Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees

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ABSTRACT

Biophysical characteristics of sapwood and outer parenchyma water storage compartments were studied in stems of eight dominant Brazilian Cerrado tree species to assess the impact of differences in tissue capacitance on whole-plant water relations. The rate of decline in tissue water potential with relative water content (*RWC*) was greater in the outer parenchyma than in the sapwood for most of the species, resulting in tissue- and species-specific differences in capacitance. Sapwood capacitance on a tissue volume basis ranged from 40 to 160 kg m^{-3} MPa^{-1}, whereas outer parenchyma capacitance ranged from 25 to only 60 kg m^{-3} MPa^{-1}. In addition, osmotic potentials at full turgor and at the turgor loss point were more negative for the outer parenchyma compared with the sapwood, and the maximum bulk elastic modulus was higher for the outer parenchyma than for the sapwood. Sapwood capacitance decreased linearly with increasing sapwood density across species, but there was no significant correlation between outer parenchyma capacitance and tissue density. Midday leaf water potential, the total hydraulic conductance of the soil/leaf pathway and stomatal conductance to water vapour (*g*$_s$) all increased with stem volumetric capacitance, or with the relative contribution of stored water to total daily transpiration. However, the difference between the pre-dawn water potential of non-transpiring leaves and the weighted average soil water potential, a measure of the water potential disequilibrium between the plant and soil, increased asymptotically with total stem capacitance across species, implying that overnight recharge of water storage compartments was incomplete in species with greater capacitance. Overall, stem capacitance contributes to homeostasis in the diurnal and seasonal water balance of Cerrado trees.

Key-words: capacitance; *Cerrado*; hydraulic architecture; stomatal conductance; water potential.

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INTRODUCTION

The flow of water along the soil–plant–atmosphere continuum depends on the atmospheric evaporative demand, soil water availability and the limiting vapour or liquid phase resistances. However, characterization of water transport in terms of soil water as the only source and the atmosphere as the only sink with the plant's xylem linking the two is an oversimplification. Different portions of the water transport pathway inside the plant, such as roots, trunks, branches and even leaves, can also serve as water storage compartments and therefore act transiently as intermediate sources of water for transpiring leaves (Meinzer, Clearwater & Goldstein 2001). Thus, true steady-state rates of water transport rarely, if ever, occur because both the properties of the water transport pathway and the environmental variables that drive transpiration change dynamically throughout the day. Recent studies have acknowledged this situation by formally incorporating capacitances and variable resistances into models of whole-plant water transport to account for transient behavior (e.g. Edwards *et al*. 1986; Tyree 1988; Williams *et al*. 1996; Phillips *et al*. 1997; Perämäki *et al*. 2001; Zweifel & Hasler 2001).

Water stored in stem tissues has long been recognized as an important factor in plant–water relations. In trees, seasonal courses of discharge and recharge of water stored in sapwood have been measured and modeled (Waring, Whitehead & Jarvis 1978; Waring, Brown & Enquist 1979). Estimates of the contribution of stored water to daily transpiration vary widely, ranging from 10 to 50% of total water uptake (e.g. Holbrook & Sinclair 1992; Goldstein *et al*. 1998). Estimates of capacitance obtained from *in situ* measurements in intact trees range from 0.4 to 2.0 kg MPa^{-1} for a number of coniferous and angiosperm species (Wronski, Holmes & Turner 1985; Tyree 1988; Kobayashi & Tanaka 2001). However, it is unclear whether this range of values largely reflects intrinsic differences in the biophysical properties of the storage tissues involved or merely differences in the size of the storage compartment because none of the estimates was normalized by differences in total tissue volume.

Selective pressures acting on the flora of Neotropical savanna ecosystems have resulted in many plant characteristics that have intrigued biologists for decades (e.g. Ferri 1944; Valio *et al.* 1966; Goodland & Ferri 1979; Sarmiento 1983; Goldstein, Sarmiento & Meinzer 1986; Medina 1987). The extremely seasonal environment of savanna ecosystems of Central Brazil (Cerrado) is characterized by about five rainless months with high precipitation during the remainder of the year. Cerrado woody species have large scleromorphic leaves that are shed during the dry season and renewed before the wet season begins. Most Neotropical savanna trees are evergreen, although a few species are leafless for a relatively short period. In addition, many species allocate more than 50% of their biomass to belowground structures (Sarmiento 1983; Castro & Kauffman 1998). Because fire is a conspicuous feature during the dry season, the bark of savanna trees tends to be very thick and insulating (Hoffmann, Orthen & Nascimiento 2003). The bark of most Cerrado tree species is not only thick, but between the dead bark tissue of the periderm and the vascular cambium, there are conspicuous and complex layers of tissues that include not only the active and non-active phloem, but also several layers of parenchyma, which can represent more than 35% of the stem tissues in cross-sectional areas (Coradin 2000). The anatomical characteristics of these tissues are very distinct and easily distinguished from the internal xylem and external dead bark layers. Hereafter, we refer to these tissues as outer parenchyma, because of the prevalence of relatively large parenchyma cells, or similar cell types, which retain a living symplast at the end of their ontogenetic development. Because of its proximity to the xylem, the outer parenchyma in stems of Cerrado trees may serve as a significant source of stored water, contributing to the seasonal stability of leaf water status in these species (Bucci *et al.* 2005).

The objectives of the present study were to determine the relative contribution of sapwood and outer parenchyma tissues to overall stem water storage capacity and to assess their impact on long-distance water transport and regulation of water deficits in intact, field-grown individuals of eight dominant Cerrado woody species. We assessed differences in the water storage capacity of the sapwood and outer parenchyma from stems, and related these differences to biophysical properties of the two types of tissues. The biophysical properties investigated in the laboratory included capacitance, wood density, relative water content (*RWC*), water potential, osmotic potential and maximum bulk elastic modulus. Diurnal variations in sapwood water potential, leaf water potential, stomatal conductance to water vapour (g_s) and sap flow were measured in the field.

MATERIALS AND METHODS

Study site and plant material

The study was conducted in a savanna site with high tree density (*cerrado denso*) at the Instituto Brasileiro de Geografia e Estatística (IBGE) Ecological Reserve, a field experimental station located 33 km south of Brasilia (15°56′S, 47°53′W, altitude 1100 m), Brazil. Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August are often devoid of precipitation. Mean monthly temperature ranges from 19 to 23 °C with diurnal temperature ranges of 20 °C being common during the dry season. The soils are deep oxisols and despite their high percentage of clay, the soils are extremely well drained.

