Plant, Cell and Environment (2003) **26**, 1633–1645

Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels

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ABSTRACT

Diel variation in specific hydraulic conductivity (k_s) was recorded in petioles of two savanna tree species, *Schefflera macrocarpa* and *Caryocar brasiliense*, from central Brazil. These two species have compound leaves with long petioles (10–30 cm). In both species, petiole k_s decreased sharply with increasing transpiration rates and declining leaf water potentials (ψ_L) during the morning. Petiole k_s increased during the afternoon while the plants were still transpiring and the water in the non-embolized vessels was still under tension. Dye experiments confirmed that in both species diel variation in k_s was associated with embolism formation and repair. When transpiration was prevented in individual leaves, their petiole k_s and water potential remained close to their maximum values during the day. When minimum daily ψ_L on selected branches was experimentally lowered by 0.2–0.6 MPa, the rate of k_s recovery during the afternoon was slower in comparison with control branches. Several field manipulations were performed to identify potential mechanisms involved in the refilling of embolized petiole vessels. Removal of the cortex or longitudinal incisions in the cortex prevented afternoon recovery of k_s and refilling of embolized vessels. When distilled water was added to petiole surfaces that had been abraded to partially remove the cuticle, k_s increased sharply during the morning and early afternoon. Evidence of starch to sugar conversion in the starch sheath cells surrounding the vascular bundles of the petioles was observed during periods of rapid transpiration when the abundance of starch granules in the starch sheath cells surrounding the vascular bundles decreased. Consistent with this, petiole sugar content was highest in the early afternoon. The most parsimonious explanation of the field observations and the experimental results was that an increase in osmotically active solutes in cells outside the vascular bundles at around midday leads to water uptake by these cells. However, the concurrent increase in tissue volume is partially constrained by the cortex, resulting in a transient pressure imbalance that may drive radial water movement in the direction of the embolized vessels, thereby refilling them and restoring water flow. This study thus presents evidence that embolism formation and repair are two distinct phenomena controlled by different variables. The degree of embolism is a function of tension, and the rate of refilling a function of internal pressure imbalances.

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Key-words: embolism repair; pressure-confining barriers; refilling mechanisms; starch to sugar conversion; xylem embolism.

INTRODUCTION

Hydraulic conductivity in plants, and therefore long distance water transport, may be affected by embolism in xylem conduits. Theoretically, air emboli should reduce hydraulic conductivity by decreasing the number of active xylem elements. It has been suggested that the reverse process, refilling of conduits, cannot take place unless positive pressure is present in the xylem (Tyree & Sperry 1989; Yang & Tyree 1992; Lewis, Harnden & Tyree 1994). However, recent studies have shown evidence of dynamic changes in xylem hydraulic conductivity even when the xylem water is under considerable tension (Salleo *et al*. 1996; Zwieniecki & Holbrook 1998; Tyree *et al*. 1999; McCully 1999; Zwieniecki *et al*. 2000; Faccette, Canny & McCully 2001; Melcher *et al*. 2001). Increases in hydraulic conductivity due to embolism repair can occur over very short time scales (e.g. overnight, Zwieniecki & Holbrook 1998) or over periods of several hours after transpiration is prevented (Salleo *et al*. 1996; Tyree *et al*. 1999). Studies carried out in intact plants using cryogenic scanning electron microscopy have also found that the number of embolized vessels can decrease during the daytime, suggesting that embolism repair can occur concurrently with transpiration (McCully, Huang & Ling 1998; Tyree *et al*. 1999; Melcher *et al*. 2001).

The mechanisms responsible for embolism repair are largely unknown (Tyree *et al*. 1999). It has been suggested that vessels may refill by reverse osmosis driven by an increase in pressure within the stem tissue achieved

through the hydrolysis of starch into osmotically active sugars (Canny 1997). Holbrook & Zwieniecki (1999) have provided evidence for the important role that the hydrophobic regions around pit pores may play during refilling of embolized vessels. They have outlined a mechanism for embolism removal in which the hydraulic compartmentation required for local pressurization is permitted by a non-zero contact angle of water on the interior vessel surface and the formation of a convex meniscus within bordered pits. Excretion of osmotically active solutes, either organic or inorganic, from living cells into the embolized vessel could also facilitate refilling (Salleo *et al*. 1996). However more recent studies using radiographic probe microanalysis of refilling vessels have found that the amount of solutes is too small to act as a driving force for water movement into embolized vessels (Tyree *et al*. 1999).

Most evergreen neotropical savanna tree species are isohydric with respect to regulation of seasonal variation in minimum leaf water potential. Despite a 4–5 month dry season, most of the species maintain minimum leaf water potentials at levels similar to those observed during the rainy season when soil water availability is high (Medina & Francisco 1994; Franco 1998; Meinzer *et al*. 1999). Strong stomatal limitation of both maximum daily transpiration rates and total daily transpiration are particularly evident during the dry season in Brazilian savanna (Cerrado) trees (Meinzer *et al*. 1999; Bucci 2001). Substantial decreases in total leaf surface area during the dry season also contribute to isohydric behaviour exhibited by most Cerrado woody species (Bucci 2001). Although the regulation of xylem tension by partial stomatal closure and leaf area adjustments may limit cavitation, embolism and consequent loss of hydraulic conductivity within the plant, diurnal embolism repair, particularly under the high evaporative demand conditions that prevail during the long dry season, may be a prerequisite for maintaining efficient long-distance movement of water to the transpiring leaves.

We have previously observed substantial diel variation in specific hydraulic conductivity (k_s) in petioles of two Brazilian Cerrado tree species with compound leaves and long petioles. The main objectives of the present study were to determine whether short-term variations in k_s in these species are an indication of embolism formation and repair, and if so, what are the biophysical and physiological factors contributing to embolism formation and repair? The use of petioles in this type of study has several advantages: (1) they represent sections of the water conducting pathway that have the same hydraulic hierarchy within the plant; (2), the age of the pathway section can be kept constant by using leaves of known age; (3) the size of the material used is uniform; and (4) petioles are adjacent to the terminal, transpiring portion of the hydraulic pathway. Manipulations were performed to modify the diurnal range of variation in leaf water potential in order to obtain a better phenomenological description of the relationship between k_s and water potential. Other field experiments were performed to alter the components of water potential of living cells in the petiole tissues and to uncouple changes in leaf water potential from changes in k_s by altering biomechanical relationships among petiole tissues. It was hoped that the resulting information on how physical and physiological factors influence the capacity for embolism repair would increase our understanding of the underlying processes responsible for refilling of embolized conduits.

MATERIALS AND METHODS

Study site and plant material

The study was carried out at the Instituto Brasileiro de Geografia e Estadistica (IBGE) reserve, a field experimental station located 35 km from Brazilia (15°56′ S, 47°53′ W, altitude 1100 m). Average annual precipitation is 1500 mm with a pronounced dry season from May to September The months of June to August are often completely rainless. Relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10%. Mean monthly temperatures range from 19 to 23 °C. The soils are very deep and well-drained oxisols. The IBGE reserve contains all major physiognomic types of savannas from very open to closed savannas. The trees studied were mostly located in savannas with intermediate tree density.

The two woody species selected were very abundant on the study site. *Schefflera macrocarpa* (C. & S) Seem. is an evergreen tree with compound leaves containing five to eight large leaflets and *Caryocar brasiliense* Camb. is a brevideciduous tree with large compound leaves containing three leaflets. The petioles of both species are 10–30 cm long. Total leaf surface area of these species decreases by about 60–70% from the wet to the dry season. Stomatal conductance also decreases from the wet to the dry season, but the average leaf-specific hydraulic conductivity remains approximately constant. Consequently, minimum leaf water potentials are similar or even higher (more positive) during the dry season in comparison with the wet season (Bucci 2001).

