the four species over the range of estimated minimum root water potentials. Bars represent standard errors.

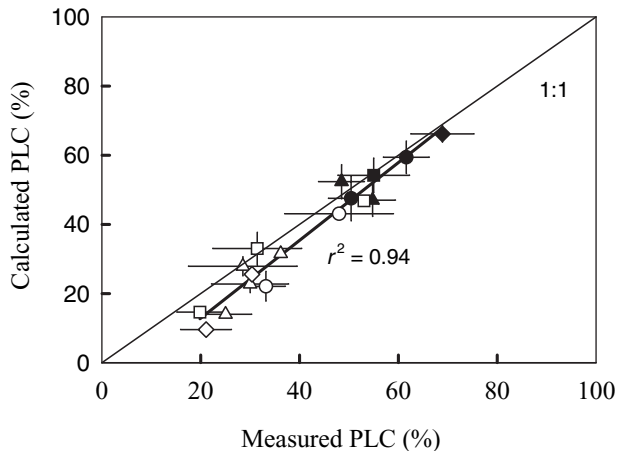


Figure 4. Relationships between percentage loss of conductivity (PLC) calculated from covered leaf water potentials (Ψ_{leaf}) and vulnerability curves, and PLC measured on roots excised at the times covered Ψ_{leaf} was measured (predawn = open symbols; mid-afternoon = closed symbols). Symbols are as in Fig. 1.

was also a significant ($P = 0.01$, $n = 6$) negative relationship between $k_{s(\text{max})}$ and P_{50} (Table 2). The minimum estimated Ψ_{root} determined from native PLC and the vulnerability curves increased with rooting depth, and ranged from -2.6 MPa in *B. salicifolius* to -1.1 MPa in *K. coriacea* (Table 2). The rate of decrease in RWC with the applied pressure differed between species and increased gradually with rooting depth (Fig. 3b). This was a consequence of the xylem capacitances increasing significantly ($P < 0.02$) with rooting depth in either the beginning or the end of the dry season (Table 2). The minimum RWC reached by each species at full embolism ranged from 75% in *B. salicifolius* to 50% in *K. coriacea*. However at the estimated minimum Ψ_{root} experienced by each species, the minimum RWC values only ranged from 68 to 77% (Fig. 3b).

Values of PLC estimated from the vulnerability curves and covered Ψ_{leaf} were in agreement with those measured on roots excised at the times covered Ψ_{leaf} was measured (Fig. 4).

Relationships between water potentials, stomatal conductance and root hydraulic properties

The estimated difference between predawn and afternoon root water potential ($\Delta\Psi_{\text{root}}$) decreased with rooting depth and varied from 0.4 to 1.0 MPa in May and from 0.5 to 1.8 MPa in July and August. There was a similar increase in root capacitance with rooting depth at the estimated Ψ_{root} encountered for each species at the beginning of the dry season, in May, and at the peak of the dry season, in July and August (Table 2). However, at the peak of the dry season, when $\Delta\Psi_{\text{root}}$ is taken into account, each root released a similar amount of water per volume of root xylem (Table 2). At midday, root xylem capacitance across species ranged between 4 and 28%RWC MPa $^{-1}$ (35 and

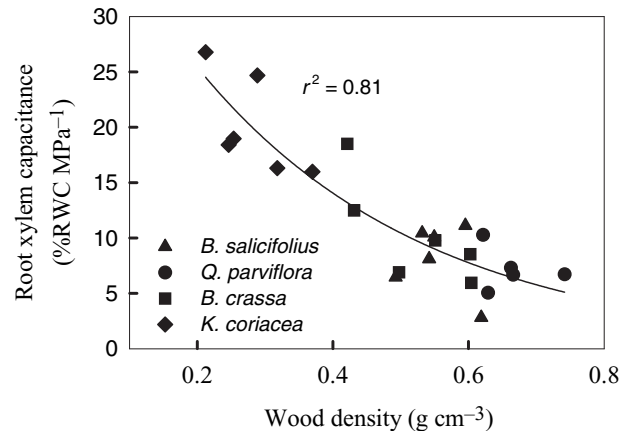


Figure 5. Relationships between root xylem capacitance taken between -0.5 and -1.5 MPa and root wood density in four Cerrado woody species.

$250 \times 10^{-3} \text{ g cm}^{-3} \text{ MPa}^{-1}$), and was inversely related to root wood density, which ranged between 0.22 and 0.72 g cm^{-3} (Fig. 5).

