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Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water

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Summary Relationships between diel changes in stem expansion and contraction and discharge and refilling of stem water storage tissues were studied in six dominant Neotropical savanna (cerrado) tree species from central Brazil. Two stem tissues were studied, the active xylem or sapwood and the living tissues located between the cambium and the cork, made up predominantly of parenchyma cells (outer parenchyma). Outer parenchyma and sapwood density ranged from 320 to 410 kg m^{-3} and from 420 to 620 kg m^{-3}, respectively, depending on the species. The denser sapwood tissues exhibited smaller relative changes in cross-sectional area per unit change in water potential compared with the outer parenchyma. Despite undergoing smaller relative changes in cross-sectional area, the sapwood released about 3.5 times as much stored water for a given change in area as the outer parenchyma. Cross-sectional area decreased earlier in the morning in the outer parenchyma than in the sapwood with lag times up to 30 min for most species. The relatively small lag time between dimensional changes of the two tissues suggested that they were hydraulically well connected. The initial morning increase in basal sap flow lagged about 10 to 130 min behind that of branch sap flow. Species-specific lag times between morning declines in branch and main stem cross-sectional area were a function of relative stem water storage capacity, which ranged from 16 to 31% of total diurnal water loss. Reliance on stored water to temporarily replace transpirational losses is one of the homeostatic mechanisms that constrain the magnitude of leaf water deficits in cerrado trees.

Keywords: capacitance, cerrado, electronic dendrometers, plant-water relations, sap flow, sapwood.

Introduction

Daily variations in stem diameter of trees may reflect any of four processes: irreversible radial growth, reversible dehydration/rehydration of living-cells, thermal expansion and contraction, and expansion of xylem tissue due to relaxation of internal tensions (Daudet et al. 2005). Recurrent shrinking and swelling associated with fluctuating hydration may greatly exceed dimensional changes associated with the other three processes (Kozlowski 1971). Short-term reversible changes in tree stem diameter have previously been attributed to variation in stem water content and have been used to draw inferences about water flow and tensions in the xylem (Garnier and Berger 1986, Irvine and Grace 1997, Zweifel et al. 2001, Perämäki 2005). Under steady-state environmental conditions, variations in stem diameter and leaf water potential should be closely related, but under non-steady-state conditions typical of the field environment, diurnal changes in stem diameter may lag changes in leaf water potential by minutes or hours (Garnier and Berger 1986, Génard et al. 2001). The time lag between the onset of transpiration and the initiation of sap flow or stem shrinkage at the base of the tree has been attributed to the time required for the capacitive release of water from stem water storage compartments (Schulze et al. 1985, Steinberg et al. 1990, Goldstein et al. 1998, Zweifel and Häsler 2001, Perämäki et al. 2001, Steppe and Lemeur 2004).

Information on the relative contributions of different stem tissues to reversible changes in diameter is scarce. Some authors have assumed that diurnal changes in stem diameter are localized in the phloem and bark rather than in the xylem (Moltz and Klepper 1973, Hinckley and Bruckerhoff 1975, Zweifel et al. 2001). Early work by Dobbs and Scott (1971) suggested that all short-term diameter changes in Douglas-fir stems originated in the living tissues external to the xylem. Richards (1973) and Brough et al. (1986) estimated that at least 75% of the observed daily diameter changes in Sitka spruce and apple trees could be attributed to the extensible tissues external to the xylem. In contrast, Neher (1993) suggested

that a more substantial proportion of reversible stem diameter changes were localized in the xylem. These reversible changes in xylem dimensions can result from diurnal cycles of dehydration/rehydration as well as elastic deformation involving little water loss, particularly in the non-conducting xylem tissue (Irvine and Grace 1997). To our knowledge, no studies have attempted to quantitatively partition rapidly reversible, hydration-based changes in stem diameter between the xylem and the more elastic tissues surrounding it (but see Sevanto et al. 2002). This information is of importance for understanding the relative roles of different stem tissues in long-distance water transport and storage. The trees of the central Brazilian savannas (cerrado) are ideal model systems for this type of study because most species have conspicuous layers of complex tissues surrounding the xylem that include not only the active and inactive phloem but also several layers of parenchyma between the dead bark tissue of the periderm and the cambium, which can comprise more than 35% of the stem cross-sectional area (Coradin 2000). The anatomical characteristics of these living tissues are distinct from the internal xylem and external dead bark layers. Hereafter, this tissue will be called outer parenchyma, because of the prevalence of relatively large parenchyma cells or similar cell types that retain a living symplast on completion of ontogeny.

The objective of our study was to assess the magnitude and dynamics of diurnal changes in stem expansion and contraction in relation to the discharge and refilling of stem water storage compartments in six dominant cerrado tree species. In particular, two stem regions were studied, the active xylem or sapwood, and the living outer parenchyma between the cambium and the cork. Diurnal as well as seasonal changes in stem diameter were continuously monitored with electronic dendrometers. In addition, biophysical properties of both tissues such as water potential, density, pressure–volume relationships, water content and osmolarity were measured and used to estimate the water storage capacity of these potential storage compartments and their contribution to total diurnal water loss. The general validity of these estimates was assessed by studying time lags in diameter changes between terminal branches and the base of the main stem and between sapwood and outer parenchyma tissues in relation to water fluxes measured with sap flow probes. The potential consequences of changes in water storage capacity for apparent soil-to-leaf hydraulic resistances were explored with numerical simulations.

Materials and methods

Study site and plant material

The study was conducted in a savanna site with high tree density (cerrado denso, ~2880 trees ha^{-1}) and a savanna site with low tree density (campo cerrado, ~1300 trees ha^{-1}) 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). Mean annual precipitation is about 1400 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 old deep oxisols consisting of about 72% clay. Despite their high percentage of clay, the soils are extremely well drained.

Six 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* var. *subvelutinum* Vog., which flushes new leaves during the wet season. The brevideciduous trees (*Blepharocalyx salicifolius* (H.B. & K.) Berg and *Caryocar brasiliense* Camb.) are functionally evergreen because they seldom remain leafless for more than a few days. However, most evergreen species also show progressive leaf senescence and abscission during the dry season. *Kielmeyera coriaceae* (Spr.) Mart. remains leafless for about a month depending on the severity of the dry season. Measurements were carried out during the wet and dry seasons of 2002–2003. Basal diameter, outer parenchyma and sapwood area and height of the study trees are indicated in Table 1.

Partitioning of stem diameter changes

The xylem and outer parenchyma were considered as coaxial cylinders that were able to swell and shrink in response to radial water flow without being constrained by the dead tissue external to the bark. Electronic dendrometers (Models DEX70 and DEX100, Dynamax, Houston, TX) were installed near the base of the main stem and on a terminal branch of one tree per species. To monitor whole-stem diameter fluctuations, the

Table 1. Leaf phenology, basal diameter, outer parenchyma area, sapwood area and height of the trees studied. Values are means ± SE (n = 3).

