Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees

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Summary   Under certain environmental conditions, nocturnal transpiration can be relatively high in temperate and tropical woody species. We have previously shown that nocturnal sap flow accounts for up to 28% of total daily transpiration in woody species growing in a nutrient-poor Brazilian Cerrado ecosystem. In the present study, we assessed the effect of increased nutrient supply on nocturnal transpiration in three dominant Cerrado tree species to explore the hypothesis that, in nutrient-poor systems, continued transpiration at night may enhance delivery of nutrients to root-absorbing surfaces. We compared nocturnal transpiration of trees growing in unfertilized plots and plots to which nitrogen (N) and phosphorus (P) had been added twice yearly from 1998 to 2005. Three independent indicators of nocturnal transpiration were evaluated: sap flow in terminal branches, stomatal conductance (g_s), and disequilibrium in water potential between covered and exposed leaves ($\Delta \Psi_L$). In the unfertilized trees, about 25% of the total daily sap flow occurred at night. Nocturnal sap flow was consistently lower in the N- and P-fertilized trees, significantly so in trees in the N treatment. Similarly, nocturnal g_s was consistently lower in fertilized trees than in unfertilized trees where it sometimes reached values of 150 mmol m^{-2} s^{-1} by the end of the dark period. Predawn g_s and the percentage of nocturnal sap flow were linearly related. Nocturnal $\Delta \Psi_L$ was significantly greater in the unfertilized trees than in N- and P-fertilized trees. The absolute magnitude of $\Delta \Psi_L$ increased linearly with the percentage of nocturnal sap flow. These results are consistent with the idea that enhancing nutrient uptake by allowing additional transpiration to occur at night when evaporative demand is lower may avoid excessive dehydration associated with increased stomatal opening during the day when evaporative demand is high.

Keywords: Cerrado, leaf water potential, stomatal conductance, transpiration.

Introduction

Nitrogen or phosphorus limitations on growth are widespread in tropical regions, and are particularly severe in the neotropical savannas of central Brazil (Cerrado) characterized by well-drained, old oxisols. These soils are not only highly nutrient deficient, and their pH and cation exchange capacities low, but their aluminum saturation levels are high, which may affect phosphorylation and reduce availability of calcium and phosphorus (Furley and Ratter 1988, Haridasan 2000). Moreover, seasonal drought, high irradiances and high evaporative demand of the atmosphere are characteristic of the Cerrado region. Although Cerrado trees can achieve high transpiration rates and high stomatal conductances, which could potentially increase nutrient uptake, hydraulic limitations and the high evaporative demand of the atmosphere during the daytime, particularly during the dry season, impose strong stomatal control of transpiration (Meinzer et al. 1999; Bucci et al. 2005). Imbalances in water uptake and loss during the daytime may substantially constrain plant capacity to optimize nutrient uptake.

Nocturnal transpiration has been studied in relatively few species because it is generally assumed that stomata are uniformly closed at night or that water vapor near the leaf surface is either at or near saturation. However, some recent studies with temperate and tropical woody species indicate that, under certain environmental conditions, nocturnal water loss can be relatively high (Benyon 1999, Sellin 1999, Donovan et al. 1999, Oren et al. 1999, Bucci et al. 2004, Mark and Lechowicz 2007, Cavender-Bares et al. 2007, Dawson et al. 2007, Fisher et al. 2007, Hubbart et al. 2007, Kavanagh et al. 2007, Scholz et al. 2007). In one extreme case, the contribution of nocturnal water loss to total daily water loss was about 50% (Feild and Holbrook 2000). In Brazilian savanna sites, nighttime values of relative humidity as low as 40–50% during the dry season

result in substantial nocturnal transpiration rates in woody species (Bucci et al. 2004).

Nutrient supply rates in the soil ultimately govern the rates of nutrient acquisition by plants (Clarkson 1985). As roots grow through the soil, they intercept some nutrients. However, the amount is often less than that contained in the growing roots and therefore cannot serve as a net source of nutrients to the rest of the plant. Consequently, nutrients must arrive by mass flow or diffusion to the root surface. Transpiration may result in substantial nutrient transport from the bulk soil to the root surfaces. The extent to which mass flow is responsible for ion transport to the roots depends on the concentration of the different ions in the bulk solution relative to the requirement for plant growth. When the nutrient arrives at the root surface, its transport across the plasma membrane may occur either by diffusion down an electrochemical potential gradient or mainly by active transport against an electrochemical potential gradient. For many ions, it is their mobility in the soil, rather than the maximum inflow rate, that determines the rate at which roots can acquire them from the rhizosphere (Clarkson 1981).

There is evidence that the transpiration stream is largely responsible for supplying soil-mobile nutrients (e.g., nitrate) to plant roots. One of the assumptions underlying our study is that maintenance of water flux through the soil–plant–atmosphere continuum via nighttime transpiration can enhance nutrient supply to plant roots and hence improve the nutrient balance of Cerrado trees. If this assumption is correct, then lower rates of nocturnal water loss should be observed in plants growing in nutrient-deficient soils where nutrient limitations have been relieved. Alternative interpretations of the adaptive significance of nocturnal water loss can also be hypothesized. Here we have made use of a long-term fertilization experiment in savanna ecosystems of Central Brazil in which N, P and N + P nutrients have been added twice a year since 1998. Three dominant tree species were targeted in this phenomenological study of soil nutrient status, leaf water potentials and patterns of nocturnal transpiration.