Eight woody species ranging from evergreen to brevideciduous and deciduous were selected for the study (Table 1). These species are commonly found throughout the Cerrado region. All species renew leaves during the dry season with the exception of *Schefflera macrocarpa* C. & S., and *Vochysia thyrsoidea* Pohl., which produce new leaves continuously throughout the year and *Sclerolobium paniculatum* Vog., which renews its leaves during the wet season. The brevideciduous species [*Byrsonima crassa* Nied., *Blepharocalyx salicifolius* (H.B. and K.) Berg and *Caryocar brasiliense* Camb.] are functionally evergreen because they seldom remain leafless for more than a few days. Most evergreen species also show progressive leaf senescence and abscission during the dry season. *Kielmeyera coriaceae* (Spr.) Mart. and *Qualea parviflora* Mart. remain leafless for

Table 1. Leaf phenology, basal diameter, height and the ratio between outer parenchyma and sapwood cross-sectional areas of the trees studied

Species	Family	Phenology	Basal diameter (cm)	Height (m)	Outer parenchyma area/sapwood area (A_{op}/A_{sw})
Byrsonima crassa	Malphigiaceae	Brevideciduous	6.5 ± 1.2	2.1 ± 0.1	0.17
Blepharocalyx salicifolius	Myrtaceae	Brevideciduous	8.5 ± 0.6	3.6 ± 0.1	1.21
Caryocar brasiliense	Caryocaraceae	Brevideciduous	13.5 ± 0.9	3.9 ± 0.1	1.87
Schefflera macrocarpa	Araliaceae	Evergreen	14.3 ± 0.4	4.4 ± 0.3	1.16
Sclerolobium paniculatum	Leguminosae	Evergreen	10.2 ± 0.7	5.5 ± 0.6	2.24
Vochysia thyrsoidea	Vochyseaceae	Evergreen	9.3 ± 0.4	6.3 ± 0.9	0.32
Kielmeyera coriaceae	Guttiferae	Deciduous	5.7 ± 0.4	3.1 ± 0.2	0.98
Qualea parviflora	Vochyseaceae	Deciduous	4.9 ± 0.3	2.8 ± 0.3	0.69

Diameter and the ratio between outer parenchyma and sapwood (active xylem) cross-sectional area (A_{op}/A_{sw}) were measured at 30–50 cm above the soil surface. The values are means ± SE of three trees per species.

about a month depending on the severity of the dry season. All measurements were carried out during the dry season of 2003. Leaf phenology, basal diameter and height of the study trees are indicated in Table 1.

Sapwood, stem and leaf water potential

Daily courses of sapwood water potential (Ψ_{sw}) were measured in 1 tree per species with *in situ* stem psychrometers (Plant Water Status Instruments, Guelph, Ontario, Canada) and recorded with a data logger (CR-7; Campbell Scientific, Logan, UT, USA) at 10-min intervals. Care was taken to install the psychrometers on the most shaded portion of the trunk to minimize temperature gradients. To allow for dissipation of residual thermal gradients, the values obtained during the first day after installation were not used. The psychrometers were calibrated against salt solutions of known osmolality.

Leaf water potential (Ψ_L) was measured with a pressure chamber (PMS Instruments, Corvallis, OR, USA). Leaf samples were immediately sealed in plastic bags upon excision and were kept in a cooler until balancing pressures were determined in the laboratory within 1 h of sample collection. Stem water potential was estimated from the water potential of attached leaves that had been enclosed in a plastic bag and wrapped in aluminum foil overnight to prevent nocturnal transpiration that would generate a water potential gradient between the leaf and the stem (Bucci *et al.* 2004). Measurements were obtained on 3–5 leaves per tree ($n = 3$) at dawn, mid-morning, midday, mid-afternoon and afternoon.

Outer parenchyma and sapwood biophysical properties

Cylinders of outer parenchyma (the parenchyma and phloem tissues outside the xylem and inside dead suberized tissues protecting the stem) and sapwood were obtained with a 5 mm increment borer near the base of the trunk of 3 trees per species and were sealed in glass vials for transport to the laboratory where they were allowed to hydrate in distilled water for < 2 h to avoid oversaturation; the tissue samples were quickly blotted to remove excess water, placed in the caps of thermocouple psychrometer chambers (JRD Merrill Specialty Equipment, Logan, UT, USA), weighed and then scaled inside the rest of the chamber for determination of water potential isotherms. Each chamber contained three cylindrical tissue samples. The psychrometer chambers were placed in an insulated water bath and allowed to equilibrate for at least 3 h before measurements with a dew point microvoltmeter (HR-33T; Wescor, Logan, UT, USA). Measurements were repeated at frequent intervals until the water potential values stabilized. The chambers were opened and the samples were allowed to dehydrate for different time intervals, re-weighed in the psychrometer caps, resealed inside the psychrometer chambers and allowed to equilibrate for another determination

of water potential. Moisture release curves for outer parenchyma and sapwood were generated by plotting water potential against *RWC*. The tissue water relation parameters calculated from moisture release curves were osmotic potential at full and zero turgor, and maximum bulk elastic modulus. Data points from 3 replicate curves per species were pooled.

Outer parenchyma and sapwood *RWC* was calculated as

$$RWC = (W_f - W_d)/(W_s - W_d),$$

where W_f is the fresh mass; W_d is the dry mass determined after oven drying at 80 °C for 72 h, and W_s is the saturated weight determined after overnight hydration and blotting of excess water. Curves fitted to the relationships between sapwood or outer parenchyma water potential and *RWC* were used to calculate sapwood and outer parenchyma capacitance as described in Meinzer *et al.* (2003). Capacitance was normalized by the outer parenchyma and sapwood tissue volume to facilitate comparison of absolute amounts of water released per unit decline in water potential. Tissue capacitance (C, kg m^{-3} MPa^{-1}) was calculated as

$$C = dW/d\Psi_t,$$

where W is weight of water per unit volume of tissue, and Ψ_t is the water potential of the tissue (either outer parenchyma or sapwood). Weight of water per unit tissue volume at saturation (kg m^{-3}) was calculated by multiplying the saturated/dry weight ratio of each tissue by tissue density (kg m^{-3}) and by subtracting tissue density. The cumulative weight of water released per unit tissue volume was then calculated by multiplying the tissue relative water deficit (1–*RWC*) at a given value of tissue water potential by the weight of water per unit tissue volume at saturation. Capacitance values were determined from instantaneous slopes of plots of cumulative weight of water released against tissue water potential. Species-specific values of tissue capacitance were taken as the slope of linear regressions fitted to the initial phase of each water release curve. To calculate the contributions of stored water, either in the sapwood or in the outer parenchyma, to the daily water used per tree, the amount of water released by each tissue was estimated using each species-specific water release curve and the minimum (most negative) tissue water potential measured near the base of the trunk with *in situ* stem psychrometers, as explained earlier. To scale up the total daily amount of water released by each tissue to the tree level, the amount of water released per unit of tissue volume was multiplied by the volume of each tissue per tree from the base of the main stem to the beginning of the crown. The tissue volume was calculated assuming that the trunks were tapered cylinders. These calculations were not performed for *Byrsonima crassa* and *Qualea parviflora* because the lowest branches occurred near the base of the main stem. The total volume of water released per tree on a daily basis by the outer parenchyma or by the sapwood was compared to the total amount of water loss per individual measured with the heat dissipation method (see next discussion), resulting

in an estimate of the contribution of tissue water storage to total daily transpiration per tree.