Hydraulic conductivity, xylem vulnerability curves and leaf water potential

Petiole hydraulic conductivity (k_h) was measured throughout the day on both species. At each sampling time, three to six leaves with petioles were excised in the field. A small portion of the petiole cut end was then immediately removed by re-cutting under water. The leaves were then tightly covered with black plastic bags and transported back to the laboratory with the cut ends of the petioles under water. Measurements were done shortly after material collection because the plants were located within a few hundred metres from the laboratory. Immediately after arriving at the laboratory, 10- to 15-cm-long petiole segments were rapidly cut under water and attached to a hydraulic conductivity apparatus (Tyree & Sperry 1989). Water exuding from the open end of the petiole drained into test tubes containing damp paper towels touching the petiole ends.

Following a short equilibration period, water flow, generated by a constant hydraulic head of 50 cm, was measured gravimetrically. Hydraulic conductivity (kg m s^{-1} MPa^{-1}) was calculated as $k_h = J_v/(\Delta P/\Delta X)$, where J_v is the flow rate through the petiole segment (kg s^{-1}) and $\Delta P/\Delta X$ is the pressure gradient across the segment (MPa m^{-1}). Specific conductivity (k_s: kg m^{-1} s^{-1} MPa^{-1}) was obtained as the ratio of k_h and the xylem cross-sectional area of the petiole segment. Diurnal courses of petiole k_s measured using a 0.5-mmol KCl solution as a perfusion fluid instead of distilled water (Zwieniecki, Melcher & Holbrook 2001a), consistently yielded k_s values that were approximately 0.1 kg m^{-1} s^{-1} MPa^{-1} higher without altering the daily course of hydraulic conductivity as measured using distilled water. Distilled/degassed water was used as perfusion fluid in all measurements reported in this study. The xylem cross-sectional area was measured with calipers and a magnifying glass. After hydraulic conductivity measurements, some of the petiole segments were perfused with a solution of safranin. After dye perfusion, thin sections were cut by hand from the middle of the petiole segment, and the number of stained vessels was determined using a light microscope.

During some sampling days, maximum hydraulic conductivity was determined after initial k_h measurements by flushing the petioles with the perfusion fluid (distilled and de-gassed water) at a pressure of 0.2 MPa for 15 min, to remove air bubbles from embolized vessels. The flushing solution had been passed through a 0.22 μm membrane filter to prevent particulate matter from blocking the conduits. After the 15-min flush, k_h was measured again as described above. The process was repeated until maximum conductivity (k_{max}) was achieved. The initial k_h measurement (k_i) was expressed as a percentage of k_{max}, and the percentage loss of conductivity (*PLC*) was calculated as $(1 - k_i/k_{max}) \times 100$. Specific conductivity was also determined on petioles of non-transpiring leaves that were sealed in plastic bags and wrapped in aluminium foil on the afternoon prior to the measurement day. Leaf water potential (Ψ_L) was measured with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA) on both freely transpiring and covered leaves.

Hydraulic vulnerability curves were constructed for the petioles of the two species by plotting the percentage loss of hydraulic conductivity (*PLC*) against ψ_L. Different *PLC* values were obtained by allowing excised branches to dehydrate slowly in air for different time periods. The branch length used was longer than the longest xylem vessel measured. Measurements of vessel length were done according to Zimmermann & Jeje (1981). In *S. macrocarpa,* maximum vessel length was between 54 and 67 cm ($n = 10$ branches) and between 35 and 52 cm ($n = 10$ branches) in *C. brasiliense*. After allowing time for partial dehydration, measurements of ψ_L and the corresponding k_h were taken on five branches. Both ends of the petiole were recut under water, connected to the hydraulic conductivity apparatus and k_i and k_{max} were determined as described above. Leaf water potentials were measured on the same branch on leaves adjacent to those used for petiole k_h determinations.

Sugar and starch content

At each sampling time, 15 petioles per species were excised from different trees and stored in liquid N_2. The petioles were dried at 80 °C for 48 h, then ground into fine powder with a mortar and pestle and 1 mL distilled water was added. Total non-structural carbohydrates were extracted for 60 min at 100 °C in a water bath. A sample (10 μL) of hot water extract was mixed with 1 mL of anthrone reagent (0.2 g anthrone in 100 mL of 95% sulphuric acid). The mixture was heated in a boiling water bath for 15 min and then the absorption was measured in a spectrophotometer (Genesys®; ThermoSpectronic, Rochester, NY, USA) at 620 nm against a blank solution. Glucose solutions were used as standards.

Fifteen petioles per species were also collected at each sampling time during the course of a day and stored in 50% ethanol. Sections were then cut by hand, stained with I_2/KI and observed under a microscope for qualitative assessment of starch content based on intracellular location and intensity of the dye.

Stomatal conductance and sap flow

Stomatal conductance (g_s) was measured with a steady-state porometer (Model LI-1600; LiCor Inc., Lincoln, NE, USA) on three leaves of the same plants used for hydraulic conductivity measurements. Sap flow near the base of the main stem was measured during 2–3 consecutive days in each of three individuals per species. The constant heating method (Granier 1985, 1987) was used. Two 20-mm-long, 2-mm-diameter probes were inserted radially near the base of each tree. Each pair of probes was separated vertically by a distance of 10 cm. The higher (downstream) probe was continuously heated by a constant current power supply, with the lower (upstream) probe serving as a temperature reference. Probe temperatures were recorded at 10 s intervals by a datalogger (CR10X; Campbell Scientific, Logan, UT, USA), and 10 min averages were stored in a solid-state storage module (SM196; Campbell Scientific). Sap flux density was calculated from the temperature difference between the probes based on a standard empirical relationship developed by Granier (1987) and recently re-validated by Clearwater *et al*. (1999). Mass flow of sap was obtained by multiplying sap flux density by the active xylem cross-sectional area obtained by injection of indigo carmine dye into stems with diameters similar to those of the measured plants. Mean area per leaf was calculated from subsamples of 20 leaves of each tree measured. Total leaf area was determined by counting all leaves and multiplying the total number of leaves by mean area per leaf. Sap flow per unit leaf area (transpiration) was calculated by dividing mass flow of sap by the total leaf area downstream to the stem section where sap flow was measured.

Field manipulations

During January 2002, a manipulation experiment was conducted to examine the effects of removal of stem xylem

tissue on petiole hydraulic conductivity. The objective of this manipulation was to increase the diurnal range of variations in leaf water potential. Partial incisions of the xylem were made along the terminal branches that contained the petioles used for k_h measurements with the objective of removing approximately 50% of the stem xylem conducting area upstream from the petioles. Three plants per species were used and the treatment was performed on the afternoon prior to measurements. The following day, k_h, *PLC* and Ψ_L were measured on both treated and control branches. Another field manipulation was conducted during August 2002 to examine the effects of petiole cortex removal on diel variation in petiole k_h. External tissues of the petiole cortex were carefully removed from several leaves of both species with a razor blade between 0600 and 0900 h and k_h was measured on treated and control petioles throughout the rest of the day. After partial cortex removal, exposed surfaces were immediately covered with silicone grease and wrapped with plastic and aluminium foil to limit air penetration and prevent water loss. Responses to subsequent manipulations involving application of silicone grease and covering indicated that these two procedures alone had no effect on time courses of k_h (see below).