Materials and methods

Study site and plant material

The study was conducted in a savanna site with intermediate tree density (Cerrado *sensu stricto*) 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). At the IBGE reserve, mean annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August are often devoid of precipitation. Mean monthly temperature ranges from 19 to 23 °C with diurnal temperature ranges of 20 °C being common during the dry season. The soils are deep oxisols containing about 70% clay. The development of micro aggregate structures (e.g., cementation by iron oxides) allows Cerrado soils to be generally porous and well drained despite their high clay content. Soil pH values were low (~ 4.2), and concentrations of P and N and organic matter were 0.4 mg kg^{-1} and 1.9 g kg^{-1} and 4 g kg^{-1}, respectively, in the upper 50 cm of soil in the study sites before the fertilization treatments began. The four fertilization treatments were: Control (no added fertilizer), N (100 kg ha^{-1} year^{-1} as ammonium sulfate), P (100 kg ha^{-1} year^{-1}, as super phosphate) and N plus P (100 kg N ha^{-1} year^{-1} + 100 kg P ha^{-1} year^{-1}). Plots were located in a homogeneous Cerrado stand with similar soil and vegetation characteristics. The field design consisted of 15 × 15 m plots with a buffer zone of 10 m between plots, and four replicates per treatment. The treatments were randomly assigned to each plot. An additional 1-m-wide border surrounding the 15 × 15 m treatment plots was also fertilized. Fertilizers were applied in October at the beginning of the wet season, and in March, before the end of the wet season, every year from October 1998 to March 2005.

Three dominant woody species with different leaf phenologies were selected for study: *Ouratea hexasperma* (St. Hil.) Baill (Ochnaceae) evergreen, *Blepharocalyx salicifolius* (H.B. & K.) Berg. (Myrtaceae) brevideciduous, and *Qualea grandiflora* Sims, (Vochyseaceae) deciduous. All of the species renew their leaves during the dry season. The brevideciduous species is functionally evergreen because it seldom remains leafless for more than a few days. The deciduous species remains leafless for about a month depending on the severity of the dry season. *Ouratea hexasperma* is a leaf-exchanger, in the sense that it shows progressive leaf senescence and abscission during the dry season and simultaneously produces the new leaves. Six trees of similar size of each species were selected in each treatment. The study trees were located as close to the center of each plot as possible to avoid edge effects and were fully exposed. Mean height (m; ± SE) and the stem diameter near the soil surface (cm; ± SE) of each of the study species are indicated in Table 1.

Leaf water potential and stomatal conductance

Leaf water potential (Ψ_L) was measured with a pressure cham-

Table 1. Characteristics of the study trees (height and basal diameter), and mean sapwood area and leaf area per branch. Values are means ± 1 SE (*n* = 6).

Species	Height (m)	Basal diameter (cm)	Mean sapwood area per branch (cm^2)	Mean leaf area per branch (m^2)
O. hexasperma	2.10 ± 0.19	7.13 ± 0.20	3.14 ± 0.14	0.20 ± 0.03
Q. grandiflora	3.30 ± 0.22	5.43 ± 0.50	2.78 ± 0.12	0.52 ± 0.06
B. salicifolius	3.50 ± 0.66	7.70 ± 1.04	3.56 ± 0.11	0.44 ± 0.04

ber (PMS Instruments, Corvallis, OR) in three covered (non-transpiring) and three exposed leaves on each of three individuals per species and treatment every 2 to 3 h throughout a 24-h cycle. To measure Ψ_L of covered leaves, all branches of three trees of each species and treatment were enclosed in both plastic bags and aluminum foil to minimize transpiration. Because differences in Ψ_L between trees of the same species and treatment were not statistically significant, data from all trees were pooled. Stomatal conductance (g_s) was measured with a steady-state porometer (Model LI-1600, Li-Cor, Lincoln, NE) in 5–7 leaves on the same trees used to measure Ψ_L of exposed leaves every 2 to 3 h throughout a 24-h cycle. New, fully expanded leaves from sun-exposed areas of the trees were measured. Cerrado trees tend to have crowns with low LAI and therefore there is little self-shading among leaves within the crown.

Sap flow

A heat pulse system based on the early work of Marshall (1958) and recently further developed by Burgess et al. (1998) and modified by Scholz et al. (2002) was used to measure sap flow in the terminal branches on two trees of each species per treatment. These trees were the same plants used to measure Ψ_L and g_s. A 6-s pulse of heat was produced by applying a known voltage across a 38 Ω line heater fitted inside a 10-μl glass micropipette and inserted in a hypodermic needle. The resulting increase in temperature was measured by two copper-constantan temperature probes placed at equal distance (0.6 cm) up- and downstream from the heater (Burgess et al. 1998). The thermocouples were connected by duplex insulated copper-constantan extension cable to a data logger (CR10X, Campbell Scientific, Inc.) sealed inside an insulated box to minimize temperature differences between the thermocouple inputs. The data logger controlled the duration of the heat pulse. The temperature probes and heater were coated with a thermal dissipation paste and inserted in holes drilled radially into stems using a template to minimize spacing errors. The heat pulse velocity (V_h, cm h^{-1}) was calculated according to Marshall (1958) as:

$$V_h = \frac{D_t}{x} \ln\left(\frac{v_1}{v_2}\right) \tag{1}$$

where D_t is thermal diffusivity of wet wood, x is distance between the heat source (line heater) and the temperature sensors, and v_1 and v_2 are the increase in temperature after the heat pulse, at equidistant points downstream and upstream, respectively, from the heater. Calculations were based on measurements made between 60 and 100 s after the release of the heat pulse, when the ratio of v_1 and v_2 was most stable (Burgess et al. 2001). Details of estimates of thermal diffusivity and other parameters and correction factors necessary for sap flow calculations can be found in Burgess et al. (2001) and Scholz et al. (2002). Heat pulses were applied every 30 min, after xylem temperatures around the temperature probes returned to ambient. Sap flow toward the leaves was considered to be positive

and flow toward the roots negative.