Species	Family	Phenology	Basal diameter (mm)	Outer parenchyma area (10^{-4} m^2)	Sapwood area (10^{-4} m^2)	Height (m)
K. coriaceae	Guttifferae	Deciduous	57 ± 4	10.6 ± 0.3	10.8 ± 0.4	3.1 ± 0.2
B. salicifolius	Myrtaceae	Brevideciduous	85 ± 6	29.5 ± 0.5	24.4 ± 0.6	3.6 ± 0.1
C. brasiliense	Caryocaraceae	Brevideciduous	135 ± 9	77.1 ± 0.8	41.0 ± 0.7	3.9 ± 0.1
S. macrocarpa	Araliaceae	Evergreen	143 ± 4	63.8 ± 0.4	54.8 ± 0.4	4.4 ± 0.3
S. paniculatum	Leguminosae	Evergreen	102 ± 7	50.3 ± 0.5	22.4 ± 0.7	5.5 ± 0.6
V. thyrsoidea	Vochyseaceae	Evergreen	93 ± 4	15.1 ± 0.4	22.0 ± 0.4	6.3 ± 0.9

bark was smoothed before dendrometer attachment and then covered with rubber foam insulation and aluminum foil to minimize heating by direct solar radiation. To monitor sapwood diameter fluctuations, the sapwood surface was exposed by removing the outer parenchyma from two small areas on opposite sides of the stem. A third dendrometer was installed on a terminal branch to measure whole-stem diameter fluctuations without a distinction between sapwood and outer cortex. In *K. coriaceae*, a dendrometer was also installed on a lateral root. Changes in diameter were measured during several days in the wet and dry seasons. Data were obtained every 10 s, and 10-min means were recorded with a data logger (CR10X, Campbell Scientific, Logan, UT), taking the signals from the full-bridge strain gage attached to the flexible frame of the caliper-style dendrometers. The frame temperature was measured with copper-constantan thermocouples to correct for the effect of temperature on the expansion of the frame. The temperature correction factor obtained in the laboratory was 0.046 mm per 20 °C, similar to the temperature correction specified by the manufacturer. To asses the potential importance of the thermal expansion of green wood on the estimated diurnal dimensional changes, dendrometers were installed on cut stems with the ends sealed and the whole stem wrapped in aluminum foil to prevent water loss. These stem sections with dendrometers installed were placed next to the dendrometers installed on intact stems to ensure similar temperature and light conditions. The temperature-corrected diameter measurements were converted to areas and normalized by the initial area (normalized ΔA) of each tissue to obtain comparable, size-independent measurements of daily dimensional changes. The patterns of variation of the cut stems and intact stems were practically identical, indicating that the thermal expansion of green wood was negligible and consequently it was not considered. Because of limitations on the availability of equipment, dendrometer measurements were made on two individuals simultaneously before removing the dendrometers and reinstalling them on another pair of individuals.

Tissue water content, solute concentration, density and symplastic water fraction

Water content of the sapwood and the outer parenchyma was measured near the base of the trunk in three individuals per species. Samples were collected with an increment borer between 0600 and 2100 h day during the dry season, sealed in aluminum foil and plastic bags and taken to the laboratory. Fresh mass was determined and the sample oven dried at 80 °C for 72 h to determine dry mass. Water content was calculated as $(W_f - W_d)/W_d$, where W_f is fresh mass and W_d is dry mass of the sample.

Solute concentration (osmolality) of the outer parenchyma was estimated with a vapor pressure osmometer (VAPRO 5520, Wescor, Logan, UT). Three samples of outer parenchyma per species were collected with an increment borer in the early morning and afternoon of the same day, sealed in aluminum foil and plastic bags and taken to the laboratory. The samples were frozen and thawed, then crushed, and an aliquot of the expressed sap was sealed in the chamber of the osmometer for osmolality determinations.

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 dry mass of the sample and V is sample volume. Volume was estimated by submerging the sample in a container filled with distilled water resting on a digital balance with 0.001 g precision. The sample was kept submerged during measurements until saturation with the help of a small needle and care was taken to ensure that it did not touch the walls of the container.

To determine the symplastic water fraction, cylinders of outer parenchyma and sapwood were obtained with a 5-mm increment borer near the base of the trunk of three trees per species and sealed in glass vials. In the laboratory, the samples were allowed to hydrate in distilled water. After 2 h, the tissue samples were cut into 10-mm segments, quickly blotted to remove excess water, placed in the caps of thermocouple psychrometer chambers (JRD Merrill Specialty Equipment, Logan, UT), weighed, and then sealed 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). 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, reweighed in the psychrometer caps, resealed inside the psychrometer chambers and allowed to equilibrate before another determination of water potential. The inverse of water potential was plotted against relative water content to create a pressure–volume curve (Tyree and Hammel 1972) and the symplastic water fraction was determined by extrapolating the linear portion of the curve to the x-axis. Values from three replicate curves per species were pooled.

Sapwood, stem and leaf water potential

Daily courses of sapwood water potential (Ψ_{sw}) were measured during the dry season in one tree per species with in situ stem psychrometers (Plant Water Status Instruments, Guelph, Ontario, Canada) and recorded with a data logger (Model CR-7, Campbell Scientific) at 10-min intervals. Care was taken to install the psychrometers on the most shaded portion of the trunk to minimize temperature gradients. The values obtained during the first day after installations 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, Corvallis, OR). Leaf samples were immediately sealed in plastic bags after excision and kept in a cooler until balancing pressures were determined in the laboratory within 1 h of sample collection. Measurements were obtained on three to five leaves per tree ($n = 3$) at dawn, midday and throughout the day.

Sap flow and hydraulic resistance

Whole-plant sap flow was measured by the heat dissipation method (Granier 1985, 1987) on several consecutive days during the dry season in the same trees on which dendrometers were installed. 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 of each plant. The upper (downstream) probe was continuously heated at a constant power by the Joule effect, whereas 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 means were stored in solid-state storage modules (Model SM192, Campbell Scientific) connected to data loggers (Model CR 10X, Campbell Scientific).

Sap flow was calculated from the temperature difference between the probes based on an empirical calibration (Granier 1985, 1987), revalidated for tropical trees (Clearwater et al. 1999). The measured temperature differences between the probes were corrected for natural temperature gradients (Do and Rocheteau 2002). Mass flow of sap per individual was obtained by multiplying sap flow 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 cm above the point of dye injection and the area of conducting tissue was determined from the pattern of staining by the dye as it moved in the transpiration stream (Meinzer et al. 1999). The sap flow probes were also installed in upper branches that were at least 40 mm in diameter. The mean cross-sectional area of active xylem in all trees studied was 0.0029 m^2, and the mean thickness of the sapwood was 30 mm, indicating that the 20-mm probes used spanned most of the hydro-active portion of the xylem.