The densities of sapwood and outer parenchyma were measured near the base of the trunk in three individuals per species. Samples were obtained with an increment borer, sealed in aluminum foil and plastic bags and taken to the laboratory. Density (ρ) was calculated as

$$\rho = M/V,$$

where M is the mass of the sample, and V is the sample volume. Volume was estimated by submerging the sample in a container with distilled water resting on a digital balance with a 0.001 g precision. The sample was kept submerged during measurements until saturation with the help of a very small needle and did not touch the walls of the container.

To determine the daily course of *RWC*, one sample of sapwood and outer parenchyma per tree ($n = 3$) was collected at frequent intervals between 6 and 21 h during a typical dry season day. Fresh mass was determined, and the sample was saturated overnight. Then samples were oven dried at 80 °C for 72 h to determine the dry mass. *RWC* was calculated as previously mentioned.

Sap flow

Whole-plant sap flow was measured with the heat dissipation method (Granier 1985, 1987) during several consecutive days in the same plants used to measure sapwood water potential during the dry season. Briefly, a pair of 20-mm-long, 2-mm-diameter hypodermic needles containing a copper-constantan thermocouple inside a glass capillary tube and a heating element of constantan coiled around the glass tube was inserted into the sapwood near the base of the main stem in each plant. The upper (downstream) probe was continuously heated at a constant power by the Joule effect, while the unheated upstream probe served as a temperature reference. Temperature differences between the upstream and downstream probes were recorded every 10 s and 10 min averages were stored in solid-state storage modules (SM192, Campbell Scientific) connected to data loggers (CR 10X, Campbell Scientific).

Sap flux density was calculated from the temperature difference between the two probes using an empirical calibration (Granier 1985, 1987) revalidated for tropical trees (Clearwater *et al*. 1999). The measured temperature differences between both probes were corrected for natural temperature gradients between the probes (Do & Rocheteau 2002). Mass flow of sap per individual was obtained by multiplying flux density by sapwood cross-sectional area. The relationship between sapwood cross-sectional area and stem diameter was obtained by injecting dye near the base of the main stem for several individuals of each species representing a range of diameters. After 2 h, the plants were decapitated a few centimeters above the point of dye injection, and the area of active conducting tissue (sapwood) was determined from the pattern of staining by the dye as it

moved in the transpiration stream (Meinzer *et al*. 1999). Transpiration per unit leaf area was obtained by dividing mass flow of sap by the total leaf area per plant. Total leaf area per plant was obtained by counting the total number of leaves per plant then multiplying by the average area per leaf determined from fresh leaf samples for each tree. Ten to fifty fully expanded leaves, depending on the total number of leaves per plant, were collected from 3 trees per species, and its fresh leaf area was determined with a scanner.

Stomatal and hydraulic conductance

A steady-state porometer (model LI-1600; Li-Cor Inc., Lincoln, NE, USA) was used to measure maximum g_s on three leaves of the same plants used for sap flow measurements, leaf water potential and stem tissue biophysical properties. Fully expanded leaves from sun-exposed areas of the trees were used for measurements.

The apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) was determined as

$$G_t = E/\Delta\Psi,$$

where $\Delta\Psi$ is the difference between the current Ψ_L and the weighted average Ψ of the soil, and E is the average transpiration rate per unit leaf area determined from sap flow measurements at the time of Ψ_L measurements. Soil Ψ in the rooting zone of each tree was estimated by extrapolating to $E = 0$ the $\Psi_{sw} - E$ relationships obtained by simultaneous measurements of Ψ_{sw} and E, from pre-dawn through afternoon, for each individual (Sperry *et al*. 2002; Bucci *et al*. 2005). The linear regressions fitted to the $\Psi_{sw} - E$ relationships were all significant at $P < 0.1$. Additional information on the technique for estimating soil Ψ in the rooting zone can be found in Bucci *et al*. (2005). The influence of capacitive exchange of water between internal storage compartments and the transpiration streams on estimates of G_t was minimized by calculating G_t with sap flow and water potential data obtained between 1200 and 1300 h. Related observations of the temporal dynamics of sap flow and stem dimensional changes suggested that most of the diurnal stem water storage capacity was exhausted by 1200 h, resulting in a quasi-steady state relationship between sap flow and $\Delta\Psi$ (Scholz, unpublished results).

RESULTS

The *RWC* varied during the day in both the outer parenchyma and sapwood, decreasing during the morning and increasing during the afternoon (Fig. 1). The magnitude of the daily fluctuation in *RWC* tended to be larger for the sapwood. In *B. crassa*, for example, the outer parenchyma *RWC* decreased by about 8% with respect to its maximum values, while the sapwood *RWC* decreased by 17%. *RWC* was higher in the outer parenchyma than in the sapwood in five of the species. Maximum values of tissue water content were achieved during the early morning (0600–0800 h) and, in general, the minimum values were observed at midday.
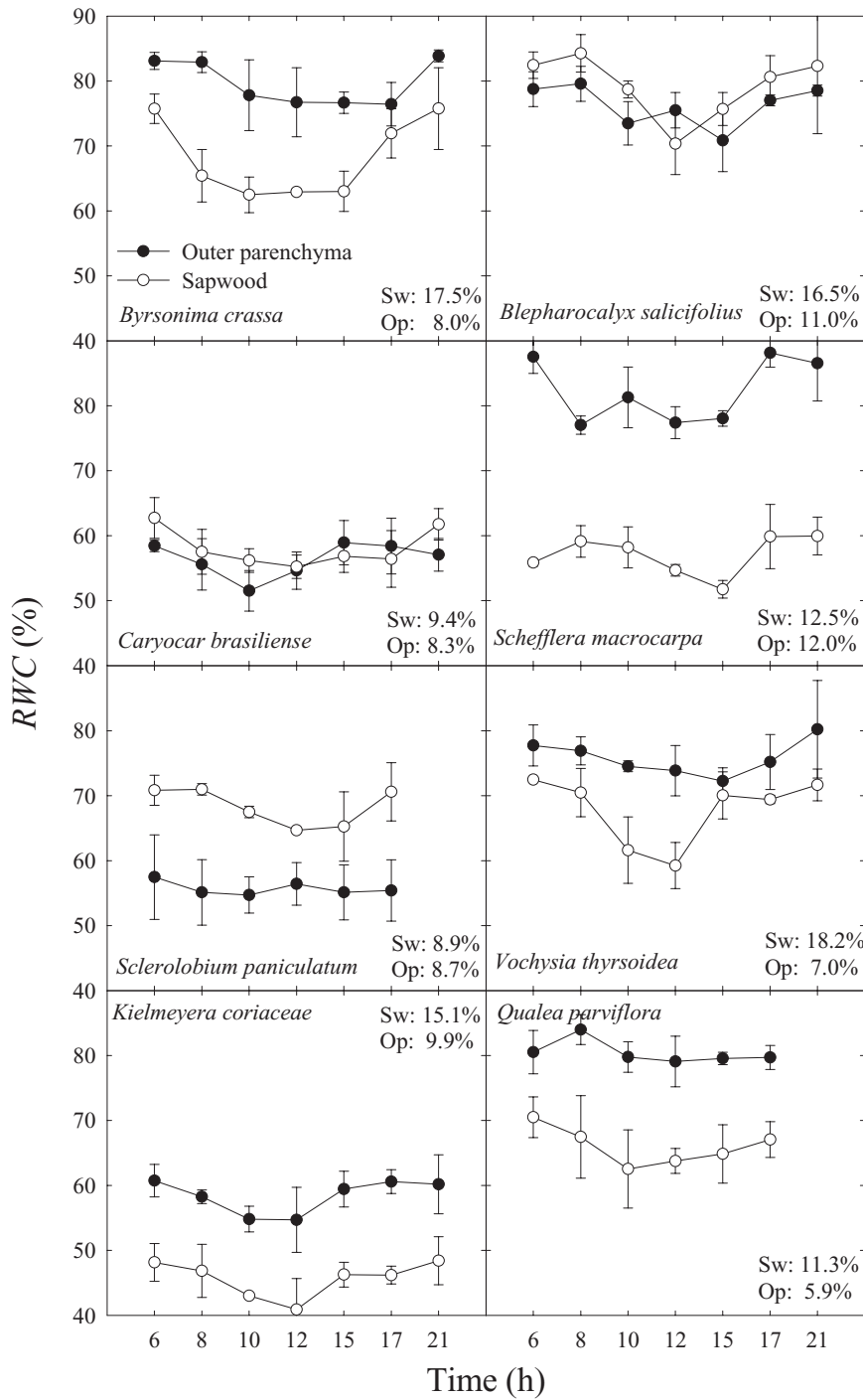
Figure 1. Daily variations in sapwood (sw, open symbols) and outer parenchyma (op, closed symbols) relative water content (*RWC*) during the dry season in eight representative Cerrado woody species. Each point represents mean values (± SE) of 3 individuals per species. The percent decrease in *RWC* from its maximum value attained during the morning to the minimum afternoon value is indicated in each panel.