Further trials showed that responses of k_h to cortex removal were similar when petioles were covered with parafilm instead of silicone grease.

Two girdling manipulations were also performed. In one experiment, a section of cortex about 1 cm wide was removed upstream from the petiole segment used for k_h measurements. In another experiment, a section of cortex about 1 cm wide was removed downstream from the petiole segment used for k_h measurements. Exposed surfaces were immediately covered with silicone grease and wrapped with plastic and aluminium foil. Girdling was performed between 0600 and 0900 h and petiole k_h and Ψ_L of treated and untreated (control) leaves were determined throughout the day.

Another manipulation, performed during September 2002, consisted of making four or five shallow longitudinal incisions in the cortex tissues along petioles of several leaves of five plants of *S. macrocarpa* between 0600 and 0900 h. The longitudinal incisions were longer than the petiole segments eventually used for conductivity measurements. Immediately after the incisions were made, the petioles were covered with silicone grease and wrapped with plastic and aluminium foil. Hydraulic conductivity of treated and control petioles was measured throughout the day. In a final series of experimental manipulations, dry filter paper (control), or saturated filter paper containing either a –1.3 MPa solution of KCl or distilled water was applied to the petiole surface where the cuticle had previously been partially removed. The cuticle was left intact in an additional control treatment. Partial cuticle removal was achieved by carefully scraping the petiole surface with a scalpel between 0600 and 0900 h. All petioles were covered with plastic and aluminium foil to prevent evaporative water loss. Hydraulic conductivity of treated and untreated (control) petioles was determined throughout the day.

Treatment effects (covered versus exposed leaves) on minimum leaf water potential was tested using Student's *t*-test. A two-way analysis of variance (ANOVA) was used to test the effect of time, treatment and their interactions on the diurnal patterns of hydraulic conductivity.

RESULTS

Petiole k_s initially increased in the morning, then decreased sharply beginning around late morning to midday, and then increased again during the late afternoon to levels close to its predawn values (Fig. 1). Petiole k_s began to decrease sharply at approximately the same time maximum values of sap flux density (or E) were attained. Late afternoon recovery of k_s was slower in *S. macrocarpa* than in *C. brasiliense*, but recovery was complete by the following morning as indicated by similar values of predawn k_s on consecutive days (data not shown). Leaf water potential typically ranged from –0.25 to –1.25 MPa in *S. macrocarpa* and from –0.4 to –1.4 in *C. brasiliense*. The lowest values of both Ψ_L and k_s were usually observed between 1300 and 1500 h and recovery of k_s was usually complete by the end of the daytime period, both during the dry and wet seasons (data not shown).

When transpiration was prevented by enclosing the leaves in plastic bags and aluminium foil, both k_s and Ψ_L remained nearly constant over the course of the day (Fig. 2). Water potentials decreased by only 0.2–0.3 MPa in bagged leaves compared with 1–1.5 MPa in freely transpiring leaves. Significant differences in Ψ_L between covered and exposed leaves of about 1.0 MPa were observed between 1200 and 1400 h, in both species ($P < 0.01$). Petiole k_s was consistently lower in exposed compared to covered leaves in both species (Fig. 2a & b). Significant differences for time and treatment effects ($P < 0.01$), as well as between the two factors (time and treatment) interactions ($P < 0.05$), were observed. Manipulations to increase leaf water deficits by removing approximately 50% of the stem xylem conducting area upstream from the petioles lowered minimum Ψ_L by 0.2–0.6 MPa in comparison with the control (Fig. 3e & f), however, the differences were only significant in *S macrocarpa* ($P < 0.1$) at 1100 h. Although neither the rate of k_s decline during the morning nor the daily minimum value of k_s appeared to be significantly affected by this treatment, the rate of recovery of k_s during the afternoon was noticeably slower in treated plants (Fig. 3a & b). Significant differences for time and treatment effects were observed in both species ($P < 0.05$). Interactions of time and treatment (removal of 50% of xylem tissue) were not significant (*C. brasiliense*; $P = 0.3$ and *S. macrocarpa*; $P = 0.4$). Because maximum k_s after high-pressure removal of emboli in the conductivity apparatus was essentially constant during the day (data not shown), the percentage loss of conductivity was higher in the treated plants at the end of the day for both species (Fig. 3c & d). Petiole k_s increased linearly and the percentage loss of conductivity decreased linearly with increasing Ψ_L in freely transpiring leaves of both species (Fig. 4). Similar relationships

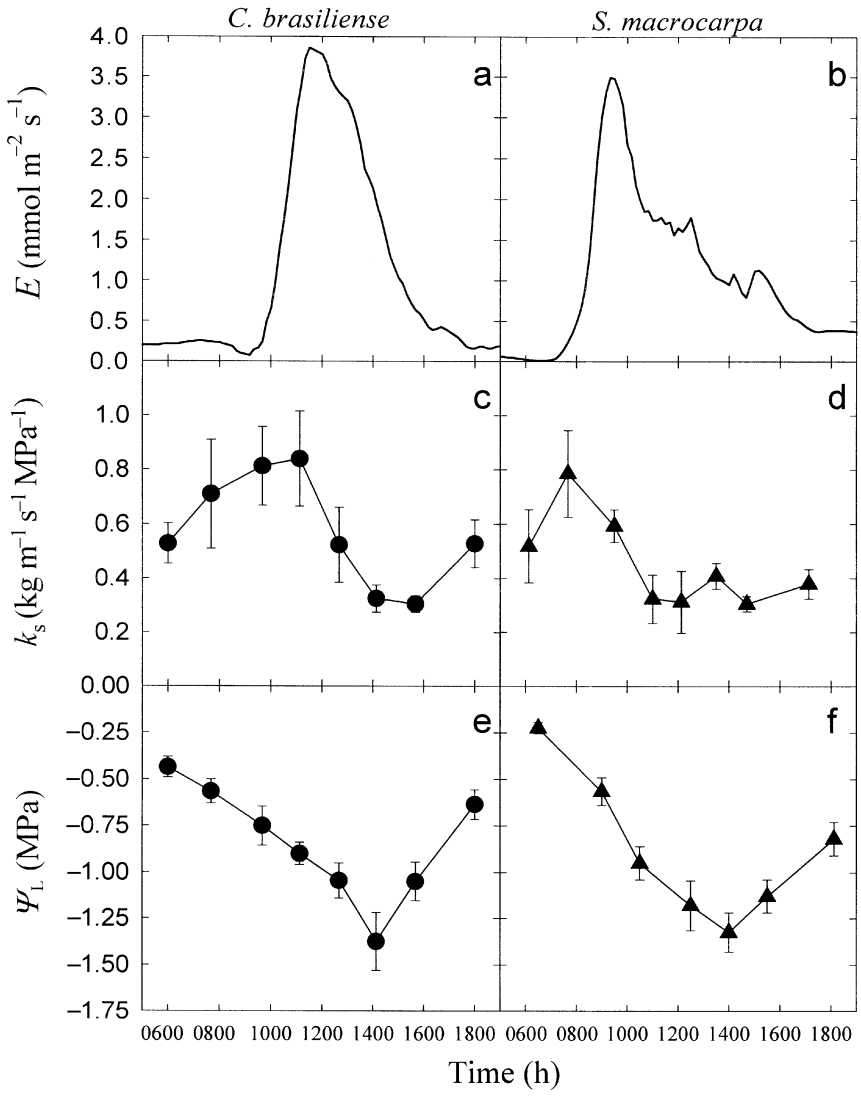

Figure 1. Diurnal courses of transpiration (E), petiole specific hydraulic conductivity (k_s) and leaf water potential (Ψ_L) in *C. brasiliense* and *S. macrocarpa* during the dry season (September 2000). Values of k_s and Ψ_L are means ±1 SE ($n = 6$, one petiole or leaf per tree for each data point). The same six trees were used throughout the day.

between k_s and Ψ_L were observed in covered leaves, but their r^2 values were lower (data not shown).