Transpiration per unit leaf area was obtained by dividing the mass flow of sap by the total leaf area per tree. Total leaf area per tree was obtained by counting the total number of leaves per tree then multiplying by the mean area per leaf determined from fresh leaf samples for each tree. Ten to 50 fully expanded sun leaves, depending on the total number of leaves per tree, were collected from three trees per species and their area was determined with a scanner.

Species-specific values of soil to leaf hydraulic resistance ($R_{soil-leaf}$) were calculated as $R_{soil-leaf} = \Delta\Psi/E$, where $\Delta\Psi$ is the difference between the current Ψ_L and the weighted mean Ψ of the soil, and E is the mean 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 = zero based on the Ψ_{sw}–E relationships obtained by simultaneous measurements of Ψ_{sw} and E from predawn through mid-afternoon for each individual (Sperry et al. 2002, Bucci et al. 2005). The linear regressions fitted to the Ψ_{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 stream on estimates of $R_{soil-leaf}$ was minimized by calculating $R_{soil-leaf}$ with information obtained between 1200 and 1300 h.

Stem water storage and time lag calculations

Branch sap flow rates were used to obtain whole-crown transpiration according to Goldstein et al. (1998). Whole-crown transpiration was estimated by normalizing branch sap flow by the mean daily maximum value. Normalized branch sap flow was then divided by the daily sum of the normalized 10-min means divided by six (for the six 10-min intervals per hour), then multiplied by the total daily sap flow measured at the base of the tree, which was assumed to be equal to total daily transpiration. This procedure yielded estimated rates of crown transpiration on an hourly basis. Total diurnal stem water storage capacity was estimated by subtracting 10-min means of basal sap flow from whole-crown sap flow when basal sap flow was less than whole-crown flow, summing the differences, then dividing by six. This value was used to calculate daily use of stored water as a percentage of total daily water use per tree. Time lags were calculated by finding the highest cross correlation of time series between (1) diameter changes of cortex and sapwood, (2) diameter changes of cortex or sapwood and basal sap flow and (3) between stem diameter changes at the base of the tree and in terminal branches (Sevanto et al. 2002).

Results

Stem diameter exhibited marked diurnal variations during the wet season characterized by continuous decreases in size during the morning, with minimum stem diameter occuring in the afternoon (Figure 1). Subsequently, the stem expanded to reach its initial maximum diameter again in the early morning of the next day. These temporal variations reflected mostly water utilization from, and recharge of, the storage compartments in the region of the stem where the dendrometers were attached. During the dry season, however, overnight recharge of internal water storage was apparently incomplete. Diurnal stem shrinkage and expansion were observed in all species with similar patterns to those observed during the wet season, but maximum stem diameter decreased with time and never recovered to the previous nighttime maximum diameter (Figure 1). Diel fluctuations in stem diameter were more pronounced during the dry season than during the wet season. In *Kielmeyera coriaceae*, for example, the maximum diel fluctuation in diameter was about 275 µm during the dry season and only 110 µm during the wet season.

Diurnal fluctuations in stem cross-sectional area (normalized by the initial cross-sectional area to remove differences due to stem sizes) of two deciduous tree species were substantially smaller in leafless trees during the dry season compared with the same trees with full leaf crowns during the wet season (Figure 2). A large decrease in stem size began at about 0900 h in the wet season, 1 to 2 h after the increases in evaporative demand at sunrise. Normalized variations in root, trunk and

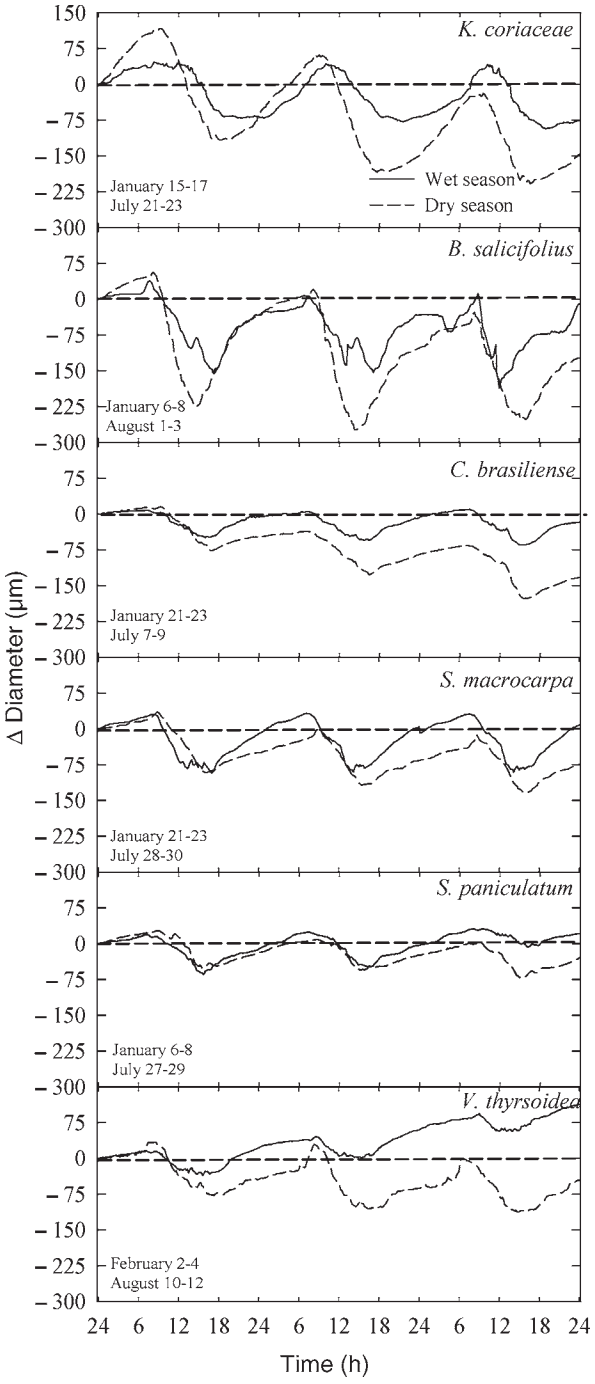

Figure 1. Diurnal changes in basal stem diameter (outer parenchyma + sapwood) in six dominant cerrado tree species during 3 days of the dry (July and August) and wet (January and February) seasons of 2003. Positive values indicate stem expansion and negative values indicate stem contraction.