The relationship between $\Delta\Psi_{\text{root}}$ and native root k_s appeared to be identical across species regardless whether we used covered Ψ_{leaf} or predicted Ψ_{root} to estimate $\Delta\Psi_{\text{root}}$ (Fig. 6). There was a linear increase of $\Delta\Psi_{\text{root}}$ with increasing specific resistivity (inverse of k_s), and extrapolation to the x -axis (zero specific resistivity) yielded a predicted minimum value of 0.4 MPa for $\Delta\Psi_{\text{root}}$ (Fig. 6, inset). The estimated Ψ_{root} , determined from the vulnerability curves and native PLC, decreased linearly with Ψ_{soil} measured on the same day the roots were collected (Fig. 7). In the morning, estimated Ψ_{root} was lower than Ψ_{soil} early in the season, but then became higher than Ψ_{soil} below -0.65 MPa. In the

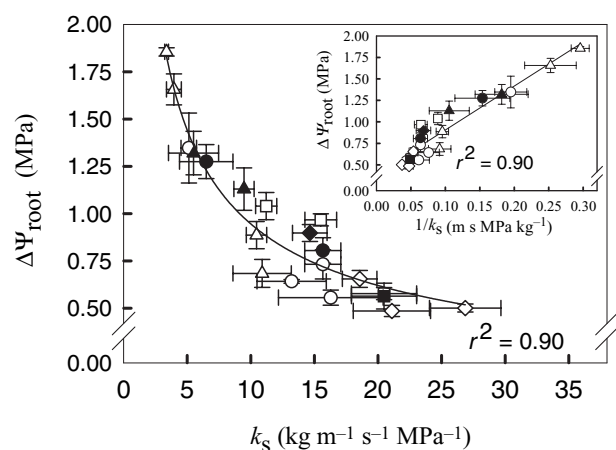


Figure 6. Relationship between the difference in root water potential between predawn and midday ($\Delta\Psi_{\text{root}}$) and specific conductivity (k_s) in roots of four tropical savanna species. Closed symbols represent $\Delta\Psi_{\text{root}}$ estimated from covered leaf water potentials, and open symbols represent $\Delta\Psi_{\text{root}}$ estimated from the measured root PLC and the vulnerability curves. The insert represents $\Delta\Psi_{\text{root}}$ versus the inverse of k_s (specific resistivity). Symbols are as in Fig. 1.

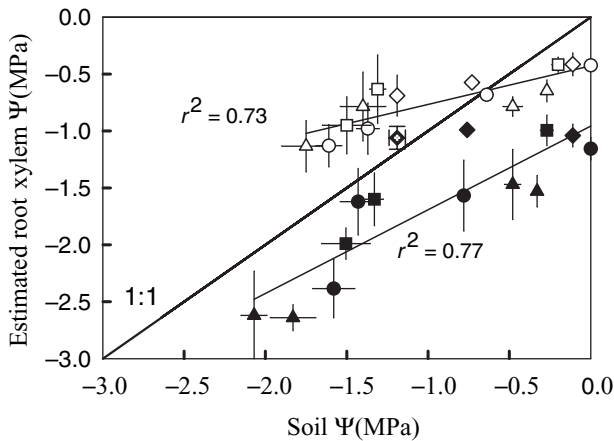


Figure 7. Relationships between estimated root water potential and measured soil water potential at predawn (open symbols) and in the mid-afternoon (closed symbols) for four savanna tree species. Symbols are as in Fig. 1. The solid diamond with the open centre represents *K. coriacea* in August when the trees were leafless, and was not included in the linear regression.

afternoon, estimated Ψ_{root} was always lower than Ψ_{soil} , but extrapolation of the linear relationship between Ψ_{soil} and estimated Ψ_{root} suggested that with continued seasonal decline in Ψ_{soil} , Ψ_{root} and Ψ_{soil} would reach the same value at around -3.2 MPa, at which point the roots would have lost 90–100% of their conductivity (Fig. 3).

Daily maximum g_s decreased linearly with increasing afternoon PLC between May and August (Fig. 8). The negative relationship between the degree of root embolism and g_s appeared to be identical across species with a seasonal increase of 40 PLC resulting in a 45% reduction in stomatal conductance.

DISCUSSION

The results of this study provide evidence that a substantial fraction of the functional root xylem of several tropical savanna tree species is dynamically emptied and refilled throughout the day independent of their rooting depth or resistance to embolism. Our findings are consistent with reports of short-term changes in hydraulic conductivity in stems, petioles and leaf blades of several woody species (Salleo *et al.* 1996; Zwieniecki & Holbrook 1998; Bucci *et al.* 2003; Brodribb & Holbrook 2004; Meinzer, James & Goldstein 2004) as well as with a report of daily change in k_s in roots of non-woody plants (McCully *et al.* 1998). However, the present study appears to be the first showing evidence for short-term changes in root conductivity in woody species. Our results also confirm previous studies that embolism reversal can occur when the water in adjacent non-embolized xylem is under considerable tension (Salleo *et al.* 1996; Bucci *et al.* 2003). The refilling of embolized xylem vessels during the day under negative water potentials of stems presents an apparent paradox (Tyree *et al.* 1999; Pickard 2003a). Water will not move from a vessel at negative pressure into an empty vessel at atmospheric pres-

sure. Yet, in the roots studied here refilling did not occur by bubble dissolution at low pressure, but against a difference in pressure of as much as 1.1 MPa (Fig. 7).