Xylem vulnerability curves were similar in both species (Fig. 5). Percentage loss of conductivity began to increase more rapidly below about –1.0 MPa and reached 100% at about –3.5 MPa. The 50% conductivity loss point occurred at about –1.7 MPa, a Ψ_L value seldom observed in intact leaves, even during the dry season. Values of *PLC* and Ψ_L measured on leaves excised at different times of day in the field were consistent with those obtained by dehydration of excised leaves in laboratory experiments. The number of vessels stained by the perfusion solution during determination of k_s was linearly correlated with k_s suggesting that the diurnal variation in k_s observed in both species was associated with embolism formation and repair (Fig. 6).

Several experiments were performed in an attempt to identify some of the potential mechanisms involved in refilling of embolized vessels. In both species, removal of the petiole cortex altered patterns of diurnal variation in k_s compared with those observed in untreated petioles (Fig. 7). Treated and control petioles exhibited similar time courses of k_s until about 1800 h after which k_s of control petioles continued to increase until about 2300 h, whereas k_s of treated petioles began to decline. By dawn of the following day, k_s of treated petioles was similar to that observed at midday, when k_s was at its minimum (Fig. 7). Significant differences for time and treatment effects were observed in both species ($P < 0.05$), but the interactions were not significant ($P = 0.5$ both species). Removal of the petiole cortex did not result in consistent differences in Ψ_L, except near the end of the night when Ψ_L of treated leaves was greater than that of control leaves (Fig. 7). In order to assess whether patterns of variation in k_s following cortex removal reflected wound responses, shallow longitudinal incisions were made along the petiole. Time courses of k_s in petioles with longitudinal incisions were similar to those observed following cortex removal. Hydraulic conductivity began to decline by the late afternoon and by dawn of the following day it was similar to that observed at midday (Fig. 8). In addition, upstream and downstream girdling of

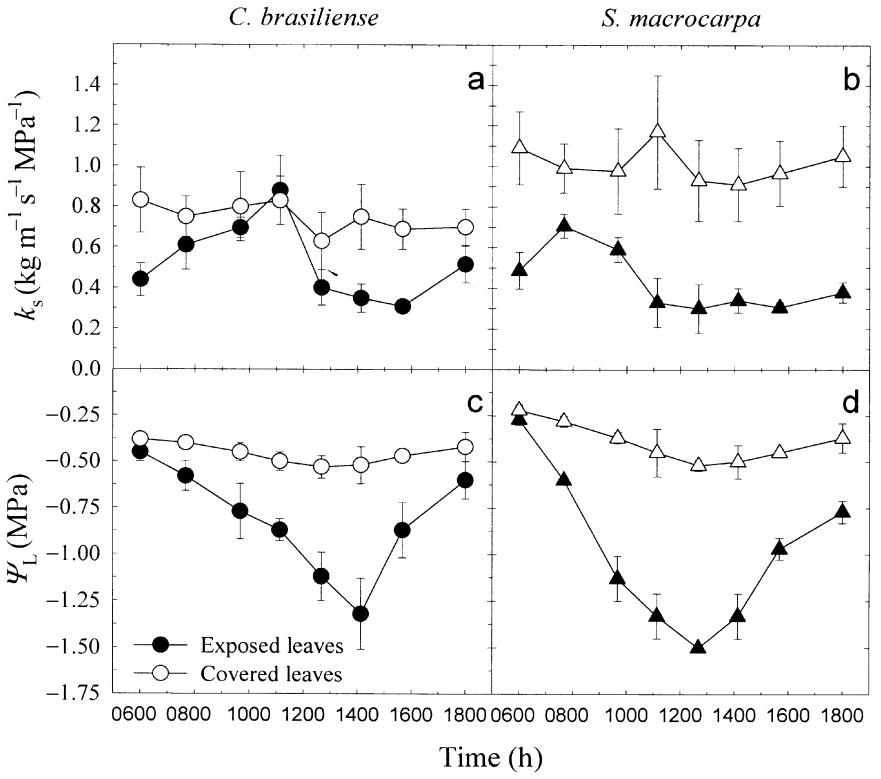

Figure 2. Diurnal courses of petiole specific hydraulic conductivity (k_s) and water potential (Ψ_L) of freely transpiring (exposed), or covered leaves during September, 2000. Values for k_s and Ψ_L are means ±1 SE ($n = 6$, one leaf or petiole per tree for each data point). The same six trees were used throughout the day.

the petiole cortex (with respect to the petiole segment used during measurements) had no effect on k_s recovery during the afternoon and night (Fig. 9). In another related field experiment, in which only the cuticle of the petioles was removed, the addition of distilled water to the petiole surface resulted in a large increase in k_s during the day with a peak at 1530 h (Fig. 10). In contrast, the time course of k_s in treated petioles exposed to a –1.3 MPa KCl

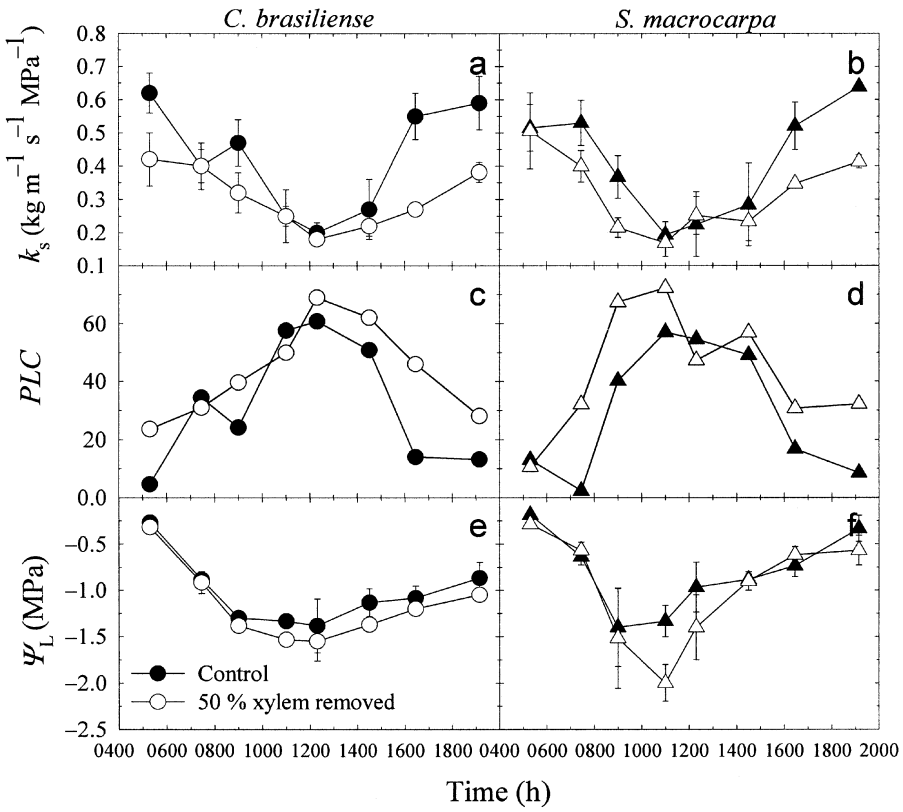

Figure 3. Diurnal courses of petiole specific hydraulic conductivity (k_s), percentage loss of specific conductivity (PLC) and leaf water potential (Ψ_L) in *C. brasiliense* and *S. macrocarpa* on 27 January 2002 for control branches (closed symbols) and branches with 50% of the xylem removed (open symbols). Values of k_s and Ψ_L are means ±1 SE ($n = 3$, one petiole or leaf per tree for each data point).