At 2100 h, the *RWC* for nearly all species and both tissues was similar to the initial pre-dawn values. Significant differences ($P < 0.1$) between maximum and minimum outer parenchyma *RWC* were found in two of the species (*B. crassa* and *S. macrocarpa*), and significant differences ($P < 0.1$) between maximum and minimum sapwood *RWC* were found in four of the species studied (*B. crassa*, *Blepharocalyx salicifolius*, *Sclerolobium paniculatum* and *Vochysia thyrsoidea*).

Sapwood water potential (Ψ_{sw}) measured near the base of the trunk varied throughout the day in all species studied (Fig. 2). For example, in *V. thyrsoidea* Ψ_{sw} was −0.1 MPa at night and −0.85 MPa at midday. Leaf water potential (Ψ_L was always more negative than sapwood water potential. The largest differences between sapwood and leaf Ψ were observed between 1300 and 1500 h and ranged from 0.7 MPa in *V. thyrsoidea* to 1.7 MPa in *B. salicifolius* (Fig. 2, insets).

The rate of decline in Ψ with *RWC* (moisture release curves) differed markedly between outer parenchyma and sapwood for most of the species (Fig. 3, insets). The water potential in the outer parenchyma (Ψ_{op}) decreased in a nearly linear fashion with decreasing *RWC*, whereas
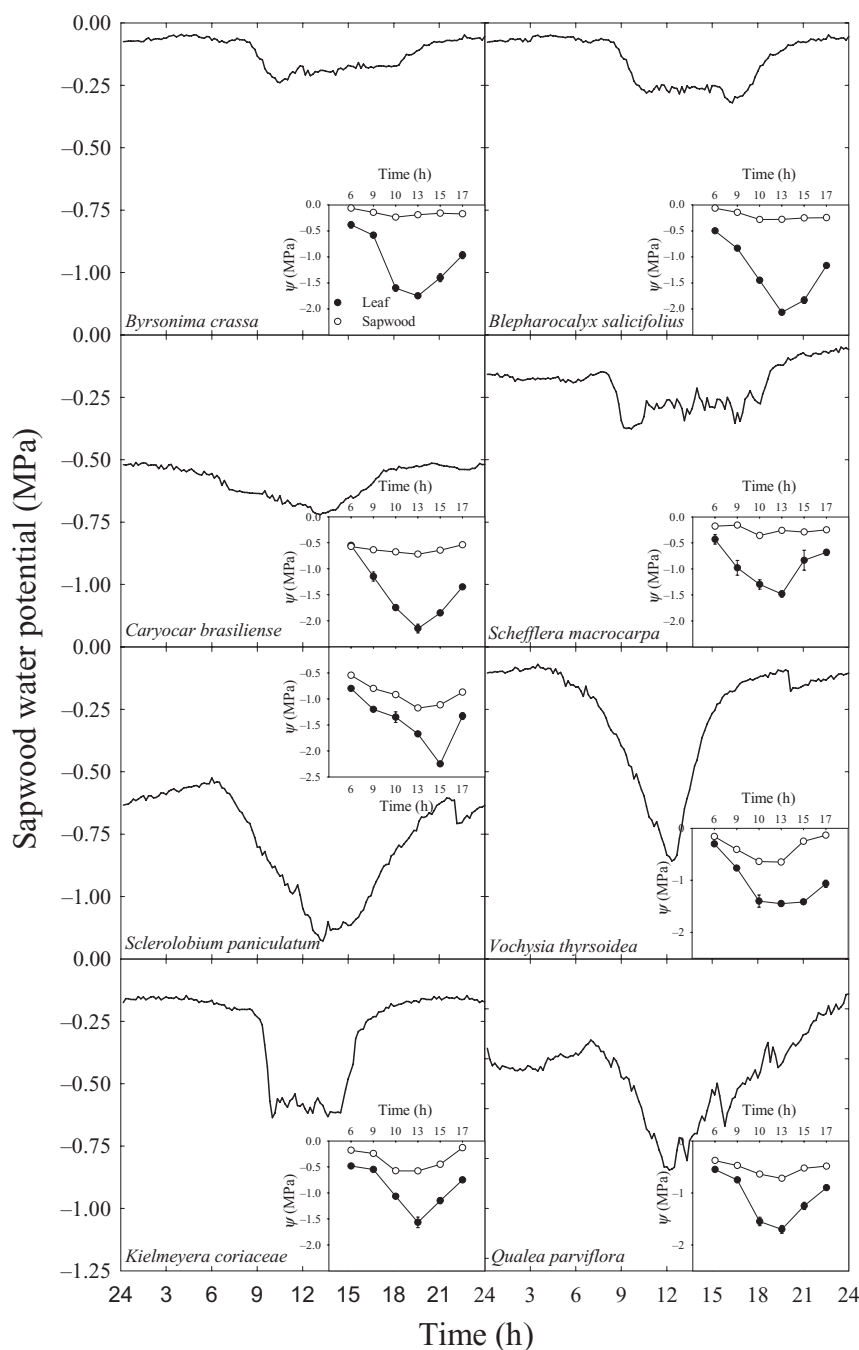
Figure 2. Daily courses of sapwood water potentials measured near the base of the trunk during the dry season in eight representative Cerrado woody species. The insets show leaf (closed symbols) and sapwood (open symbols) water potential changes (ψ) from 0600 to 1700 h. Values of leaf water potential are means $\pm$ SE ($n = 3$ and 3–5 leaves per tree). The data points for sapwood water potential were taken from the daily course measurements at the time when leaf water potential was measured.