The results of the field manipulation involving shallow longitudinal incisions along roots (Fig. 2) were consistent with those reported for petioles of other species growing in the same study site (Bucci *et al.* 2003). Refilling may have been the result of local transient pressure imbalances that drive radial water movement in the direction of the embolized vessels (Bucci *et al.* 2003). Canny (1998) proposed that the refilling process at the whole tree scale is regulated by hydrolysis of starch into sugars during periods of increased tension and cavitation events. Instead, we propose that this refilling mechanism only operates on a local scale, causing dynamic changes in root conductivity. Even though measurements of solute concentration in xylem sap during refilling have not shown an increase in osmotic concentration sufficient to drive refilling (Tyree *et al.* 1999), to create a transient pressure imbalance, it would be critical to demonstrate that in roots the mechanism leading to xylem recovery is associated with an increase in osmotically active solutes (Milburn 1996; Bucci *et al.* 2003). This increase in xylem solute concentration may have occurred in *Q. parviflora* in August, which would explain the increase in k_s and the decrease in PLC in mid-afternoon compared with predawn (Fig. 1).

One of the objectives of our study was to evaluate a hypothesized correlation between g_s and root embolism. Indeed, the seasonal decline in maximum g_s was strongly related to the seasonal decline in root conductivity, with a common relationship found among all four species despite important differences in rooting depths (Fig. 8). The linkages between stomatal behaviour and the degree of embolism in roots allow us reconsider the ecological implications of species-specific strategies with regard to embolism and stomatal opening. We suggest that embolism in roots and

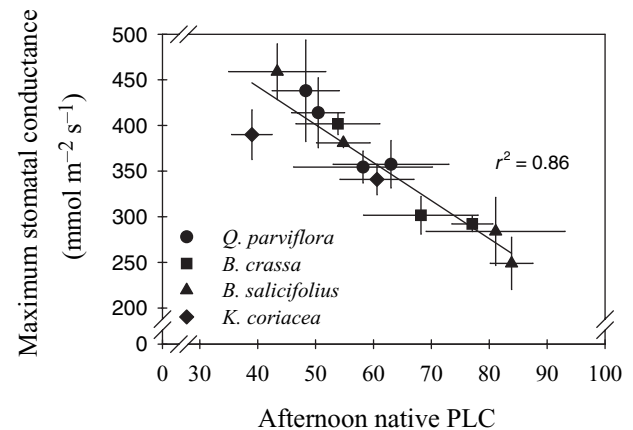


Figure 8. Relationship between seasonal variation in maximum stomatal conductance (g_s , average of measurements taken between 1000 and 1200 h) and afternoon percentage loss of conductivity (PLC) in roots of four Cerrado woody species. Standard errors represent the mean of the maximum values measured on each tree ($n = 4$).

probably leaves (Brodribb & Holbrook 2004; Meinzer *et al.* 2004) constitutes part of a hydraulic signal involved in normal daily stomatal regulation to prevent Ψ from declining to values that could provoke embolism in stems where embolism reversal may not be as vigorous as in the roots (Nardini & Salleo 2000). It is then logical to infer that the magnitude of daily refilling of root xylem forms part of this hydraulic signal between roots and shoots. Our results do not preclude the interaction of hydraulic and chemical signals such as abscisic acid (Gowing, Davies & Jones 1990; Lemichez *et al.* 2001) in governing stomatal response to variation in root embolism. The strong relationship between native k_s and the daily fluctuation in Ψ_{root} (Fig. 6) indicates that partial loss of root k_s through embolism would decrease the minimum value of Ψ_{root} required to maintain equal flow (Fig. 6, insert). As a result, the generation and export of similar amounts of abscisic acid or other chemical signal by each species could explain the common stomatal sensitivity to a decrease in hydraulic conductivity (Aasamaa *et al.* 2002). It has also been shown that roots synthesize abscisic acid when their RWC drops below 90% (Zhang & Davis 1987). This RWC value, corresponding to a common Ψ_{root} of -0.5 MPa, associated with the narrow range of decrease in RWC probably encountered by each species (Fig. 3b) may also explain the similarity in stomatal control across species.