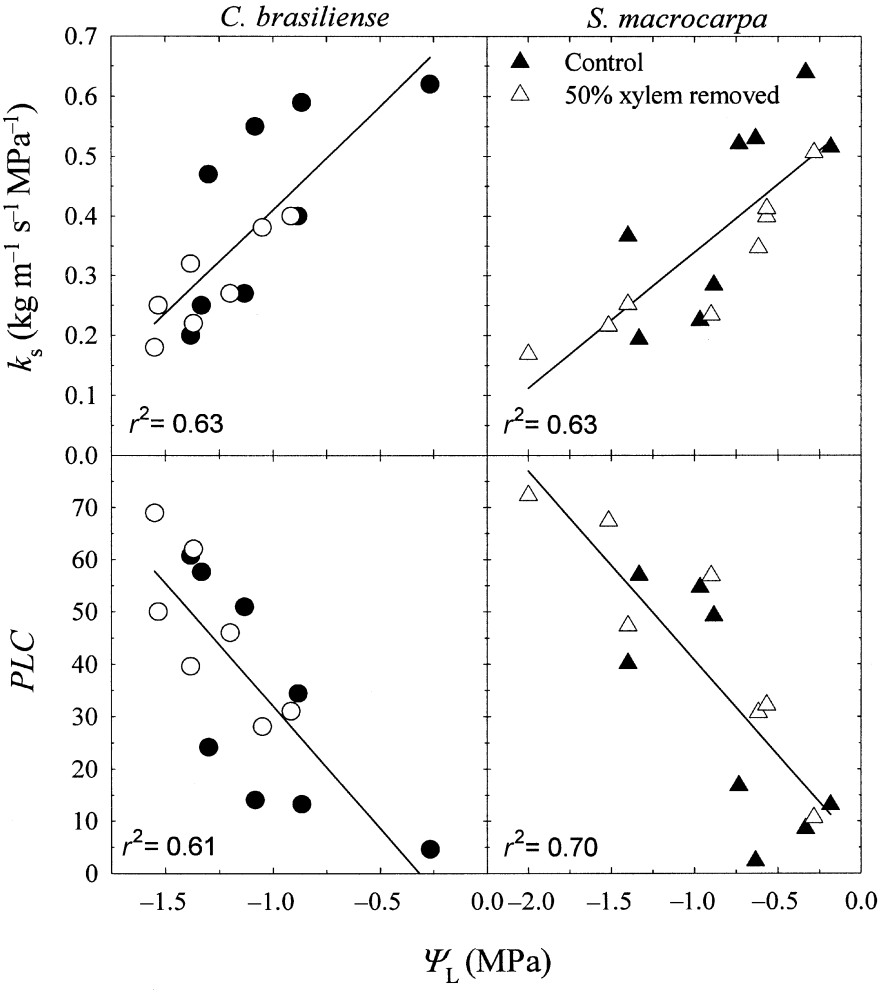

Figure 4. Relationships between petiole specific conductivity (k_s) or percentage loss of specific conductivity (*PLC*) and leaf water potential (Ψ_L) in *C. brasiliense* and *S. macrocarpa* for control branches (closed symbols) and branches with 50% of the xylem removed upstream of the petioles studied (open symbols). Data are from the diurnal courses in Fig. 3. Control and treated samples, in each case, were pooled for the linear regression analysis because there were not significant statistical differences between the slopes of the linear regressions when fitted to either control branches or to branches with 50% of the xylem removed ($P < 0.05$).

solution was similar to that observed in control petioles (Fig. 10).

Diurnal variation in the amount of osmotically active sugars was observed in petioles of freely transpiring leaves (Fig. 11). Maximum sugar content was observed at about 1330 h in the transpiring leaves of both species whereas sugar content remained constant in bagged leaves of *S. macrocarpa* throughout the day (results not shown). Abundant starch granules were observed in the starch sheath cells surrounding the vascular bundles of the petioles and in parenchyma cells of the phloem tissue, particularly at night in *S. macrocarpa* (Fig. 12, 2130 h). On days of rapid transpiration, a large fraction of petiole starch disappeared by the early afternoon (Fig. 12, 1330 h). Starch granules were consistently abundant in petioles of covered leaves throughout the day (results not shown).

DISCUSSION

Variation in petiole k_s for the two savanna species was strongly correlated with the number of vessels stained by the perfusion solution used to determine k_s, suggesting that diel changes in k_s were associated with embolism formation and removal. It was also observed that in the afternoon, k_s and water potential increased concurrently, a trend that continued during the night, implying that at least initially, embolism repair occurred when the water in nearby xylem conduits was under considerable tension. These results are consistent with recent reports of short-term changes in hydraulic conductivity in woody species (Salleo *et al.* 1996; Zwieniecki & Holbrook 1998; Tyree *et al.* 1999; Zwieniecki *et al.* 2000) as well as with diurnal changes in the number of water-filled conduits as determined by cryo-scanning electron microscopy techniques (Canny 1997; McCully *et al.* 1998; Melcher *et al.* 2001).

A strong, positive correlation between k_s and Ψ_L was observed in petioles of *S. macrocarpa* and *C. brasiliense* during both dry and wet season days. Because correlation does not necessarily imply causation, we cannot confirm that Ψ_L was directly linked with embolism formation and repair. Nevertheless, this study presents evidence that diel changes in petiole conductivity are somehow related to diel changes in leaf water status. It is clear that cavitation and embolism were linked during periods of increasing tension, with higher frequency of embolism formation at high tensions. Even though pressure chamber measurements of Ψ_L on freely transpiring leaves will overestimate tension in the petiole xylem (Balling & Zimmermann 1990; Zimmermann

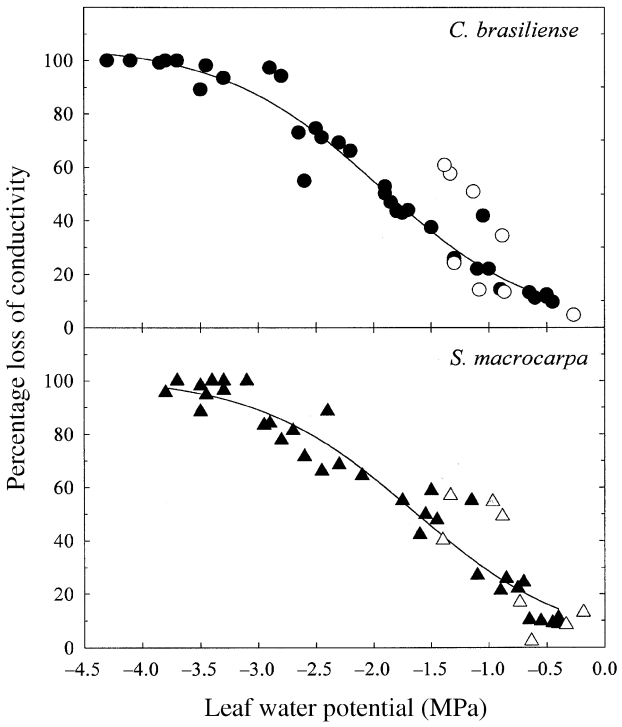

Figure 5. Xylem vulnerability curves obtained in bench dehydration experiments (closed symbols), and percent loss of conductivity-leaf water potential values determined *in situ* (open symbols). Each point for the bench dehydration experiments represents results for individual leaves. Leaves from five trees per species were used. Sigmoidal functions with three parameters were fitted to the xylem vulnerability curves with $r^2 = 0.95$ and 0.96 ($P < 0.01$) for *C. brasiliense* and *S. macrocarpa*, respectively.

et al. 1994; Melcher *et al*. 1998), the values obtained are still proportional to the tension that causes cavitation or embolism. When Ψ_L was induced to drop 0.2–0.6 MPa below the normal Ψ_L attained at midday by partial sectioning of the stem xylem, the rate of recovery of k_s in the afternoon tended to be slower. Thus, lowering the minimum water potential by partial sectioning of the sapwood apparently impeded the processes involved in embolism repair.