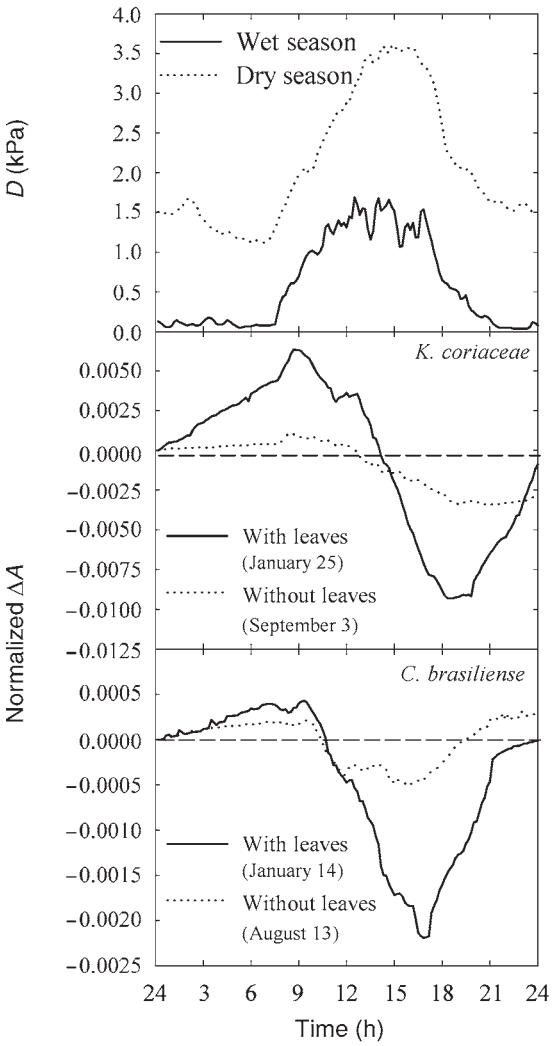

Figure 2. (a) Typical courses of air saturation deficit (*D*) during the dry and wet seasons and typical daily time courses of changes in normalized stem cross-sectional area (ΔA, outer parenchyma + sapwood) of (b) *Kielmeyera coriaceae* and (c) *Caryocar brasiliense* trees for periods of maximum leaf surface area during the wet season (January) and leafless periods during the dry season (August and September).

branch cross-sectional areas in *K. coriaceae* from July 7 to August 2, 2002 are depicted in Figure 3. The daily variations in cross-sectional area were initially similar in roots, stem and branches. However, mean root cross-sectional area increased on July 13, presumably because of the onset of root growth, whereas the branch cross-sectional area fluctuations remained about constant and the trunk cross-sectional area exhibited a small decline on July 17 but then remained constant. The morning decline in cross-sectional area started first in the branch (0800 h), then in the stem (0920 h) and finally, with a lag of about 2 h, in the root (1000 h) (inset in Figure 3).

Diurnal fluctuations in cross-sectional area of the outer parenchyma and sapwood had similar overall patterns, but the relative magnitudes of the variation were larger in the outer parenchyma than in the sapwood (Figure 4). Basal sap flow increased after sunrise between 0600 and 0900 h, depending on the species, to reach a maximum at about 1200 h (Figure 4). The decline in sap flow occurred immediately after reaching a maximum in *C. brasiliense*, *S. macrocarpa* and *V. thyrsoidea*, but in the other species sap flow remained more or less constant for about 3 h before declining later in the afternoon. In all species, with the exception of *B. salicifolius*, the morning de-

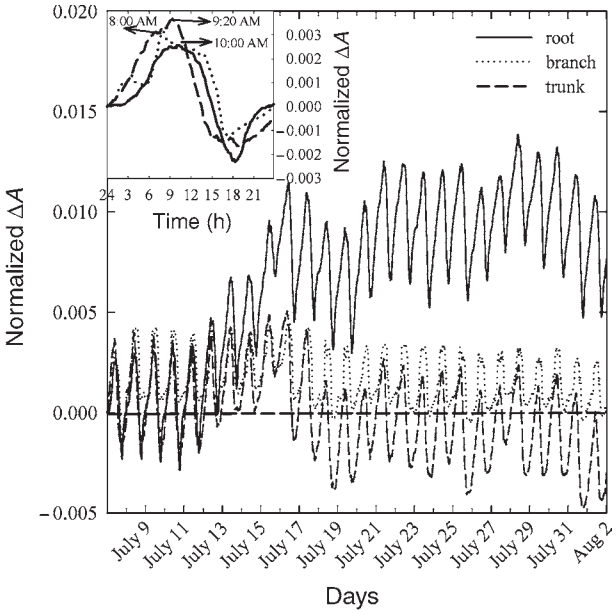

Figure 3. Normalized variations in cross-sectional area (ΔA) for a root, trunk and branch in *Kielmeyera coriaceae* during the dry season. The initial cross-sectional area on July 7, 2002 was referenced to zero. The inset shows variation in cross-sectional area on July 7, 2002.