sapwood Ψ_{sw} decreased exponentially with RWC, regardless of species. Linear regressions were fitted to the $\Psi - RWC$ relationships of the outer parenchyma tissue because of the high r^2 values of the regressions. Even though Ψ_{op} tended to decline in an exponential manner initially, the r^2 values obtained with exponential regressions were consistently lower than those obtained with linear fits. When these variables were transformed and plotted as Ψ_{op}^{-1} against 1-RWC for pressure–volume analysis, characteristic biphasic relationships were obtained that allowed osmotic potential at full and zero turgor, and maximum bulk elastic modulus to be estimated.

When wood density was used to normalize the relationships between Ψ and RWC on a volume basis, all sapwood relationships exhibited an initial phase in which cumulative water release increased in a nearly linear fashion as Ψ_{sw} declined to a threshold value of Ψ_{sw} (Fig. 3). After this threshold, water released by sapwood increased slowly and asymptotically. Cumulative water released by outer parenchyma increased linearly with Ψ_{op} (Fig. 3). The slopes of the initial nearly linear portions of water released versus Ψ relationships were taken as the species-specific capacitance values over the normal physiological operating range of tissue water potential.
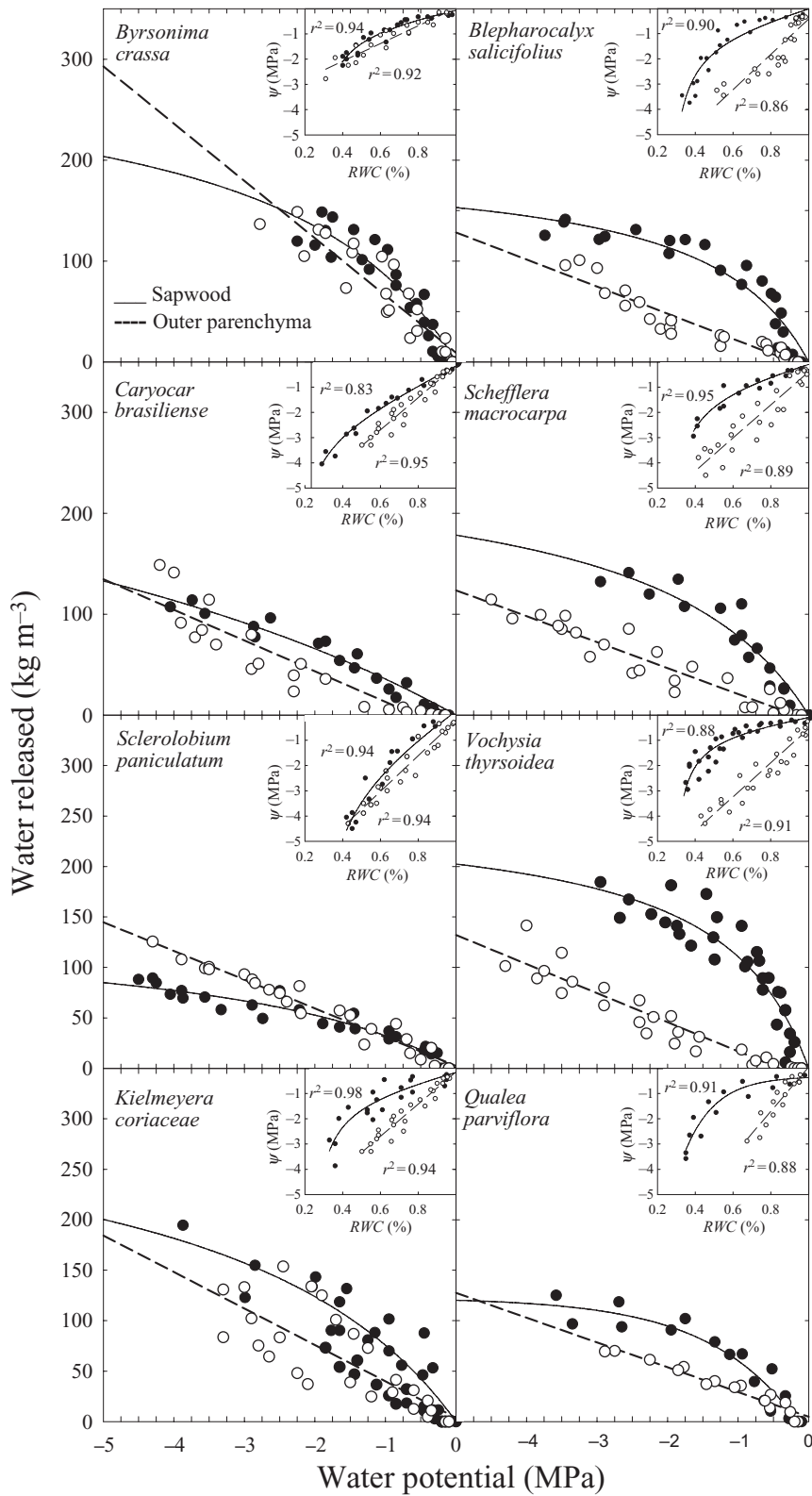
Figure 3. Cumulative amount of water released per unit tissue volume as a function of tissue water potential (sapwood, closed symbols; outer parenchyma, open symbols). The insets show relationships between water potential (ψ) (sapwood, solid line; outer parenchyma, dashed line) and relative water content (*RWC*). A hyperbolic function was fitted to the sapwood versus *RWC* relationships, and a linear function was fitted to the outer parenchyma versus *RWC* relationships. The r^2 values of the fitted functions are indicated.
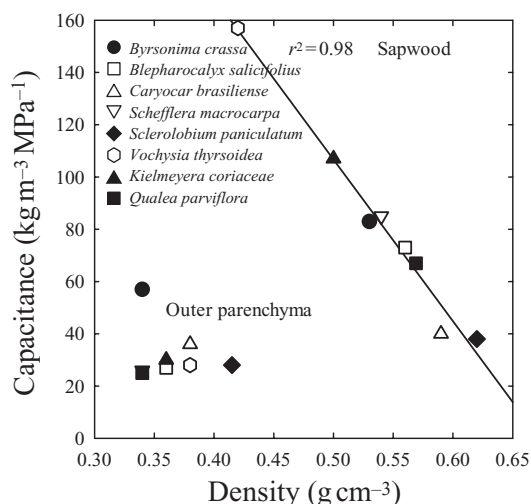
Figure 4. Relationships between tissue capacitance and tissue density in eight Cerrado woody species. Values of sapwood capacitance were estimated from the slopes of the lines fitted to the initial linear portions of the water release curves and values of outer parenchyma capacitance were estimated from the slopes of the linear function fitted to the data from Fig. 3. The line is the linear regression fitted to the sapwood data ($y = 416 - 619x$, $P < 0.001$). No significant relationship between outer parenchyma density and outer parenchyma capacitance was observed.