Refilling of xylem conduits when water in adjacent conduits is under tension appears difficult to explain given the physical requirement of positive pressure to force the air bubbles back into solution (Tyree 1997). However, Holbrook & Zwieniecki (1999) outlined different potential scenarios in which hydraulic compartmentalization required for local pressurization, apparently a prerequisite for embolism repair under tension, is physically plausible in the water transport system of transpiring plants. In our study, removal of the petiole outer tissue layers, consisting of epidermis, parenchyma, and fibres, inhibited the recovery of k_s during the afternoon, and caused k_s to decline throughout the subsequent night to an early morning value equivalent to the midday minimum value observed during the previous day. In contrast, recovery of Ψ_L was not impeded by cortex removal. In fact, the treated petioles tended to exhibit more positive water potentials than control petioles at the end of the night. Cortex removal thus uncoupled k_s from its dependence on Ψ_L observed in intact leaves, similar to the uncoupling observed between k_s and Ψ_L when partial sectioning of the stem xylem was experimentally performed. Shallow longitudinal incisions along petioles also prevented recovery of k_s during the afternoon and night, but upstream and downstream girdling of petioles had no effect on recovery of k_s, suggesting that the effects of petiole cortex removal and longitudinal incisions on diurnal patterns of k_s did not constitute a direct wound response to the treatments. The results of these experimental manipulations suggest that the integrity of the outer cell layers of the petiole is critical for repair of embolized vessels. It is possible that the cortex acts as a pressure-confining barrier, mechanically constraining the increase in petiole volume that occurs during hydration of its internal tissues when Ψ_L is recovering (becoming less negative) during the afternoon. The outer cell layers of the petiole cortex would thus be partially analogous to the cell wall that limits the volume increase of an individual cell experiencing an influx of water due to the increase of osmotically active solutes. Important effects of pressure-confining barriers on physiological processes have been documented for other tissues or plant compartments (e.g. Welbaum & Bradford 1988, 1990; Welbaum *et al*. 1992).

If both embolism formation and removal are continuously occurring when substantial tension is present in the

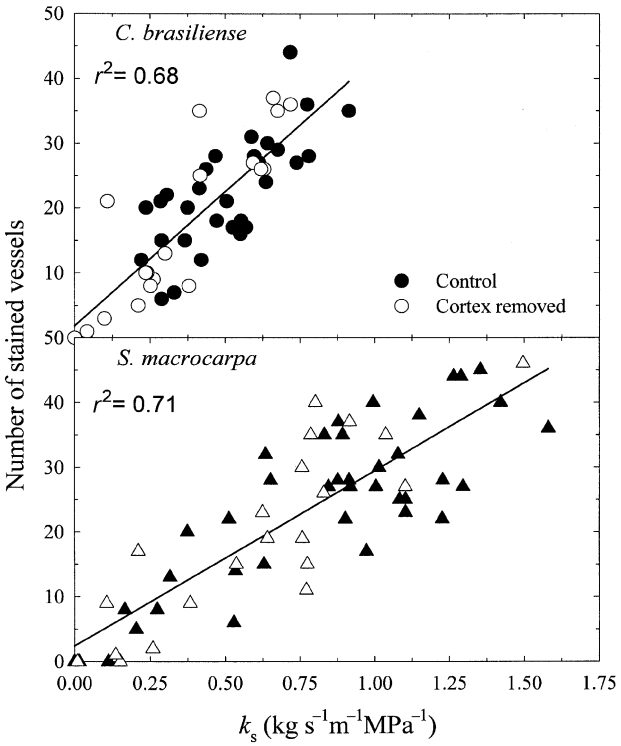

Figure 6. Number of stained vessels in relation to specific conductivity (k_s) in intact *C. brasiliense* and *S. macrocarpa* petioles (closed symbols) and petioles with the cortex removed (open symbols). Each point represents data from a single petiole.

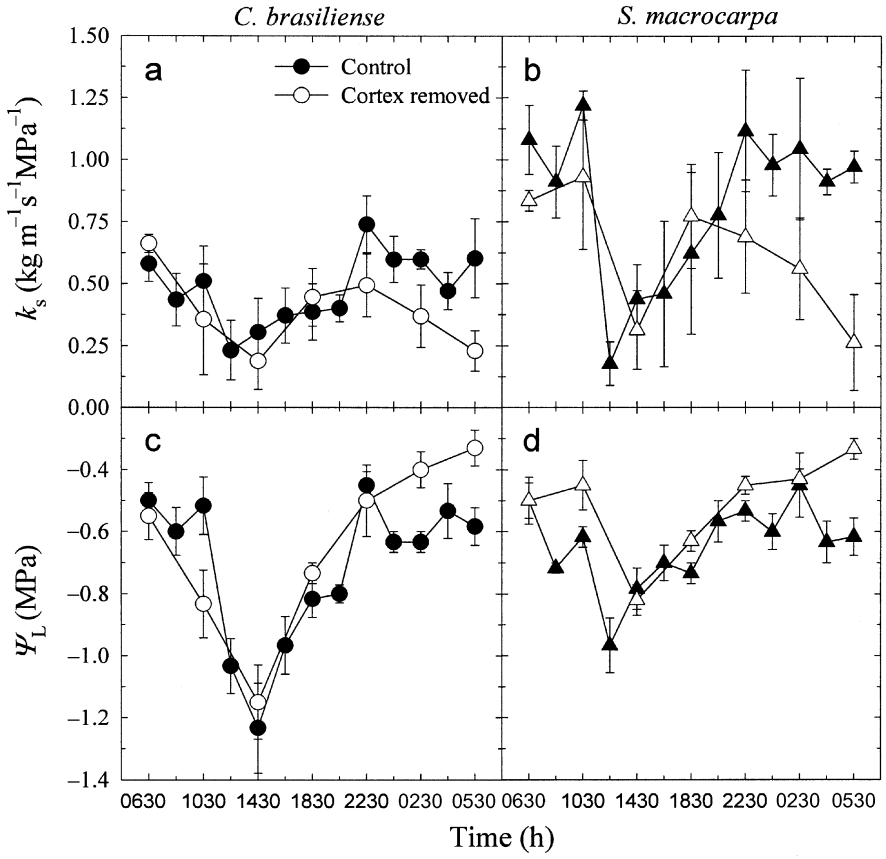

Figure 7. Diel courses of specific hydraulic conductivity (k_s) and leaf water potential (Ψ_L) in intact (control) *C. brasiliense* and *S. macrocarpa* petioles (closed symbols) and in petioles with the cortex removed (open symbols). Values are means ±1 SE ($n = 3$, one leaf or petiole per tree for each data point). Measurements were made on 22 and 23 August 2002.