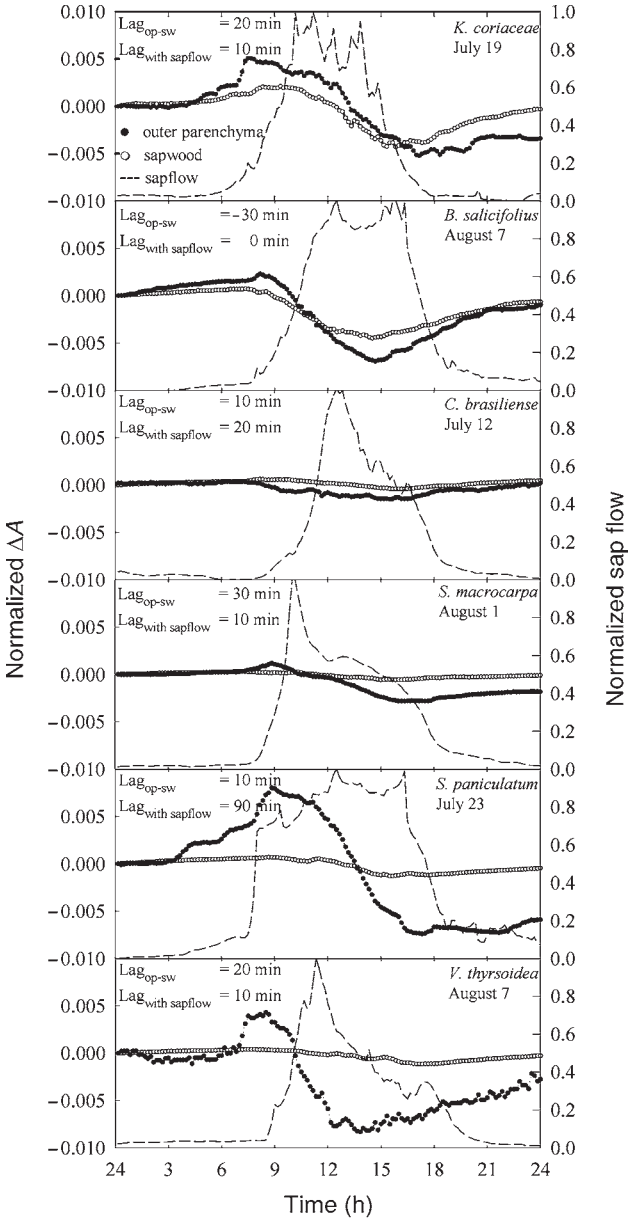

Figure 4. Variations in basal sapwood cross-sectional area (○), basal outer parenchyma cross-sectional area (●), and basal sap flow (dashed lines) in six representative cerrado woody species during one day of the dry season (July and August of 2003). Changes in cross-sectional area of each stem tissue were normalized by initial cross-sectional area, and sap flow values were normalized by the maximum value attained during the daytime. The initial cross-sectional area at midnight was referenced to zero. The lag times between initial declines in basal outer parenchyma (op) and basal sapwood (sw) cross sectional area in the morning (lag_{op-sw}), and lag time observed between basal cortex or sapwood decline and the increase in morning basal sap flow ($lag_{with\ sapflow}$) are shown in each panel. For the outer parenchyma versus sapwood lag times, a negative time means that the outer parenchyma decrease lags behind the sapwood morning decline.

cline in cross-sectional area started between 10 and 30 min earlier in the outer parenchyma than in the sapwood (Figure 4). The morning decline in cross-sectional area of the outer parenchyma lagged behind the initial morning increase in sap flow by 10 to 90 min, depending on the species (Figure 4).

The relative changes in sapwood and outer parenchyma cross-sectional area were positively correlated with sapwood and outer parenchyma water contents during the day (Figure 5). Data for all species conformed to a single relationship between outer parenchyma cross-sectional area and water content (Figure 5a). However, the dependence of changes in sapwood cross-sectional area on water content was species-specific for *Kielmeyera coriaceae*, *Schefflera macrocarpa* and *Vochysia thyrsoidea*, and nonsignificant for the remaining species (Figure 5b). Because the slopes of the regressions for *K. coriaceae*, and *S. macrocarpa* were not significantly different, only one regression was fitted for the combined data of both species. Water content was nearly always higher in the outer parenchyma than in the sapwood across all species, with sapwood water content ranging from 0.6 to 1.5 g g^{-1}, and outer parenchyma water content ranging from 1.0 to 2.2 g g^{-1} (Figure 5). Differences between the morning maximum and afternoon minimum outer parenchyma water content were significant in all species (Figure 6a). The osmolality of the outer parenchyma was significantly higher when its water content reached its minimum value in the afternoon (Figure 6b).

The rate of change in normalized cross-sectional area of sapwood or outer parenchyma per unit change in sapwood water potential (normalized $\Delta A/\Delta\Psi$) decreased exponentially with increasing tissue density (Figure 7). The outer parenchyma water potential was assumed to be in equilibrium with that of the sapwood for this analysis. In order to avoid hysteretic effects, the plot in Figure 7 contains only the data obtained during the morning when both water potential and

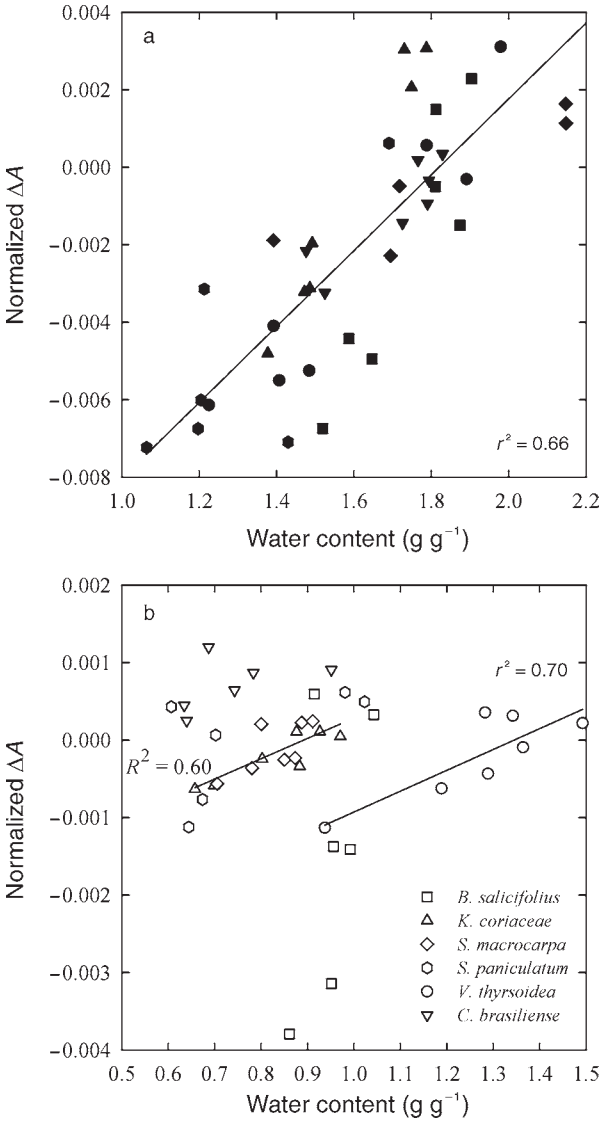

Figure 5. Relative changes in (a) normalized outer parenchyma cross-sectional area and (b) normalized sapwood cross sectional area in relation to the water content of each tissue type. Each value represents cross-sectional area ($n = 1$) and water content ($n = 3$) measured at different times during the course of one day during the dry season for six cerrado species. Only one linear relationship was fitted to the outer parenchyma data (a), whereas two linear regressions were fitted for the sapwood data (b): one for *Vochysia thyrsoidea* and another for the combined data for *Kielmeyera coriaceae* and *Schefflera macrocarpa*. In the remaining three species, the linear regressions for the sapwood data were not statistically significant ($P > 0.1$).