Volumetric sap flux (V_{sf}, cm^3 h^{-1}) through the stem was obtained by multiplying V_h by the sapwood cross-sectional area (Table 1). The active xylem area for water transport was obtained by injecting indigo carmine dye near the location where sap flow was measured (Meinzer et al. 1999). The diameters of the branches were measured with calipers. The branches were thermally insulated in the vicinity of the probes after installation. When negative flows were observed, zero flow offset values were determined at the end of the measurement period by severing the proximal and distal ends of the stem section containing the probes, thereby isolating it from the rest of the plant.

Water use per unit leaf area was obtained by dividing V_{sf} by the total leaf area per branch (Table 1). Total leaf area per branch was obtained by counting the total number of leaves per branch then multiplying by the mean area per leaf determined from fresh leaf samples for each branch.

Saturated water content

Saturated water content of sapwood in terminal branches was measured on the same branches used to measured sap flow. Sapwood samples were collected, sealed in aluminum foil and plastic bags and taken to the laboratory to determine the fresh mass and allowed to equilibrate overnight in deionized water. The samples were weighed after being lightly blotted with tissue paper to determine saturated mass and then oven-dried to a constant mass and reweighed to obtain the dry mass. Saturated water content was determined by dividing the saturated mass minus the dry mass by the dry mass

Results

During typical days at the middle and end of the dry season, the air saturation deficit (D) remained relatively high during the day and throughout the night, indicating that the driving force for nocturnal transpiration was substantial (Table 1). For example on September 10 and 11, 2005 (end the dry season), mean nocturnal D was 1.25 kPa and mean daily D was 2.07 kPa (Table 1). It is common to observe maximum D values approaching 4 kPa during the dry season. Air relative humidity can be as low as 10% during the middle of the day.

The water potentials of covered (non-transpiring) leaves were less negative than those of exposed leaves throughout the daytime and nighttime in all the species and treatments (Figure 1, Table 2). Nocturnal water potential of covered leaves ($\Psi_{L,covered}$) tended to be more negative in the N- and P-fertilized trees of all species, but the differences were not statistically significant. In contrast, nocturnal water potential of exposed leaves ($\Psi_{L,exposed}$) was more negative in trees on control plots than in trees on N and P plots, and the differences were significant for *O. hexasperma* and *Q. grandiflora* (Table 2). At night, differences between exposed and covered leaf water potential ($\Delta\Psi_L$) were smaller than during the daytime, ranging from 0 to 0.61 MPa at the end of the night (Figure 1). Unfertilized trees had higher nocturnal $\Delta\Psi_L$ than N- and P-fertilized trees. For example, $\Delta\Psi_L$ at predawn (0430–0530 h) in *O. hexasperma*
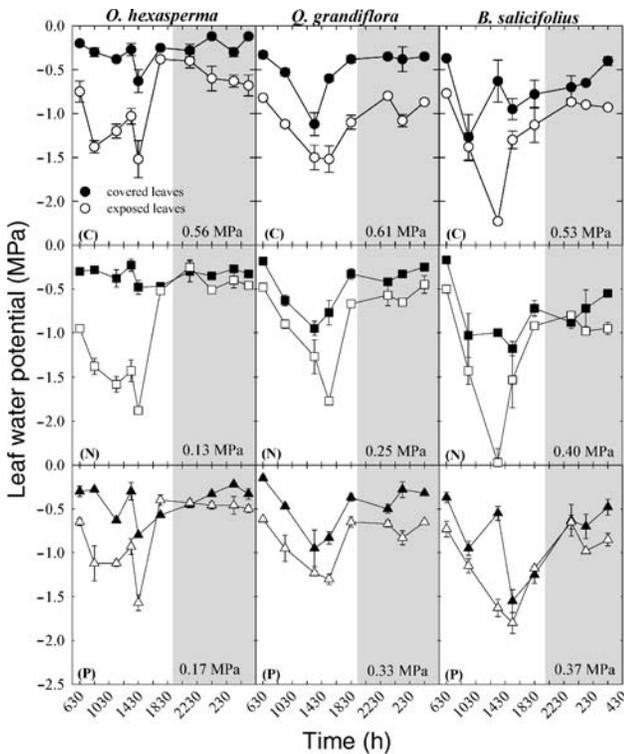
Figure 1. Daily courses of water potential of exposed (○, □, △) and covered leaves (●, ■, ▲) during the dry season for the study species in the treatments: Control (C), Nitrogen (N), Phosphorus (P). Each value represents the mean (± SE) for three leaves from three trees per species and treatment. Measurements were made on August 26 and 27, 2004 for *O. hexasperma*, on July 10 and 11, 2005 for *Q. grandiflora* and on September 10 and 11, 2005 for *B. salicifolius*. The gray area in each panel indicates the dark period. Predawn differences (0430–0530 h) between water potential of exposed and covered leaves are indicated in each panel.

was −0.56 MPa in trees in control plots and −0.13 and −0.17 MPa in trees in N and P fertilized plots, respectively (Figure 1). Significant differences between mean nocturnal

$\Delta\Psi_L$ (between 1900 and 0530 h) were observed in *O. hexasperma* and *Q. grandiflora* (Table 2).