Species-specific sapwood capacitance ranged from 40 to 160 kg m^{-3} MPa^{-1} and decreased linearly with increasing sapwood density (Fig. 4). In contrast, outer parenchyma capacitance spanned a much smaller range and was not significantly correlated with tissue density. Osmotic potentials at full turgor and at the turgor loss point were more negative for the outer parenchyma than for the sapwood, and the maximum bulk elastic modulus was higher for the outer parenchyma than for the sapwood in all species studied, with the exception of *Q. parviflora* (Table 2). The ratio of outer parenchyma to sapwood cross-sectional area varied by more than an order of magnitude from 0.17 in *B. crassa* to 2.24 in *S. paniculatum*, which exhibited more outer parenchyma than sapwood (Table 1). Species with lower proportions of outer parenchyma relative to sapwood cross-sectional area had higher total stem capacitance (outer parenchyma + sapwood, Fig. 5). Total capacitance ranged from 70 kg m^{-3} MPa^{-1} in *S. paniculatum* to 190 kg m^{-3} MPa^{-1} in *V. thyrsoidea*.

Daily minimum Ψ_L increased with increasing sapwood capacitance and total stem capacitance (Fig. 6). In contrast to the strong relationship between Ψ_L and sapwood capacitance, no significant relationship between Ψ_L and outer parenchyma capacitance was observed (data not shown). Species-specific apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) increased linearly across species with increasing relative contribution of water storage to total daily transpiration (Fig. 7a). However, g_s initially increased in a nearly linear fashion with increasing relative water storage, but the relationship became asymptotic at water storage values above 20%.

Table 2. Osmotic potentials at full turgor and at zero turgor and maximum bulk elastic modulus of the sapwood (sw) and outer parenchyma (op) obtained from pressure–volume relationships, and contribution of water storage to daily transpiration (water storage) estimated using minimum sapwood water potential and water released from tissues

| Species	Osmotic potential at full turgor (MPa)		Osmotic potential at zero turgor (MPa)		Bulk elastic modulus (MPa)		Water storage (%)
	sw	op	sw	op	sw	op	
Byrsonima crassa	−0.34	−0.50	−0.39	−0.65	2.3	2.9	–
Blepharocalyx salicifolius	−0.36	−1.90	−0.47	−2.10	4.7	13.9	12.54
Caryocar brasiliense	−0.37	−0.87	−0.65	−1.20	2.6	7.0	14.60
Schefflera macrocarpa	−0.50	−1.63	−0.55	−1.90	3.7	10.9	11.11
Sclerolobium paniculatum	−0.69	−0.69	−0.85	−1.20	2.7	9.0	10.48
Vochysia thyrsoidea	−0.37	−1.38	−0.45	−1.60	2.2	15.8	20.47
Kielmeyera coriaceae	−0.45	−1.82	−0.50	−2.30	7.56	11.9	30.64
Qualea parviflora	−0.57	−0.51	−0.63	−0.55	10.8	9.8	–
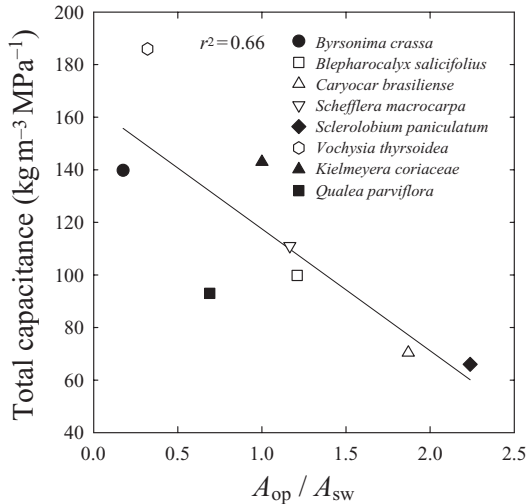
Figure 5. Relationship between species-specific total stem capacitance (outer parenchyma + sapwood) and the ratio between outer parenchyma and active sapwood cross-sectional area (A_{op}/A_{sw}) in eight Cerrado woody species. The line is the linear regression fitted to the data ($y = 163 - 46x$; $P < 0.05$).

Species with high water storage capacity, such as *V. thyrsoidea* and *Kielmeyera coriaceae*, had both the highest G_t and g_s (Fig. 7b).

The difference between the pre-dawn water potentials of non-transpiring leaves ($\Psi_{L\ covered}$) and weighted average soil water potential (Ψ_{soil}), which is a measure of water potential disequilibrium between the soil and leaves, increased in a sigmoid fashion with increasing total stem capacitance (Fig. 8). For example, *V. thyrsoidea*, *K. coriaceae* and *B. crassa*, the species with the highest total capacitance, showed the largest pre-dawn water potential disequilibrium between soil and leaf, whereas *S. paniculatum* and *Caryocar brasiliense*, the species with the lowest total capacitance, achieved complete equilibrium between Ψ_{soil} and $\Psi_{L\ covered}$ before the end of the dark period. The insert in Fig. 8 shows that the pre-dawn disequilibrium between the water potential of freely transpiring leaves ($\Psi_{L\ exposed}$) and Ψ_{soil} was not related to total capacitance. The weighted average Ψ_{soil} and pre-dawn Ψ_L were both higher for species with high capacitance (results not shown)

DISCUSSION

Water storage capacity is defined in this study as the amount of water that can be withdrawn (or released) for a given change in water potential of the water storage compartment, generally referred to as the tissue capacitance (Jarvis 1975; Nobel 1991). If the total volume of the storage compartment is known, then an alternative definition of water storage capacity can include the total amount of available water in a particular tissue compartment in relation to the total transpiring leaf area (Goldstein, Meinzer & Monasterio 1984), or in relation to total daily transpiration (Goldstein *et al.* 1998). In this study, two different types of stem tissues whose water storage characteristics were