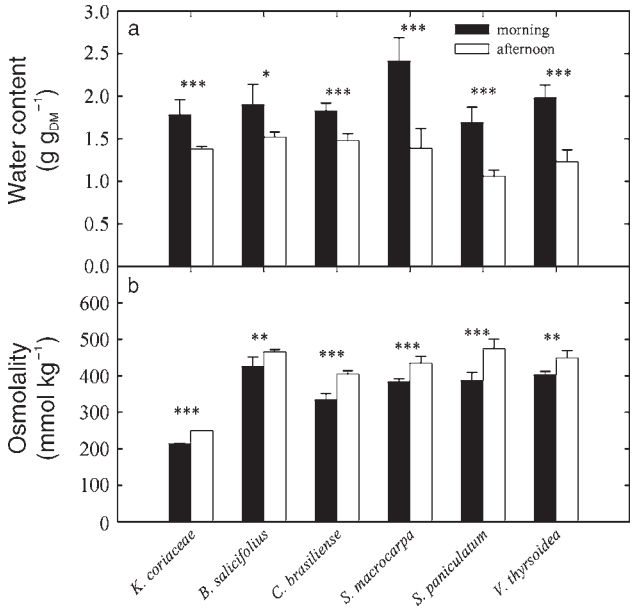

Figure 6. (a) Daily maximum and minimum outer parenchyma water content and (b) osmolality (mmol kg^{-1}) for six cerrado tree species. Bars are means values (+SE) for three replicates per species. Asterisks indicate significant differences: *, $P < 0.1$; **, $P < 0.01$; and ***, $P < 0.001$.

outer parenchyma cross-sectional area were decreasing. The denser sapwood tissue exhibited a smaller change in cross-sectional area per unit change in water potential than the less dense outer parenchyma tissue.

In all species, the cross-sectional areas of the trunk and branches increased overnight, then began to decline in the morning, reaching minimum values in the mid- to late afternoon (Figure 8, left). However, the morning decline in cross-sectional area of the trunks lagged that of the terminal branches by 10 to 100 min, depending on the species. Trunk and branch sap flows increased rapidly in the morning and decreased sharply in the afternoon (Figure 8, right). However, the initial morning increase in basal sap flow lagged behind

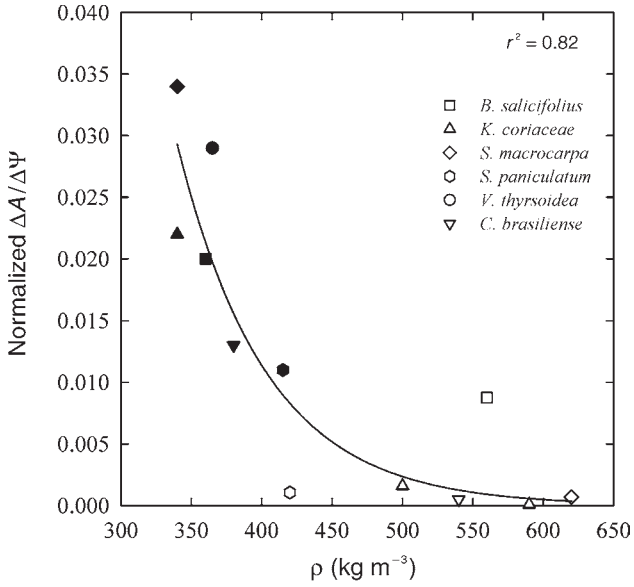

Figure 7. Rate of change in normalized cross-sectional area of sapwood or outer parenchyma per unit change in water potential (Normalized $\Delta A/\Delta\Psi$) as a function of sapwood (open symbols) or outer parenchyma (filled symbols) density (ρ) for six cerrado tree species. The line is an exponential function fitted to the data ($y = 0.0012 + 11\exp(-0.018x)$, $P < 0.001$).

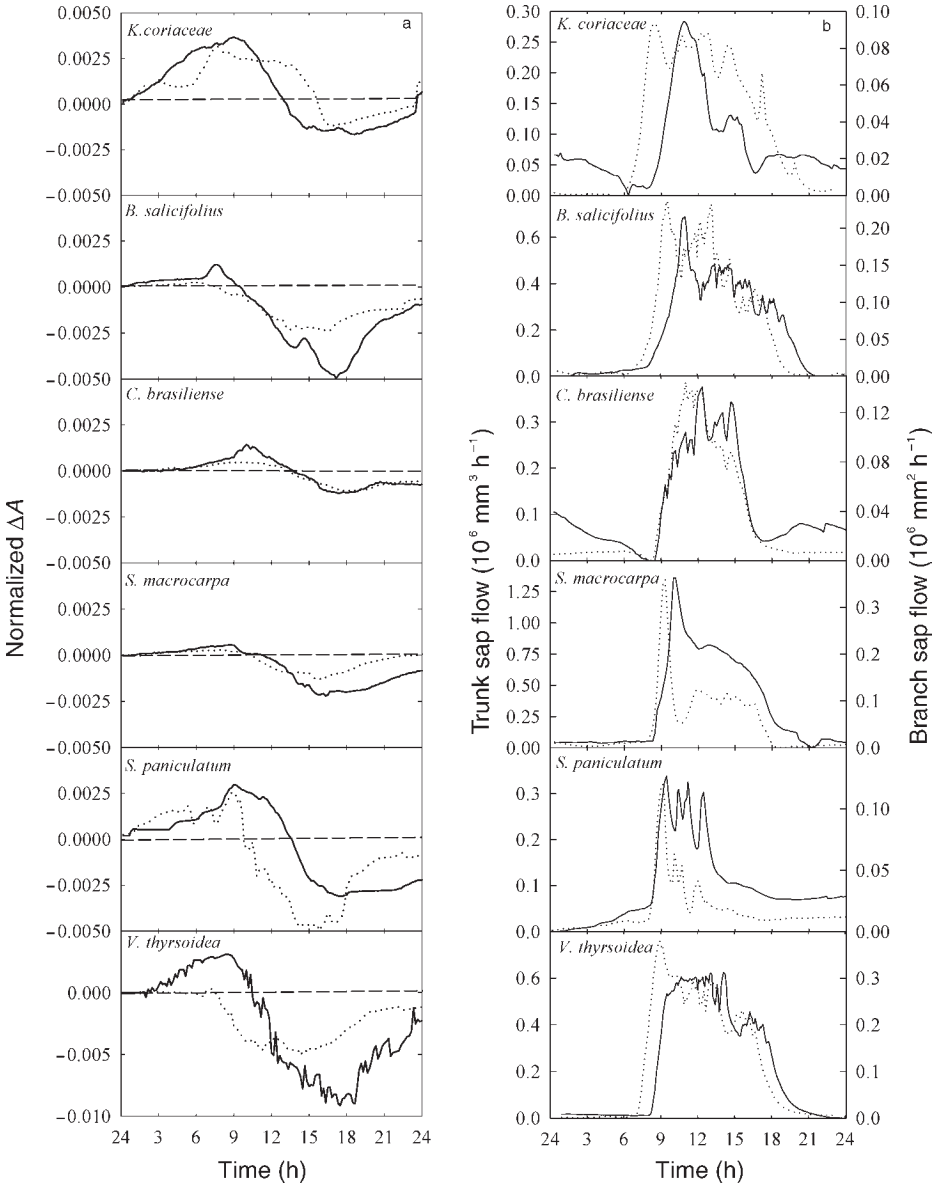

Figure 8. (a) Daily changes in trunk (solid line) and branch (dotted line) normalized cross-sectional area (ΔA), and (b) daily courses of trunk (solid line) and branch (dotted line) sap flow during one day of the dry season (July and August) in six cerrado woody species.