Stomatal conductance (g_s) reached its maximum values during the morning in all species and treatments, and declined more or less continuously after 0930 h until nightfall (Figure 2). However, g_s never dropped below 25 mmol m^{-2} s^{-1} at night in any of the species, and in some cases increased to values of 150 mmol m^{-2} s^{-1} by the end of the dark period. In general, g_s was similar among treatments during the daytime for all species, but during the nocturnal period g_s tended to be lower in trees in the N and P treatments (Figure 2). For example, for *O. hexasperma*, nocturnal g_s was about 60 mmol m^{-2} s^{-1} in trees from control plots and 30 and 39 mmol m^{-2} s^{-1} in trees from N and P fertilized plots, respectively (Table 2). Nocturnal g_s was high in all species and treatments and constituted from 21 to 41% of total daily integrated stomatal conductance. Nocturnal g_s represented a higher percent of total daily g_s in unfertilized trees than in fertilized trees. For example for unfertilized *Q. grandiflora* trees, the percentage of nocturnal g_s compared with daily g_s was 41%, whereas in N- and P-fertilized trees nocturnal g_s constituted 33 and 36% of total daily g_s, respectively. During this period, the air saturation deficit (*D*) remained relatively high during the day and throughout the night, indicating that the driving force for nocturnal transpiration was substantial (Figure 2). For example on September 10 and 11, 2005 (end the dry season), mean nocturnal *D* was 1.25 kPa and mean daily *D* was 2.07 kPa (Figure 2, bottom). It is common to observe maximum *D* values approaching 4 kPa during the dry season. Air relative humidity can be as low as 10% during the middle of the day.

Water use per unit leaf surface area exhibited diurnal fluctuations typical of freely transpiring Cerrado woody plants (Figure 3). Maximum water use per unit of leaf surface area was about 0.5 mmol m^{-2} s^{-1} in *O. hexasperma* and *Q. grandiflora* and about 0.4 mmol m^{-2} s^{-1} in *B. salicifolius*. In general, mean water use per unit leaf surface area at night was higher for trees in control plots than for trees in fertilized plots. For example, nocturnal water use per unit leaf surface area of *Q. grandiflora*

Table 2. Mean values of nocturnal leaf water potential of covered leaves ($\Psi_{L, covered}$), exposed leaves ($\Psi_{L, exposed}$), nocturnal difference between Ψ_L of exposed and covered leaves ($\Delta\Psi_L$) and nocturnal stomatal conductance (g_s) between 1900 and 0530 h. Values are means ± 1SE of three trees and three leaves per tree for Ψ_L and 5–7 leaves per tree for g_s. Measurements were obtained on August 26 and 27, 2004 for *O. hexasperma*, on July 10 and 11, 2005 for *Q. grandiflora* and on September 10 and 11, 2005 for *B. salicifolius*. Significant effects of fertilization are indicated as: * = *P* < 0.1; and ** = *P* < 0.05. The treatments were: C, control; N, nitrogen fertilization; and P, phosphorus fertilization.

Species	Treatment	Nocturnal $\Psi_{L, covered}$ (MPa)	Nocturnal $\Psi_{L, exposed}$ (MPa)	$\Delta\Psi_L$ (MPa)	Nocturnal g_s (mmol m^{-2} s^{-1})
O. hexasperma	C	−0.19 ± 0.05	−0.58 ± 0.06	0.39 ± 0.10	60 ± 9
	N	−0.31 ± 0.01	−0.42 ± 0.05*	0.11 ± 0.03**	30 ± 1*
	P	−0.33 ± 0.05	−0.46 ± 0.01*	0.13 ± 0.05**	39 ± 3
Q. grandiflora	C	−0.30 ± 0.01	−0.91 ± 0.08	0.61 ± 0.07	158 ± 6
	N	−0.33 ± 0.05	−0.56 ± 0.06**	0.22 ± 0.05**	104 ± 9
	P	−0.37 ± 0.07	−0.70 ± 0.06*	0.30 ± 0.10*	102 ± 2
B. salicifolius	C	−0.58 ± 0.01	−0.90 ± 0.20	0.32 ± 0.10	81 ± 2
	N	−0.72 ± 0.10	−0.91 ± 0.06	0.19 ± 0.14	67 ± 10
	P	−0.61 ± 0.06	−0.83 ± 0.10	0.22 ± 0.10	77 ± 3
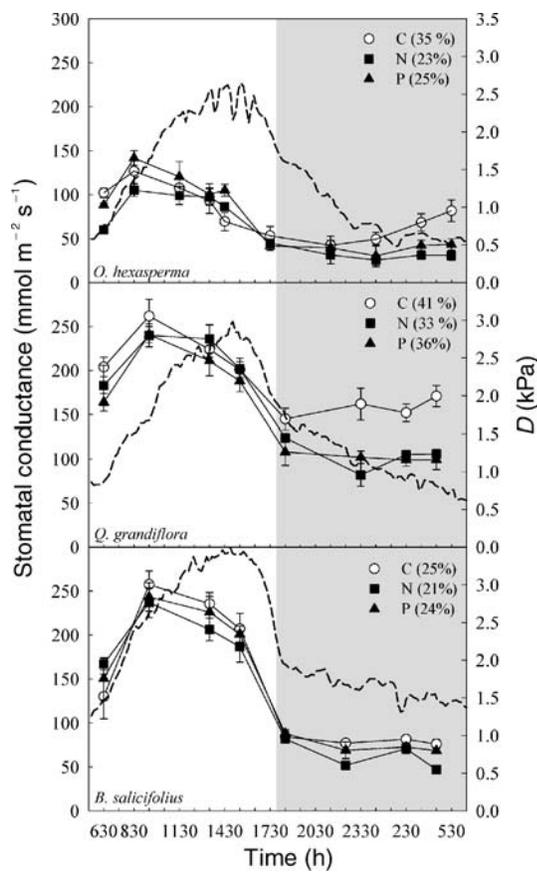
Figure 2. Daily courses of stomatal conductance and air saturation deficit (*D*) during the dry season for the study species in the treatments: Control (C), Nitrogen (N), Phosphorus (P). Each value represents the mean (± SE) for five to seven leaves from three trees per species and treatment. Measurements were obtained on August 26 and 27, 2004 for *O. hexasperma*, on July 10 and 11, 2005 for *Q. grandiflora* and on September 10 and 11, 2005 for *B. salicifolius*. The gray area indicates the dark period. Values within parentheses indicate the percent of nocturnal stomatal conductance compared with total daily stomatal conductance, calculated from the area under the curve for each species.