considered to be of potential importance for the water relations of savanna trees were studied: the active portion of the xylem, or sapwood, and the outer parenchyma tissues outside the vascular cambium that retain their cytoplasm at maturity. Both tissues are distinct anatomically and their limits are easily recognized. If both the tissue capacitance and the tissue volume per individual are known, then a comprehensive characterization of daily capacitance would take into account the *in situ* operating range of water potential of the reservoir. The amount of water released by the sapwood and outer parenchyma on a daily basis was estimated by using species-specific water release curves for each tissue and the most negative daily water potential experienced by each tissue *in situ*. To scale up the total daily amount of water released per tissue compartment to the tree level, the amount of water released per unit tissue volume was multiplied by the volume of each tissue per tree from the base of the main stem to the beginning of the crown. The total storage capacity (sapwood + outer parenchyma) according to these calculations ranged from 11 to
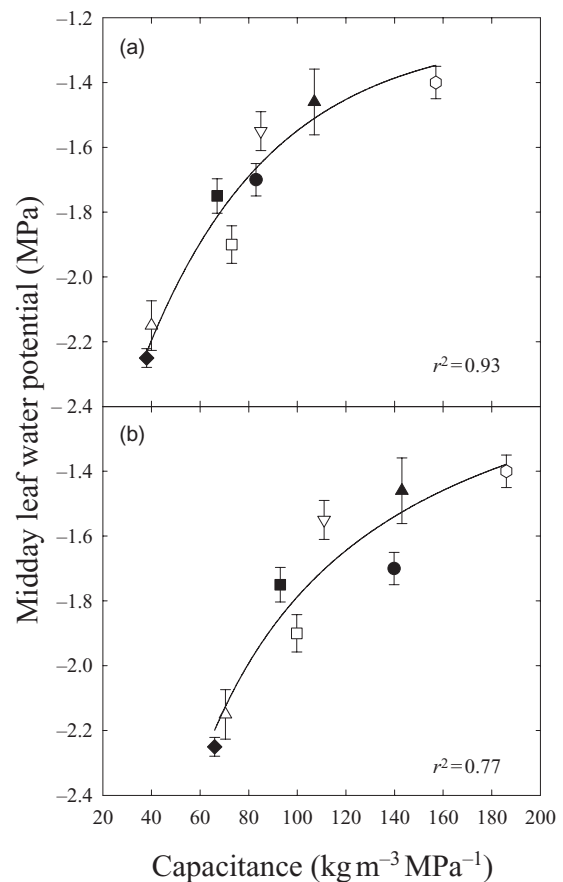

Figure 6. Midday leaf water potential in relation to (a) sapwood capacitance and (b) total capacitance (outer parenchyma + sapwood) in eight Cerrado woody species. The lines are asymptotic functions fitted to the data {[a] $y = -3.34 + 2.01[1 - \exp(-0.02x)]$, $P < 0.001$; [b] $y = -2.55 + 1441[1 - \exp(-0.000005x)]$; $P < 0.001$}. Each value of water potential represents the mean $\pm$ SE of 3–5 leaves per tree ($n = 3$). See Fig. 4 for symbols.
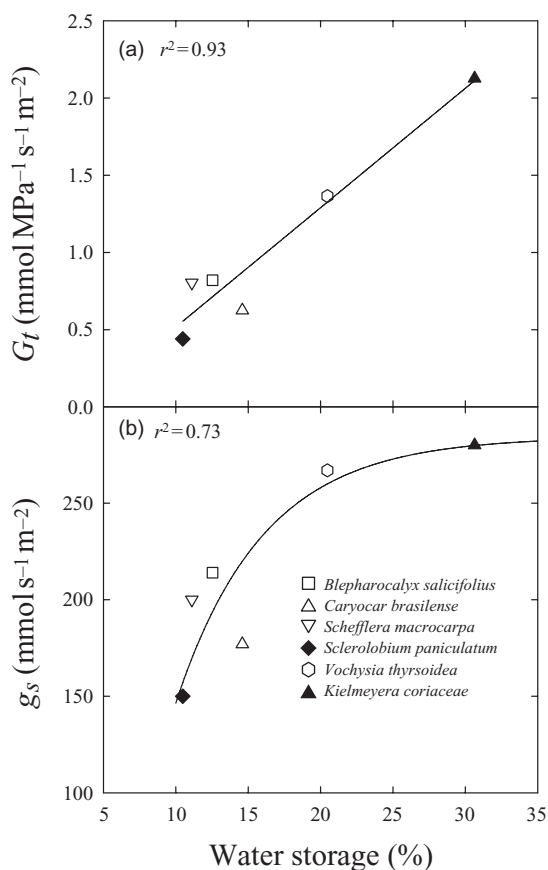
Figure 7. (a) Species-specific apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) and (b) species-specific stomatal conductance (g_s) in relation to the relative contribution of water storage to daily transpiration. G_t and g_s were determined during the midday period when the lags between upper branch and basal sap flow, and presumably the discharge capacitance of water from water storage, was minimal. The line in (a) is a linear regression fitted to the data: $y = -0.25 + 0.08x$, $P < 0.01$. The line in (b) is an asymptotic function fitted to the data: $y = 121 + 72950 \times [1 - \exp(-0.00007x)]$, $P < 0.01$.

31% of the total daily transpiration, consistent with estimates of 10–50% obtained using other approaches in different tree species and ecosystems (e.g. Holbrook & Sinclair 1992; Goldstein *et al.* 1998; Phillips *et al.* 2003).

Capacitance and tissue biophysical properties

The sapwood and the outer parenchyma exhibited different biophysical properties. The sapwood had less negative osmotic potentials at full and zero turgor, as well as a smaller bulk elastic modulus than the outer parenchyma. A lower elastic modulus implies greater elasticity according to pressure–volume theory (Tyree & Hammel 1972). According to the terminology used by Holbrook (1995), elasticity refers to the relative differences in volume change due to the contractions and expansion of the storage tissue when water is being withdrawn and recharged. In this context,

xylem tissue has been considered to be a more rigid and less elastic water storage compartment compared with parenchyma tissue, because the living cells in parenchyma tissue can presumably undergo a relatively large change in volume with changes in symplastic water content. However, in addition to the xylem conduits, the sapwood contains xylem parenchyma and ray cells among other cell types that can function in water storage. These living cells can undergo substantial changes in volume with relatively small changes in turgor and are well suited as intracellular water storage compartments (Holbrook 1995). Extracellular water stores include water retained within intercellular spaces and the lumens of cavitated xylem conduits (Tyree & Yang 1990). Water released by cavitation may account for a certain fraction of the readily available extracellular water in the sapwood (Zimmermann 1983; Tyree & Yang 1990). The effectiveness of the outer parenchyma tissue as a water reservoir was apparently limited by its high rigidity (high elastic modulus) (Table 2), which led to steeper declines in Ψ in outer parenchyma than in sapwood as water was extracted (Fig. 3). Sapwood and outer parenchyma water release curves eventually intersected, but at values of Ψ well below those experienced *in vivo*.