that of branch sap flow by about 10 to 130 min. In *Cayocar brasiliense* and *Schlerolobium paniculatum*, the initial morning increase in sap flow was observed almost simultaneously in the crown and at the stem base. The lag time between the initial declines in branch and trunk cross-sectional areas in the morning increased with increasing contribution of internal water storage to total daily water use (Figure 9a), whereas the apparent $R_{soil-leaf}$ decreased linearly with increasing relative reliance on internal water storage (Figure 9b).

Apparent $R_{soil-leaf}$ ranged from 0.5 MPa s m^2 $mmol^{-1}$ in *K. coriaceae* to 2.3 MPa s m^2 $mmol^{-1}$ in *S. paniculatum* (Table 2). To predict the apparent $R_{soil-leaf}$ when water storage capacity was partially depleted, we used the linear function fitted to the relationship between $R_{soil-leaf}$ and water storage capacity (Figure 9b), and assumed for this numerical exercise that E remained constant. With a hypothetical reduction of 30% in stem water storage capacity, $R_{soil-leaf}$ increased up to three times, depending on the species (Table 2). Saturated water content was always higher in the outer parenchyma than in the sapwood, but the symplastic water fraction (SF) tended to be higher in the sapwood. Species-specific minimum sapwood water potentials attained during the day ranged from –0.28 to –1.17 MPa (Table 2).

Discussion

Tissue expansion and contraction and radial water movement

According to the cohesion theory, when rates of water loss are substantial during the daytime, transpiration creates a tension (negative pressure) gradient that pulls water up through the xylem (Nobel 1991). The water is transported axially to the sites of evaporation in the leaves along a complex pathway that in-

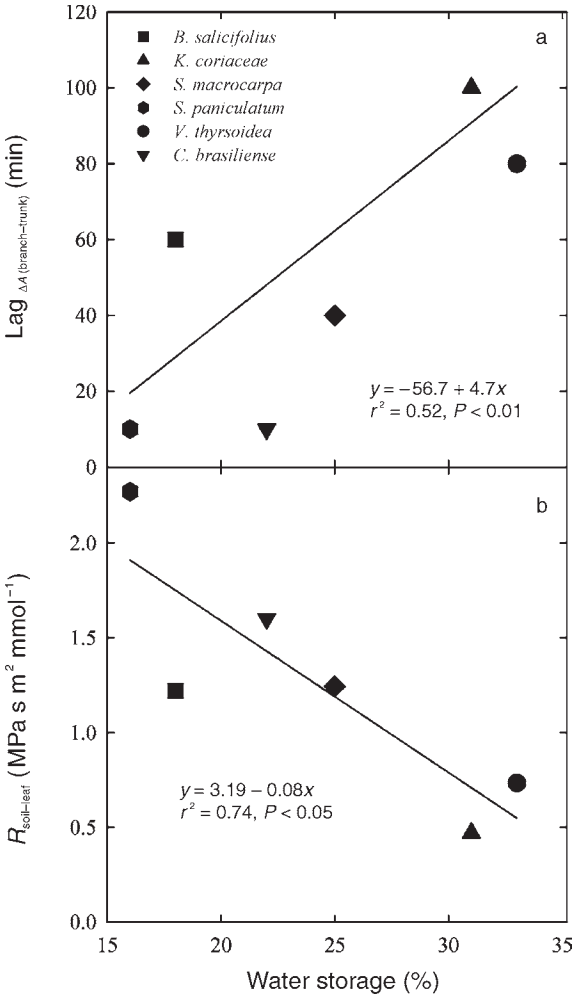

Figure 9. (a) The lag time between the initial morning decline in branch and trunk cross-sectional area and (b) apparent soil-to-leaf hydraulic resistance ($R_{soil–leaf}$) in relation to the relative contribution of internal water storage to total daily water use. The lines are linear regressions fitted to the data.

cludes not only the xylem conduits, but also radial exchange of water between the xylem and surrounding tissues. Because both the sapwood and outer parenchyma tissues exhibit a certain degree of elasticity, varying tensions should be reflected in dimensional changes of both tissues. The modulus of elasticity of wood, a measure of its stiffness, is positively correlated with its density (van Gelder et al. 2006). Thus, even though density is not a direct measure of elasticity it may reflect potential differences in radial elastic bending of a tissue subjected to a given amount of tension. In this study, outer parenchyma density ranged from 320 to 410 kg m^{-3} and sapwood density from 420 to 620 kg^{-3}, implying that the sapwood was more rigid than the outer parenchyma tissue. Consistent with this, the denser sapwood exhibited smaller changes in cross-sectional area per unit of change in water potential (normalized $\Delta A/\Delta\Psi$) compared with the less dense outer parenchyma tissue (Figure 7). If this relationship holds for all cerrado species, then tissue water potentials can be predicted with information on tissue density and changes in cross-sectional area.

The large daily amplitude of the variation in whole-stem diameter (e.g., 200 μm) in cerrado trees resulted mainly from the release or recharge of water from sapwood and outer parenchyma tissues because these variations were related to changes in tissue water content. We found that changes in tissue cross-sectional area were consistently associated with changes in outer parenchyma water content, and in three of the study species with changes in sapwood water content. Although the daily variations in sapwood cross-sectional area were relatively small compared with those of the outer parenchyma, the amount of water released per unit change in cross-sectional area was larger for the sapwood. It can be inferred from the results in Figure 5 that a 0.001 change in normalized cross-sectional area of the outer parenchyma would release 0.1 g of water, whereas for the same change in normalized sapwood cross-sectional area the amount of water released would be about 0.35 g. For the three species that exhibited no statistically significant positive relationship between changes in sapwood cross-sectional area and changes in sapwood water content, tension may have been an important determinant of the observed tissue dimensional changes. Perämäki et al. (2005) developed a non-steady-state model of water tension propagation in tree stems based on the assumption that fluctuating water tensions driven by transpiration together with the elasticity of wood cause the variations in the diameter of tree stems. In our study, however, emphasis is on the dynamics of stem water storage withdrawal and recharge rather than on the patterns of

Table 2. Saturated water content and symplastic water fraction (SWF) of the sapwood (sw) and outer parenchyma (op) tissues, minimum sapwood water potential, contribution of internal water storage to total daily water use, and soil-to-leaf hydraulic resistance ($R_{soil–leaf}$) at full (100%) and reduced (70%) water storage capacity, of the trees studied.