Figure 3. Mean daily courses of water loss per unit of leaf surface area in terminal branches during the dry season for two trees per study species in the treatments: Control (C), Nitrogen (N), Phosphorus (P). Measurements were obtained on August 25, 2004 for *O. hexasperma*, on July 9, 2005 for *Q. grandiflora* and on September 9, 2005 for *B. salicifolius*. Values within parentheses indicate the percent of nocturnal water loss compared to the total daily water loss.

reached 0.25 mmol m^{-2} s^{-1} in control trees and of 0.18 and 0.06 mmol m^{-2} s^{-1} in N- and P-fertilized trees, respectively. Nocturnal water use as a percentage of the total daily water use was higher for trees in control plots than for trees in fertilized plots (Figure 3). The greatest differences in percent of nocturnal water use between control and fertilizer treatments were observed in *O. hexasperma* and *Q. grandiflora*.

Scaling up the overall effects of nutrient additions to the whole stand by averaging the values of all the study species revealed that N and P decreased nocturnal $\Delta\Psi_L$, nocturnal g_s and nocturnal water use relative to the control values (Figure 4). Compared with the control value, mean nocturnal water use was consistently but not significantly lower in trees receiving P additions (Figure 4, bottom).

The difference in water potential between covered and exposed leaves before dawn increased linearly with increasing fraction of nocturnal water use across all species and treatments (Figure 5a). The values corresponding to the control trees tended to be distributed along the lower right-hand portion of the linear relationship, whereas the values for the fertilized trees were distributed along the upper left-hand portion of the relationship. Nocturnal water use measured as sap flow and predawn stomatal conductance were positively and linearly correlated (Figure 5b). Predawn stomatal conductance decreased exponentially with increasing sapwood saturated water content (%) in a similar fashion independent of individuals, species and treatments (Figure 6). Species and individuals with greater saturated water content in sapwood of terminal branches thus exhibited stronger stomatal control during the night than species and individuals with lower saturated water content.

Discussion

At a savanna site similar to the one we studied, Bucci et al. 2004) found that nighttime transpiration in Cerrado trees is
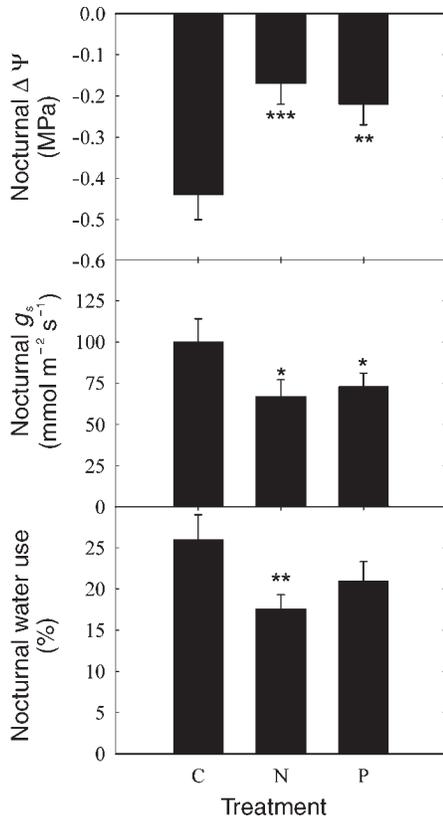
Figure 4. Pooled data for the study species showing mean differences between nocturnal (between 1900 and 0530 h) water potential of exposed and covered leaves ($\Delta\Psi_L$), nocturnal stomatal conductance (g_s) and nocturnal water use (%) in the control and fertilization treatments. Treatments are: Control (C), Nitrogen (N), and Phosphorus (P). Bars are mean values + or – 1SE. Significant effects of fertilization are indicated as: * = $P < 0.1$; ** = $P < 0.05$; and *** = $P < 0.01$.

Figure 5. (a) Differences between leaf water potential of exposed and covered leaves ($\Delta\Psi$, between 0430 and 0530 h) and (b) predawn stomatal conductance (between 0430 and 0530 h), in relation to nocturnal water use measured in terminal branches during the dry season. Each value represents an individual tree (two trees per species per treatment). Treatments are: Control (C) (open symbols), Nitrogen (N) or Phosphorus (P) (closed symbols). Species are: *O. hexasperma* (Ou, $\diamond$, $\blacklozenge$), *Q. grandiflora* (Qg, $\square$, $\blacksquare$) and *B. salicifolius* (Ble, $\triangledown$, $\blacktriangledown$). The lines were fit by linear regression: (a) $y = 0.03 – 0.018x$, $P < 0.001$; and (b) $y = –32 + 5.5x$, $P < 0.001$.