Species-specific sapwood capacitance decreased linearly with increasing sapwood density. This inverse relationship between wood density and sapwood capacitance has already been noted in other studies (Meinzer *et al.* 2003, 2006). An inverse relationship between sapwood capacitance and wood density is perhaps not surprising given the
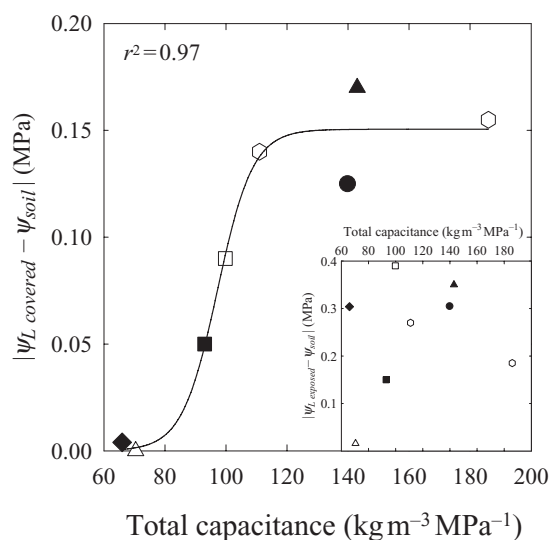

Figure 8. Species-specific pre-dawn disequilibrium between plant and soil water potential (Ψ_{soil}) calculated as the absolute value of the difference between pre-dawn leaf water potential (Ψ_L) of covered leaves ($\Psi_{L\ covered}$) and weighted average soil water potential (Ψ_{soil}) as a function of species-specific total capacitance (outer parenchyma + sapwood). The line is a sigmoidal function fitted to the data, $y = 0.15/[1 + \exp(-x - 97)/5.8]$, $P < 0.001$. The insert shows the same relationship using pre-dawn Ψ_L of exposed leaves instead of covered leaves. See Fig. 4 for symbols.

species-independent exponential decline in sapwood saturated water content with increasing wood density (Simpson 1993; Bucci *et al.* 2004). Nevertheless, capacitance increased fourfold from the highest to the lowest wood density observed in the present study (Fig. 4), whereas the corresponding increase in sapwood saturated water content would be about 70%. The species-independent scaling of sapwood capacitance with wood density found in the present study suggests that this easily measured trait is a good predictor of differences in water storage capacity among Cerrado woody species.

Capacitance in relation to Ψ_L, G_t and g_s

Midday Ψ_L, G_t and g_s all increased with stem capacitance per unit tissue volume, or with the relative contribution of stem water storage to total daily transpiration (Figs 6 & 7). Species-independent scaling of Ψ_L, G_t and g_s with capacitance, and of capacitance with wood density, implies that the biophysical properties of stem tissues constrain and regulate suites of functional characteristics leading to a limited number of compatible physiological solutions to selective pressures imposed by environmental stresses in the Brazilian Cerrado. Thus, species-specific values of wood density largely determine stem capacitance and stem water storage capacity contributing to species-specific set points for Ψ_L, G_t and g_s. Increased capacitance, greater stability of plant water balance and higher rates of gas exchange associated with reduced carbon allocation to stem wood should allow greater allocation of carbon to other structures such as roots. Indeed, in a recent phylogenetically independent comparison, Cerrado species have substantially higher root-to-shoot ratios than their nearby forest congeners (Hoffmann, Orthen & Franco 2004).

Role of outer parenchyma

It is intriguing that the contribution of outer parenchyma to total stem capacitance was relatively low compared with that of the sapwood, despite the relatively large cross-sectional area of the outer parenchyma. Besides being a somewhat effective but small water storage compartment, the outer parenchyma can also serve other functions. The generally more negative osmotic potentials in the outer parenchyma than in the sapwood suggest that the former may serve as a storage reservoir for soluble carbohydrates. Another possibility is that the outer parenchyma also serves as a thermal insulator for protecting stem tissues, such as the vascular cambium and phloem, from damage by frequent fires. The large cells of the outer parenchyma contain a relatively large amount of water with high heat capacity, which can dampen temperature fluctuations inside the outer parenchyma. This may add an additional protection against the brief periods of high temperatures, which is typical of Cerrado fires where the fuel load is mostly the herbaceous layer.

Besides Cerrado trees, other growth forms contain large amounts of parenchymatous tissue in their stems that function as sources of water for transpiring leaves. In particular, the well-developed inner and outer parenchyma tissues of *Fouquieria columnaris*, a tree species endemic to arid and semi-arid regions of Mexico, can supply the leaves and transpiring stems with sufficient water for several weeks during long dry seasons (Franco-Viscaino, Goldstein & Ting 1990; Nielsen *et al.* 1990). Caulescent giant rosette species growing in tropical alpine environments have a voluminous succulent pith consisting of parenchyma cells located in the centre of their woody stem, which is thermally insulated by a thick layer of marcescent leaves to prevent freezing overnight (Goldstein *et al.* 1984; Meinzer & Goldstein 1986). The amount of water released from pith water storage into the transpiration steam of giant rosette plants at high elevations is adequate for avoiding leaf water deficits in the early morning when soil water is frozen and root water uptake is impaired by low temperatures (Goldstein *et al.* 1984).

Capacitance and nocturnal transpiration

Nocturnal transpiration has been studied in relatively few species because until recently, it has generally been assumed that stomata are uniformly closed at night and/or that water vapour near the leaf surface is at or near saturation. However, some recent studies with temperate and tropical woody species indicate that under certain environmental conditions, nocturnal water loss can be relatively high (Benyon 1999; Donovan *et al.* 1999; Oren *et al.* 1999; Sellin 1999; Bucci *et al.* 2004; Scholz *et al.* 2006). In Brazilian savannas, night-time values of relative humidity as low as 40–50% during the dry season result in substantial nocturnal transpiration in woody species that accounts for 13–28% of their total daily transpiration and contributes to pre-dawn disequilibrium in Ψ between leaves and soil (Bucci *et al.* 2004, 2005). In this study, higher total water storage capacity was associated with larger pre-dawn water potential disequilibrium between plants and soil. At night, incompletely recharged internal water storage compartments are likely to compete with transpiring leaves as sinks for water taken up from the soil. Incomplete recharge of internal water storage may explain the failure to achieve complete equilibration in species with large water storage capacity. Cerrado species with small stem water storage capacity were able to achieve soil-to-leaf equilibration at the end of the night-time period.

CONCLUSIONS

Water stored in the sapwood appears to play a dominant role in the regulation of diurnal water deficits of Cerrado tree species. Although Cerrado trees exercise strong stomatal control of transpiration, partial stomatal closure during the day is not sufficient for preventing water stress (Meinzer *et al.* 1999; Bucci *et al.* 2005). Even though many Cerrado woody species have dimorphic root systems with roots that access deep and stable soil water sources (Jackson *et al.* 1999; Scholz *et al.* 2002; Bucci *et al.* 2005), root hydraulic resistances along the soil–stem pathway, and the

high evaporative demands in particular during the dry season, can result in imbalances between water demand by the transpiring leaves and the soil water sources. Unless there are compensatory mechanisms such as the presence of intermediate water sources in the stem and other tissues, which can help improve delivery of water to the leaves, excessive water deficits may occur. Most Cerrado species exhibit homeostasis in maximum water deficits with midday water potential being nearly identical in the wet and dry seasons (Bucci *et al.* 2005). The positive relationships between stem water storage capacity and minimum leaf water potential and G_t across the different species in this study suggest that the daily fluctuations in water deficits of Cerrado woody species are substantially constrained by the presence of stem water storage tissues.

ACKNOWLEDGMENTS

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