Species	Water content (g g^{-1})		SWF		Sapwood water potential (MPa)	Water storage (%)	$R_{soil–leaf}$ (MPa s m^2 $mmol^{-1}$)	
	sw	op	sw	op			100%	70%
K. coriaceae	1.6	3.4	0.55	0.55	−0.58	31	0.47	1.45
B. salicifolius	1.2	2.0	0.63	0.51	−0.28	18	1.22	2.18
C. brasiliense	1.2	3.3	0.57	0.53	−0.72	22	1.60	1.96
S. macrocarpa	1.5	2.1	0.66	0.55	−0.36	25	1.24	1.79
S. paniculatum	1.1	2.3	0.58	0.49	−1.17	16	2.27	2.29
V. thyrsoidea	1.9	2.2	0.68	0.47	−0.65	33	0.73	1.34

pressure fluctuations within the stem of the trees.

Total water content tended to be higher in the outer parenchyma than in the sapwood, but the symplastic water fraction was higher in the sapwood which probably includes water contained in xylem parenchyma. It is possible that, within the operational range of stem tissue water potential, the water released by the sapwood comes preferentially from the symplast, particularly in the early morning. Sapwood water potential did not drop substantially during the day. The species that exhibited the most negative sapwood water potential was *S. paniculatum* and it fell only to about –1.1 MPa. Consequently the water released by embolized sapwood conduits later in the day, when tensions are greater, may represent a small proportion of the total amount of water used from sapwood storage. This hypothesis needs to be tested.

Lag times

Cross-sectional area decreased earlier in the morning in the outer parenchyma than in the sapwood, with sapwood shrinkage lagging up to 30 min behind that of the outer parenchyma in most of the species studied. Although a larger tension may be required to induce a given dimensional change in the denser sapwood, its capacity to deliver stored water to the transpiration stream was more than three times that of the outer parenchyma for a given dimensional change. The relatively small lag times between the tissues for both shrinkage and swelling (discharge and recharge, respectively) suggest that the tissues are well connected hydraulically. Preliminary anatomical studies revealed that the xylem rays extend into the outer parenchyma, which may facilitate radial water transport (data not shown). This finding appears to contrast with observations made by Sevanto et al. (2002) who reported that that whole stem diameter shrinkage always lagged 30 to 110 min behind that of xylem diameter shrinkage in Scots pine trees.

The similarity of lag times between the morning decline in cross-sectional areas of trunks and terminal branches, and between the initial morning increase in sap flows in trunks and branches was consistent with water being withdrawn first from storage compartments closest to the sites of water loss in the crown. Furthermore, lag times between morning declines in branch and trunk cross-sectional areas were a function of the relative contribution of internal water storage to total daily water use (Figure 9). The initial morning increase in sap flow was almost simultaneous in the crown and at the stem base in *C. brasiliense* and *S. paniculatum*, two species with a small water storage capacity. The species-specific time lags of stem diameter changes were, therefore, a function of the storage capacity of the stem tissues along the pathway, similar to findings in other studies (e.g., Whitehead and Jarvis 1981, Herzog et al. 1995). Compared with differences in lag times between trunks and branches, lag times between initial declines in cross-sectional areas of branches and roots were larger. In one of the species studied, root diameter declined about 2 h after diameter changes were first observed in terminal branches and 40 min after changes were observed at the base of the main stem, suggesting that stored water is withdrawn from branches first, then from the main stem and finally from the roots. Roots of cerrado trees are therefore not only the pathway for water entry into the xylem, but also an intermediate water source replacing water loss from leaves and stems due to transpiration in the early morning.

Water storage capacity and axial water transport

The 16 to 31% of total daily water loss contributed by stem water storage compartments represents a relatively small fraction of the total daily water use of cerrado woody species, but it may be important for their water economy. Contribution of stored water estimated from minimum sapwood water potential and pressure–volume relationships of each tissue ranged between 8 to 30% of total daily water loss for the sapwood, and between 1 to 8% of total daily water loss from the parenchyma tissue, depending on the species (Scholz et al. 2007), similar to our findings based on diurnal changes in basal stem versus terminal branch sap flow. Reliance on stored water to temporarily replace transpirational losses is a homeostatic mechanism that constrains the magnitude of leaf water deficits, thereby maintaining carbon assimilation as hydraulic path length and, therefore, hydraulic resistivity increase (Goldstein et al. 1998, Meinzer et al. 2003, Phillips et al. 2003, Bucci et al. 2005). Stem water storage decreased the apparent soil-to-leaf hydraulic resistivity by shortening the pathway from source to sink, when water was being transpired during periods of increasing evaporative demand in the morning. If water storage capacity were decreased by 30%, for example, then the apparent hydraulic resistance would increase substantially (Table 2). To maintain leaf water balance under these conditions, stomatal conductance or total leaf surface area, or both, would have to decrease, which would increase marginal costs for the plant in terms of carbon assimilation and net photosynthesis.

The diurnal dynamics of utilization and recharge of stored water were mostly reflected in dimensional changes in stem tissues. During the wet season, stem diameter fully recovered overnight. During the dry season, however, the stem apparently does not completely rehydrate overnight as indicated by the gradual decline in its daily maximum diameter over several consecutive days. Incomplete overnight recharge of stem water storage during the dry season may have resulted from substantial nocturnal transpiration and from reverse flow associated with hydraulic redistribution by shallow roots, which act as competing sinks for water taken up by deep roots (Bucci et al. 2004, Scholz et al. 2002, Brooks et al. 2006). When the deciduous trees in our study were leafless, the magnitude of the diurnal variation in stem diameter was much less than during the wet season when the leaf surface area was at its maximum, consistent with the idea that several internal sinks compete for water taken up by deep roots (such as transpiring leaves and shallow roots). Similar reductions in the magnitude of stem shrinkage and expansion following leaf fall were found by Baker et al. (2002) for a deciduous species (*Celtis zenkeri*) in Ghana.

Overall, we have demonstrated that daily variations in stem diameters of six cerrado woody species primarily resulted from the depletion and refilling of their extensible tissues. Our observations support the importance of outer parenchyma and

sapwood as water storage compartments, being hydraulically well connected with one another and having relatively low radial resistance to water flow. In cerrado trees, reliance on stored water to temporally replace water loss from leaves can be an important mechanism that partially uncouples leaf water status from the frictional resistance associated with water movement, particularly during the dry season or during days with high air saturation deficits.

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