The measurements of morning native PLC in the present study were not significantly different from those predicted using the vulnerability curves and the covered Ψ_{leaf} (Fig. 4). This result was unexpected because it has been shown that refilling is faster when embolism is artificially induced by air pressure than when it is naturally induced by drought (Salleo *et al.* 1992; Hacke *et al.* 2001). Cavitation fatigue and hysteresis in the refilling processes have recently been proposed to explain the discrepancy between measured and calculated PLC from vulnerability curves (Hacke *et al.* 2001). The large vessel diameter and bordered pits of roots (Bowes 2000) may explain the lack of hysteresis in our study because large pits would be less affected by embolism and refilling cycles. The concordance between measured and calculated PLC suggests that using covered Ψ_{leaf} corrected to ground level as a surrogate of predawn and midday Ψ_{root} is justified, and that the Ψ_{root} predicted from native PLC and vulnerability curves was a reliable estimate of the actual Ψ_{root} *in situ*.

In the present study, estimated Ψ_{root} in the morning was more negative than Ψ_{soil} at the beginning of the dry season, but became substantially less negative at the end of the dry season (Fig. 7). The disequilibrium between plant and Ψ_{soil} early in the season was probably attributable to nocturnal transpiration or incomplete overnight rehydration of the above-ground portion of the trees (Bucci *et al.* 2005). It has been shown that nocturnal transpiration can prevent overnight equilibration between plant and Ψ_{soil} (Donovan, Linton & Richards 2001, Donovan, Richards & Linton 2003; Bucci *et al.* 2004a). Later in the season, Ψ_{soil} in the morning fell below that of *B. crassa* and *B. salicifolius* roots, the species with the shallowest roots (Fig. 7). This observation

is consistent with an earlier report of reversal of sap flow in roots of these two species (Scholz *et al.* 2002) associated with hydraulic redistribution (HR), the passive movement of water from moister to drier layers of the soil via roots (Richards & Caldwell 1987). It is likely that HR limited the drop in Ψ_{soil} in the afternoon (Brooks *et al.* 2002; Moreira *et al.* 2003) and prevented Ψ_{root} from falling below thresholds causing full embolism (Domec *et al.* 2004). Because water uptake by shallow roots was prevented by HR, it is logical to infer that water release from cavitation was the only water supplied by shallow roots for transpiration in the morning. However, in the afternoon a high degree of embolism in the shallow roots re-coupled their Ψ to that of the surrounding soil, causing Ψ_{root} to fall below that of the soil (Fig. 7).

For species with deep roots where no hydraulic redistribution was detected (Scholz *et al.* 2002), water stored in the xylem itself may play an important role in minimizing temporal imbalances between water supply and demand. In *K. coriacea*, the species with the deepest roots and the highest root capacitance (Table 2), the largest diurnal variation in PLC was observed in June when the soil was relatively wet and the lowest in July and August when the soil was dry. There appeared to be a cost associated with larger root water storage capacity because roots of this species were also the most vulnerable to embolism (Table 2, Fig. 3a), which may explain why it started to drop its leaves in late June to be leafless at the end of July when Ψ_{soil} fell below -1.0 MPa. This decrease in leaf area probably increased whole-tree leaf-specific hydraulic conductance, which may also explain why there was an increase in g_s during the dry season (Meinzer & Grantz 1990; Bucci *et al.* 2005).

Wood density was highly correlated with water storage capacitance indicating that wood anatomy is a major predictor of water transport properties (Meinzer 2003). The exponential decay relationship in Fig. 5 predicts that root capacitance would reach a minimum value at a root wood density of 0.8 g cm^{-3} . The increase in root capacitance with decreasing wood density was also associated with an increase in k_s (Table 2), which suggests that wood density can be taken as a surrogate for water-transport efficiency in these and other diffuse-porous species (Bucci *et al.* 2004b). The emptying of a substantial fraction of the functional root xylem and its refilling at night implied that this process was largely responsible for the root water storage capacitance observed. However, over the range of Ψ_{root} experienced by each species, the amount of water released daily on a tissue volume basis was similar across species and ranged around 10–14%, or 7 to $8 \times 10^{-2} \text{ g cm}^{-3}$ (Table 2). In species with low root k_s , a greater driving force was required compared to species with high k_s . Several studies that have explored the relation between water flow through roots and the drop in Ψ across plant and soil concluded that there is a large resistance to flow that appears to be at the interface between the plant and the soil (Brislow, Campbell & Calisendorff 1984). However, our study showed no such large resistance (Fig. 7), suggesting that the sharp increase

in resistance that appears as soil dries may originate within the plant (Blizzard & Boyer 1980; Passioura 1984).

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