xylem, improved petiole water status during the afternoon could enhance the rate of embolism removal by providing a radial supply of water to the xylem. It has been suggested that the starch sheath cells may act as a source of the pressure necessary for displacing water into the embolized xylem conduits (Canny 1998). In the petioles of the two species studied, the starch sheath cells are localized outside the vascular bundles. Hydrolysis of starch into osmotically active sugars in these cells would lower their osmotic and

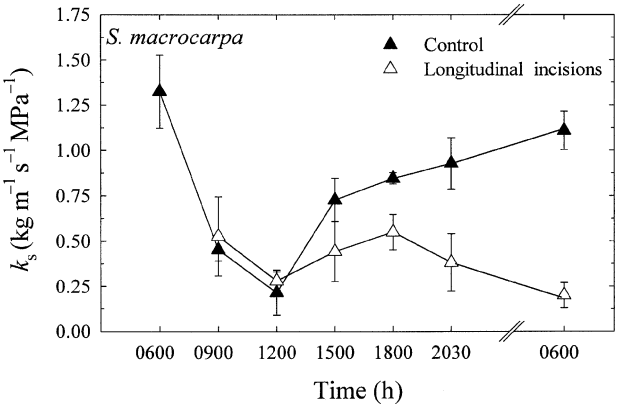

Figure 8. Diel course of specific hydraulic conductivity (k_s) in intact (control) *S. macrocarpa* petioles and in petioles with longitudinal incisions, on 17 and 18 September 2002. Values are means ±1 SE ($n = 5$) of five trees and one petiole per tree.

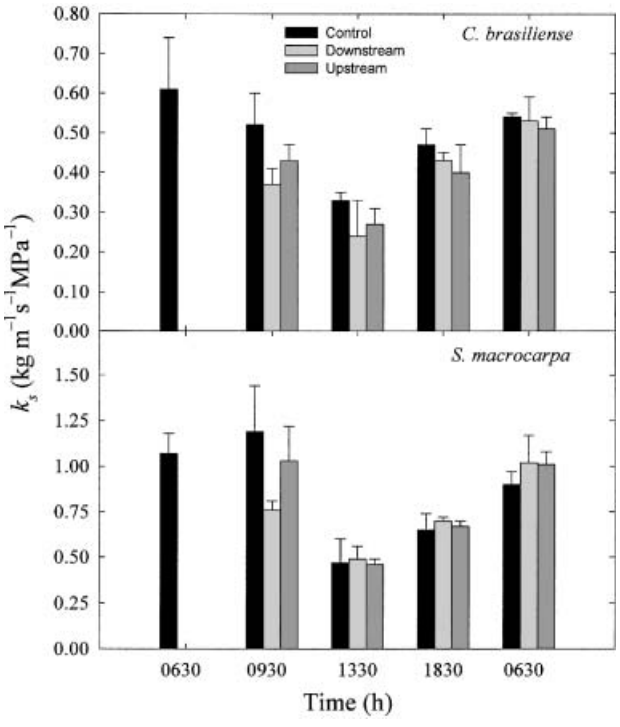

Figure 9. Time courses of specific hydraulic conductivity (k_s) in intact (control) *C. brasiliense* and *S. macrocarpa* petioles and in petioles that were girdled either upstream or downstream from the section in which k_h was measured. Bars are means +1 SE ($n = 3$) of three individuals and one petiole per individual.

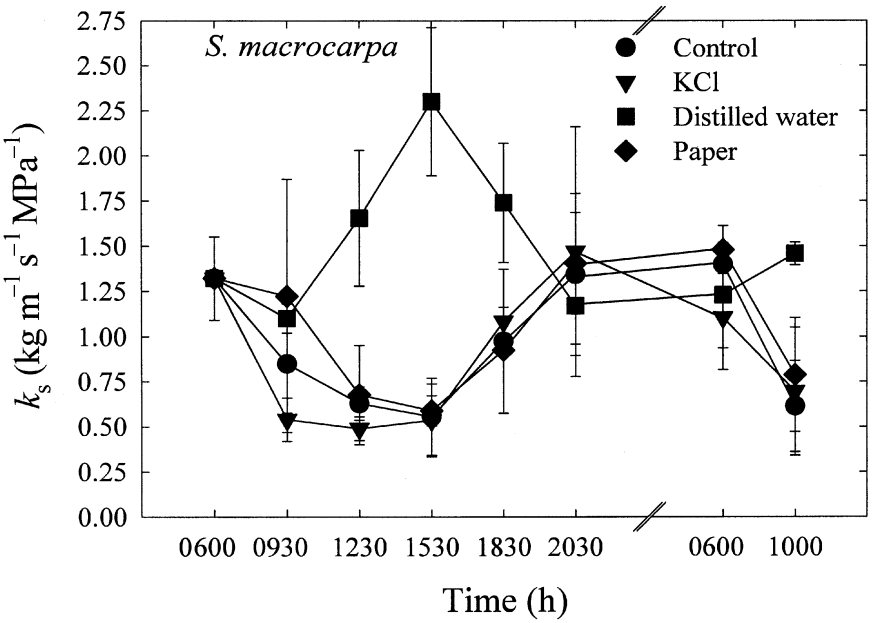

Figure 10. Time courses of specific hydraulic conductivity (k_s) in untreated (control) *S. macrocarpa* petioles, in petioles wrapped in filter paper saturated with a –1.3 MPa solution of KCl or distilled water, and in petioles wrapped in dry filter paper. The cuticle was partially removed in all treatments except the control. Values are means ±1 SE ($n = 3$) of three trees and one petiole per tree.

water potential, leading to water uptake and localized increases in pressure that would drive water to move radially into embolized conduits before a new steady-state equilibrium in water potential is attained. The source of water used for repair could be water in parenchyma cells surrounding the vascular bundles or parenchyma inside phloem tissue in close contact with the xylem. It is possible that part of the water initially taken up in response to the osmotic and water potential disequilibrium generated by the starch sheath cells may be transported axially through the intact xylem vessels. This possibility is consistent with the fact that the maximum sugar content occurs around 1300–1400 h, whereas k_s begins to recover slowly after 1400 h. Before new steady-state values of water potential inside the petiole are attained, water could move radially in direction of the embolized vessels. Preliminary results of a field experiment involving morning injections of D_2O into the cortex of petioles of both species suggested that the source water for the afternoon refilling of embolized vessels was located in tissues outside the vascular bundles (unpublished observations).