substantial, accounting for 13 to 28% of total daily transpiration, and has a series of effects on plant water relations, contributing to predawn disequilibrium in Ψ between leaves and soil. Three independent lines of evidence for nocturnal transpiration exist for Cerrado trees, (1) high nocturnal g_s measured with a steady state porometer (Bucci et al. 2004), (2) nocturnal sap flow measured with heat dissipation and heat pulse systems (Bucci et al. 2004, Bucci et al. 2005), and (3) failure to achieve equilibrium between leaf and soil water potential overnight (Bucci et al. 2004, Bucci et al. 2005). In our study, both g_s and water utilization per unit of leaf surface area were higher at nighttime in the control trees than in the fertilized trees. Differences in g_s and water use were consistent across species and treatments, with particularly large differences in g_s and water use between control trees and fertilized trees observed in *Q. grandifolia*. The large effects of N and P fertilizers on g_s in *Q. grandifolia* compared with their small effects in *B. salicifolius* are consistent with the relative differences in water use per unit leaf surface area for these species. A strong, positive linear relationship was observed between nighttime stomatal conductance and nocturnal water use

across treatments and species (Figure 5b).

Nighttime Ψ_L was more negative in exposed leaves than in covered leaves for all species, indicating that they did not achieve internal water potential equilibrium by dawn. The differences were larger in control trees than in fertilized trees, consistent with differences in water loss between treatments at night. This implies that the magnitude of predawn water potential disequilibrium was related to the amount of soil nutrients. A large portion of the liquid-phase resistance in the water transport pathway lies in small twigs, petioles and the leaf lamina itself (Brodribb and Holbrook 2003, Bucci et al. 2003). Covered leaf water potential gives an estimate of the water po-
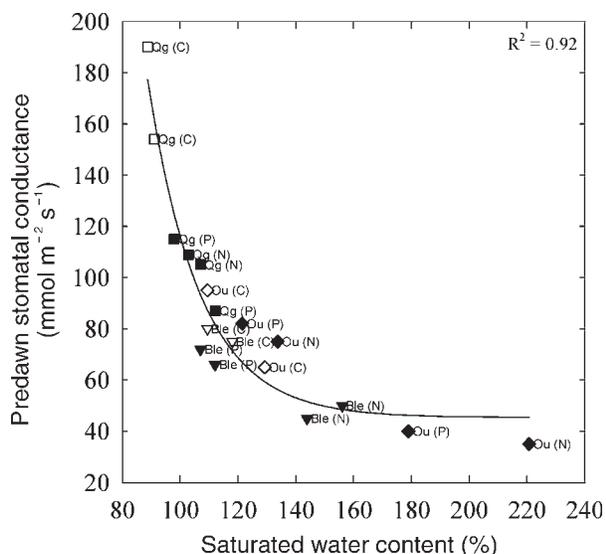
Figure 6. Relationship between predawn stomatal conductance (between 430 and 530 h) and sapwood saturated water content (%) in terminal branches, during the dry season. Each value represents an individual tree (two trees per species per treatment). Treatments are: Control (C) (open symbols), Nitrogen (N) or Phosphorus (P) (closed symbols). Species are: *O. hexasperma* (Ou, $\diamond$, $\blacklozenge$), *Q. grandiflora* (Qg, $\square$, $\blacksquare$) and *B. salicifolius* (Ble, $\triangledown$, $\blacktriangledown$). The line is an exponential decay function: $y = 45 + 17970 \exp(-0.055x)$, $P < 0.001$.

tential of the stem at the point of leaf insertion (Begg and Turner 1970). Consequently, larger nighttime fluxes should result in larger differences between stem and leaf water potentials. If the magnitude of nighttime transpiration is low, then the water potential differences should be small, assuming that the resistances encountered by the transpiration stream between the stem and the leaves are not substantially affected by the fertilization treatment. Although nighttime water fluxes were lower in N-and P-fertilized trees than in unfertilized trees, persistent differences in Ψ_L of covered and exposed leaves indicated that the fertilized trees did not achieve full internal water potential equilibration by dawn. Both control and fertilized trees were distributed along a continuum of increasing water potential disequilibrium with increasing nocturnal water use (Figure 5a).

The fertilizer treatments affected the biophysical properties of the xylem. The saturated water content of terminal branch sapwood tended to be greater in the N- and P-fertilized trees than in unfertilized trees, and nighttime g_s decreased exponentially with increasing sapwood saturated water content in a similar fashion independent of species and treatment (Figure 6). Saturated water content is inversely related to wood density. Lower wood density and higher saturated water content in stems of fertilized trees may have been the result of higher stem growth rates. Lower saturated water content, and therefore lower sapwood water storage capacity, was associated with higher nocturnal g_s, higher nocturnal water use and higher water potential disequilibrium. At night, incompletely recharged internal water storage compartments are likely to

compete with transpiring leaves as sinks for water taken up from the soil. Consequently, it may be adaptive to have a small water storage capacity if high nocturnal transpiration prevents overnight equilibration between leaf and soil Ψ in Cerrado woody species. Stem water stores tend to recharge faster when nocturnal transpiration is reduced (Bucci et al. 2004), suggesting that trees either use water from internal stem stores before dawn or the water that could be used to recharge the stem water storage is used to replace leaf level nocturnal water losses, or both. Incomplete recharge of internal water storage may explain the steady increase in Ψ_L of covered plants at night and the failure to achieve complete equilibration even though nocturnal transpiration was largely prevented (Bucci et al. 2004).