What type of signal could trigger diel starch–sugar interconversions within the petiole? One possibility is that reduced turgor pressure or volume in the starch sheath cells promotes starch to sugar conversion during periods of high transpiration and low Ψ_L. Conversely, increased turgor or volume could favour the polymerization of sugar into starch when transpiration declines and Ψ_L recovers in the

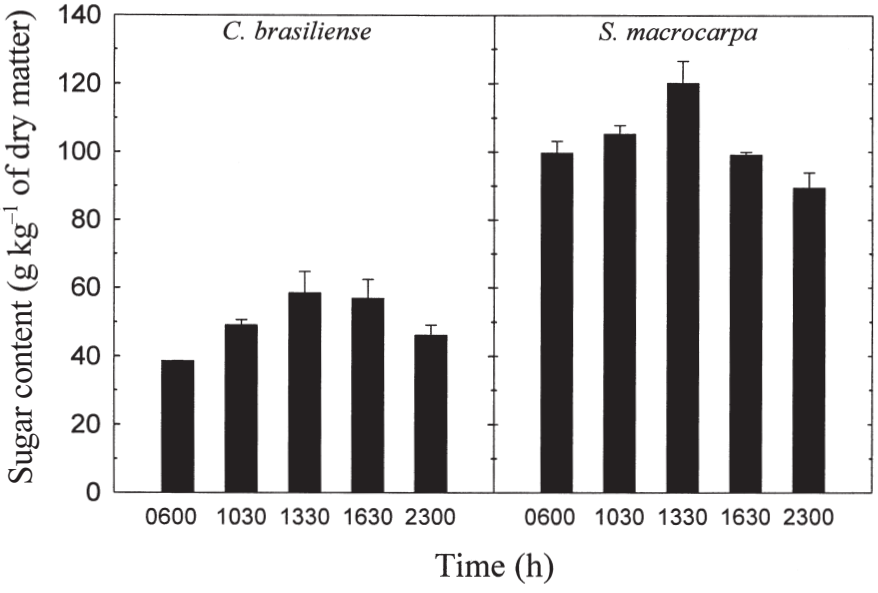

Figure 11. Time courses of sugar content in petioles of freely transpiring leaves of *S. macrocarpa* and *C. brasiliense* during January 2003. Bars are means +1 SE ($n = 3$).

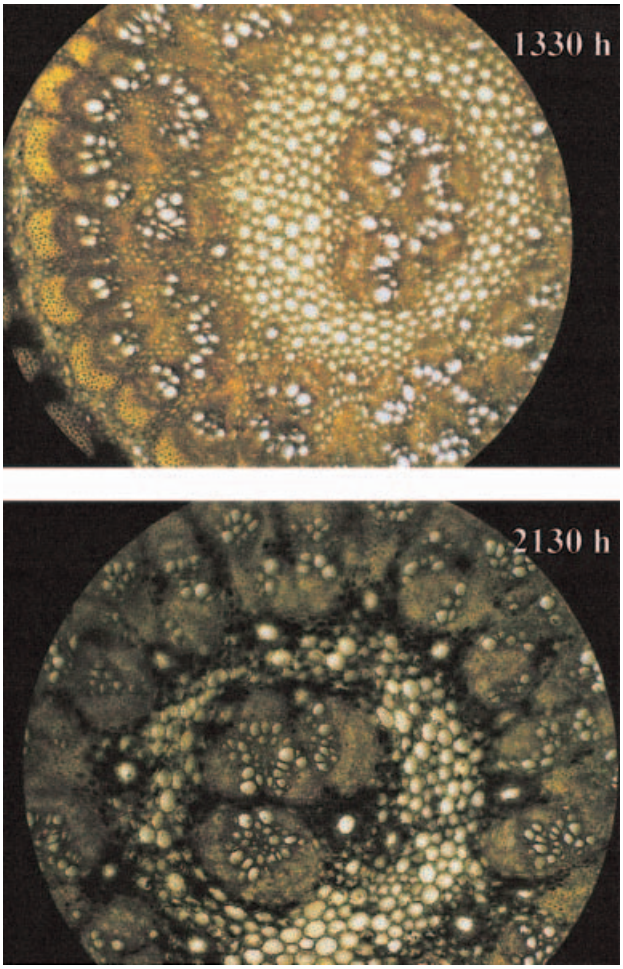

Figure 12. Cross-sections of *C. brasiliense* petioles obtained at 1330 and 2130 h on 17 September 2002. Starch granules (in black), are abundant around the vascular bundles at night but are absent at 1330 h.

afternoon and evening. Transport of sugars from the photosynthesizing leaves to the petiole via the phloem may be another mechanism by which osmotically active solutes could increase in the afternoon. Differences in wall elasticity among different cell types in the petiole may also contribute to temporary pressure imbalances that drive water movement toward embolized conduits. When Ψ_L recovers in the afternoon, large parenchyma cells with elastic walls probably expand more rapidly than smaller, rigid cells before attaining water potential equilibrium with them. Alternative or complementary mechanisms could also be involved in embolism repair such as changes in membrane reflection coefficients, water channel activity or pit membrane osmosis (e.g. Holbrook & Zwieniecki 1999; Hacke & Sperry 2003), which would affect movement of water and solutes between the parenchyma and embolized vessels. Water movement against a water potential gradient (pressure in embolized vessels is close to atmospheric whereas water potential in the liquid phase outside the vascular conduits is more negative) in a multiphase system requires a local input of energy that may come from the activities of living cells. This mechanism of embolism repair is thermodynamically plausible as long as there is a source of work (Roderick 2001), and the most obvious sources of work in a plant would be inside certain petiole cells or the result of carbohydrates transport from leaves to petioles. Regardless of the mechanisms involved in embolism repair, this study presents evidence that embolism formation and repair in petioles are two distinct phenomena controlled by different variables. Cavitation and embolism could be a function of tension, and refilling a function of the pressure imbalances that can be generated. The balance between these phenomena would determine daily courses of k_s. Several questions concerning this putative mechanism of embolism repair merit further exploration. An obvious one is whether this mechanism is applicable to the dynamic process of loss and recovery of hydraulic conductivity in branches and stems of woody plants.

Behaviour of k_s following experimental manipulations in the field was consistent with a xylem refilling mechanism involving radial movement of water into embolized conduits driven by transient pressure imbalances between the xylem and surrounding tissue. For example, complete removal and even partial disruption of the petiole epidermis and cortex, a potential pressure-confining barrier, inhibited afternoon and overnight recovery of k_s. These treatments may have caused the turgor pressure of living cells surrounding embolized vessels to relax, which may have prevented local pressurization, and therefore radial water transport towards the vascular bundles, from occurring. Perhaps the simplest explanation is that without a pressure-confining barrier, relaxation of turgor itself lowers the water potential causing water to be taken up by living cells rather than being driven into the xylem. The large increase in k_s at midday when distilled water was supplied to the outer petiole cells is consistent with the transient pressure imbalance mechanisms outlined above. Presumably, the presence of pure water in contact with the petiole cortical cells enhanced water uptake by these cells, elevating the pressure imbalance inside the petiole above that which normally occurs *in vivo*.

This study presents evidence for diel changes in petiole conductivity that are correlated with variation in leaf water status. In addition, field manipulations provided strong evidence that living cells were involved in restoring petiole hydraulic conductivity in the afternoon during periods of high transpiration. The balance between loss and recovery of xylem hydraulic conductivity may be determined by the prevailing tension in the xylem, starch to sugar conversions in localized petiole cells, delivery of sugars in the phloem, as well as by the petiole cortex acting as a pressure-confining barrier. Properties of the water transport system of vascular plants thus appear to be more dynamic than previously thought (Zwieniecki, Melcher & Holbrook 2001b). In woody plants, and in particular neotropical savanna trees, homeostatic mechanisms appear to be continuously operating to maintain the integrity of the xylem water

transport system during periods of high transpiration or prolonged seasonal soil water deficits.

ACKNOWLEDGMENTS

This work was supported by grants from Inter-American Institute for Global Change Research and the National Science Foundation (USA) grant no. 0296174. We are grateful to Birgit Orthen for help in sugar content analysis. We are also grateful to Jack Fisher from the Fairchild Tropical Garden, and personnel of the Plant Anatomy Laboratory of the Universidade de Brazilia for help with anatomical studies. Paula Campanello and Jose Hinojosa provided useful field assistance. We thank the personnel of the Reserva Ecologica do IBGE for logistic support and Michael J. Clearwater for comments on the manuscript.

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Received 23 March 2003; received in revised form 29 May 2003; accepted for publication 4 June 2003