Another potential mechanism that could prevent overnight equilibration of leaf and soil Ψ is hydraulic redistribution, the movement of water from moist to drier regions of the soil profile via plant roots (Scholz et al. 2002). Hydraulic redistribution may occur when the water potential of the soil around shallow roots is lower than that of the shoot. Soil water potentials in the range of –1.5 to –2.5 MPa are frequently observed in the upper soil layers during the dry season in Cerrado ecosystems (Scholz et al. 2002, Meinzer et al. 2004). Consequently, soil near the shallow roots can constitute a competing sink for water taken up by deep roots that otherwise could be used to replace water loss from the leaves at night or for water storage recharge. During the dry season, hydraulic redistribution is a common phenomenon in Cerrado woody plants with dimorphic root systems (Scholz et al. 2002, Moreira et al. 2003). Among the species studied, *B. salicifolius* had the lowest transpirational water loss at night not only in the fertilized plots but also in the control plots, and this species also exhibits the most frequent hydraulic redistribution during the dry season (Scholz et al. unpublished data). Hydraulic redistribution has been hypothesized to enhance nutrient capture in dry soils (Caldwell et al. 1998). Thus, a species that exhibits vigorous hydraulic redistribution may have a lower requirement for nocturnal transpiration if the latter also enhances nutrient capture in nutrient-poor systems.

There may be several alternative explanations for the dependence of nighttime g_s on soil nutrient availability. It has been shown that N and P deficiencies may reduce the amplitude of diurnal changes in root hydraulic conductivity (Clarkson et al. 2000), leading to relatively higher hydraulic conductivity at night in nutrient-limited sites. Higher conductivity may promote higher nocturnal g_s in nutrient-deficient plants compared with plants that have ample nutrient availability. Nitrogen-fertilized Cerrado plants exhibit more negative leaf water potentials and higher rates of water utilization because of an increase in total leaf surface are per plant (Bucci et al. 2006). This may result in more ABA synthesis in the roots, which when transported to the leaves may promote partial stomatal closure in the N-fertilized plants. In addition both N and P fertilization may have enhanced fine root production in the upper soil layers that have lower soil water availability. Roots exploring drier soil layers may synthesize and export more ABA to shoots. This hypothesis is consistent with the lower nighttime values of Ψ_L observed for covered leaves of all spe-

cies in the N- and P-fertilized plots compared with the control plots. Nighttime water potentials of covered leaves better reflect weighted mean soil water potentials in the rhizosphere than water potentials of exposed leaves. Fertilization-induced changes in the pH of xylem sap may also have contributed to reduced nighttime g_s in fertilized plants. The pH of the xylem sap can change dramatically in response to mineral nutrient supply in the absence of concomitant changes in either root or shoot water status (Wilkinson et al. 1998). An increase in xylem ABA concentration as well as an increase in xylem pH can act as signals for leaves to close their stomata (Wilkinson and Davies 1997). Leaf calcium concentration may also affect stomatal action, with lower leaf Ca concentrations resulting in less complete stomatal closure, possibly via interactions with ABA (Atkinson et al. 1990, Atkinson 1991). Leaves of control trees of *Q. parviflora* and *B. salicifolius* had lower foliar Ca concentrations than leaves of the same species growing in the P-fertilized plots. For example, leaves of *Q. parviflora* had 2.3 g kg^{-1}of Ca in the control plots and 4.2 g kg^{-1} in the P-fertilized plots. Foliar Ca increased in *O. hexasperma* in both the N and P treatments. All of these variables can act in isolation or synergistically to promote reductions in g_s at night in Cerrado trees.

Regardless of the mechanisms that act to maintain stomatal opening at night in nutrient-poor Cerrado sites, higher rates of nocturnal transpiration could facilitate nutrient acquisition. Soil water utilization by plants may result in substantial nutrient transport from the bulk soil to the root surfaces. From an adaptive point of view, it may be better to enhance water flux toward roots in Cerrado plants at night because vapor pressure deficits are extremely high during the daytime. Nocturnal water loss may enhance nutrient uptake without the cost of having to open the stomata widely when high evaporative demand and hydraulic constraints may result in excessive dehydration. Having stomata partially open at dawn may present an additional advantage of ensuring that diffusive resistance for entry of CO_2 is low when evaporative demand is still low. We found that Cerrado trees in nutrient-poor soils exhibited relatively high rates of nocturnal water loss compared with the same species in soils where nutrient limitations had been relieved by long-term fertilization. Although the association of soil nutrient availability with rates of nocturnal transpiration does not necessarily imply causality, it is the first indirect evidence that water loss at night may have adaptive value by enhancing nutrient acquisition in the extremely nutrient-poor soils of the Cerrado.

Acknowledgments

References

Atkinson, C.J., T.A. Mansfield and W.J. Davies. 1990. Does calcium in xylem sap regulate stomatal conductance? New Phytol. 116: 19–27.

Atkinson, C.J. 1991. The flux and distribution of xylem sap calcium to adaxial and abaxial epidermal tissue in relation to stomatal behaviour. J. Exp. Bot. 42:987–993.

Begg, J.E. and N.C. Turner. 1970. Water potential gradients in field tobacco. Plant Physiol. 46:343–346.

Benyon, R.G. 1999. Nighttime water use in an irrigated *Eucalyptus grandis* plantation. Tree Physiol. 19:853–859.

Brodribb, T.J. and N.M. Holbrook. 2003. Stomatal closure during leaf dehydration correlation with other leaf physiological traits. Plant Physiol. 132:2166–2173.

Bucci, S.J., F.G. Scholz, G. Goldstein, F.C. Meinzer and L. DA. S.L. Sternberg. 2003. Dynamic changes in hydraulic conductivity in petioles of two savannas tree species: factors and mechanisms contributing to the refilling to embolized vessels. Plant Cell Environ. 26:1633–1645.

Bucci, S.J., G. Goldstein, F.C. Meinzer, F.G. Scholz, A.C. Franco and M. Bustamante. 2004. Functional convergence in hydraulic architecture and water relations of savanna trees: from leaf to whole plant. Tree Physiol. 24:891–899.

Bucci, S.J., G. Goldstein, F.C. Meinzer, A.C. Franco, P. Campanello and F.G. Scholz. 2005. Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plants in Neotropical savanna trees. Trees 19:296–304.

Bucci, S.J., F.G. Scholz, G. Goldstein, F.C. Meinzer, A.C. Franco, P. Campanello, R. Villalobos Vega, M. Bustamante and F. Miralles-Welheim. 2006. Nutrient availability constrains the hydraulic architecture and water relations of savanna trees. Plant Cell Environ. 29:2153–2167.

Burgess, S.S.O., M.A. Adams, N.C. Turner and C.K. Ong. 1998. The redistribution of soil water by tree root systems. Oecologia 115: 306–311.

Burgess, S.S.O., M.A. Adams, N.C. Turner, C.R. Beverly, C.K Ong, H.A. Khan and T.M. Bleby. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiol. 21:589–598.

Caldwell, M.M., T.E. Dawson and J.H. Richards. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113:151–161.

Cavender-Bares, J., L. Sack and J. Savage. 2007. Atmospheric and soil drought reduce nocturnal conductance in live oaks. Tree Physiol. 27:611–620.

Clarkson, D.T. 1981. Nutrient interception and transport by root systems. *In* Physiological Factors Limiting Plant Productivity. Ed. C.B. Johnson. Butterworths, London, pp 307–314.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annu. Rev. Plant Physiol. 36:77–115.

Clarkson, D.T., M. Carvajal, T. Henzler, R.N. Waterhouse, A.J. Smyth, D.T. Cooke and E. Steudle. 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. J. Exp.Bot. 51:61–70.

Dawson, T.E., S.S.O. Burgess, K.P. Tu, R.S. Oliveira, L.S. Santiago, J.B. Fisher, K.A. Simonin and A.R. Ambrose. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiol. 27:561–575.

Donovan, L.A., D.J. Grisé, J.B. West, R.A. Papport, N.N. Alder and J.H. Richards. 1999. Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. Oecologia 120: 209–217.

Feild, T.S. and N.M. Holbrook. 2000. Xylem sap flow and stem hydraulics of the vesselness angiosperm *Drymis granadensis* (Winteraceae) in a Costa Rican elfin forest. Plant Cell Environ. 23: 1067–1077.

Fisher, J.B., T.A. Debiase, Y. Qi, M. Xu and A.H. Goldstein. 2005. Evapotranspiration models compared on a Sierra Nevada forest ecosystem. Environ. Model. Software 20:783–796.

Fisher, J.B., D.D. Baldocchi, L. Misson, T.E. Dawson and A.H. Goldstein. 2007. What the towers don't see at night: nocturnal sap flow in trees and shrubs at two AmeriFlux sites in California. Tree Physiol. 27:597–609.

Furley, P.A. and J.A. Ratter.1988. Soil resources and plant communities of the central Brazilian Cerrado and their development. J. Biogeogr. 15:97–108.

Haridasan, M. 2000. Nutrição mineral das plantas nativas do Cerrado. Rev. Brasil. Fisiol. Veget. 12:54–64.

Hubbart, J.A., K.L. Kavanagh, R. Pangle, T. Link and A. Schotzko. 2007. Cold air drainage and modeled nocturnal leaf water potential in complex forested terrain. Tree Physiol. 27:631–639.

Kavanagh, K.L., R. Pangle and A.D. Schotzko. 2007. Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. Tree Physiol. 27:621–629.

Marshall, D.C. 1958. Measurement of sap flow in conifers by heat transport. Plant Physiol. 33:385–396.

Marks, C.O. and M.J. Lechowicz. 2007. The ecological and functional correlates of nocturnal transpiration. Tree Physiol. 27: 577–584.

Meinzer, F.C., G. Goldstein, A.C. Franco, M. Bustamante, E. Igler, P. Jackson, L. Caldas and P.W. Rundel. 1999. Atmospheric and hydraulic limitations on transpiration in Brazilian Cerrado woody species. Funct. Ecol. 13:273–282.

Meinzer, F.C., J.R. Brooks, S.J. Bucci, G. Goldstein, F.G. Scholzy and J.M. Warren. 2004. Converging patterns of uptake and hydraulic redistribution of soil water in contrasting woody vegetation types. Tree Physiol. 24:919–928.

Moreira, M.Z., F.G. Scholz, S.J. Bucci, L.S. Sternberg, G. Goldstein, F.C. Meinzer and A.C. Franco. 2003. Hydraulic lift in a neotropical savannah. Funct. Ecol 17:573–581.

Oren, R., N. Phillips, B.E. Ewers, D.E. Pataki and J.P. Megonigal. 1999. Sap-flux scaled transpiration responses to light, air saturation deficit, and leaf area allocation in a flooded *Taxodium distichum* forest. Tree Physiol. 19:337–347.

Scholz, F.G., S.J. Bucci, G. Goldstein, F.C. Meinzer and A.C. Franco. 2002. Hydraulic redistribution of soil water by neotropical savanna trees. Tree Physiol. 22:660–612.

Sellin, A. 1999. Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? Acta Oecol. 20:51–69.

Wilkinson, S., J.E. Corlett, L. Oger and W.J. Davies. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves. Plant Physiol. 117:703–709.

Wilkinson, S.J. and W.J. Davies. 1997. Xylem sap pH increase a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol. 113:559–573.