

## SHORT COMMUNICATION

# Leaf tissue water relations in tree species from contrasting habitats within the upper Rio Negro forests of the Amazon region

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The landscape of the upper Rio Negro basin (North Amazon) exhibits distinctive habitats that are associated with differential soil characteristics and topographical conditions as well as species composition (Herrera *et al.* 1978). The mixed forests thrive on well-structured oxisols on slightly more elevated areas. The valleys with sandy podzols are occupied by the ‘Amazon caatinga’ complex with three distinct zones: the bottom valley and the gentle slopes, both of which have closed forests, and the sandy domes with open forests (‘bana’ or sclerophyllous forest; Breimer 1985). From the mixed forest towards the caatinga valley-slope-dome habitats, the leaf  $\delta^{15}\text{N}$  signatures become increasingly negative, suggesting a trend in N limitation in the same direction (Sobrado 2010). Thus, negative leaf  $\delta^{15}\text{N}$  signatures depleted in  $^{15}\text{N}$  compared with the soil indicate a very tight N cycle in all of the habitats. Water availability follows a similar pattern from the top of the oxisol towards the flooded valley bottom of the caatinga, with extreme water-table fluctuations in the sandy domes (Klinge 1978). Thus, parallel variation in nutrient and water availabilities exist in this area that are associated with soil characteristics and topography. Under such contrasting habitats, species-specific responses would be linked to particular conditions of the habitat at a local scale (Comita & Engelbrecht 2009, Engelbrecht *et al.* 2007). A number of studies in these habitats have shown that this is the case for soil fertility (Coomes 1997, Medina

*et al.* 1990, Sobrado 2010, Sobrado & Medina 1980). Similarly, the hydraulic characteristics and long-term water use are species specific and related to particular conditions of the habitat at the local scale (Sobrado 2010). In this report, it was hypothesized that the leaf tissue water relations of species thriving in different habitats may reflect the water availability at the particular sites as well. The leaf tissue water relations of species thriving in the extreme nutrient and water-supply conditions of the sandy domes from the caatinga complex have been previously studied in detail (Sobrado 2009a). However, these data are currently not available for the species that thrive in the surrounding area of the closed forests, and importantly, such information would allow for a comparison across habitats. Therefore, the present study assessed the minimum leaf water potential (midday) under field conditions as well as the leaf tissue water relations by using pressure-volume analysis of dominant tree species in the top canopy of these high-stature forests.

The study area is located 4 km north-east of San Carlos de Rio Negro (Amazonas, Venezuela; 1°54'N; 67°03'W) within the upper Rio Negro basin. The zone features a mean temperature of 26.2 °C and an annual rainfall of 3500 mm. No month receives less than 100 mm of precipitation. Similar to previous studies conducted in these habitats (Sobrado 2010, 2011), the experiments assessed *Caryocar glabrum* (Aubl.) Pers. (Caryocaraceae) and *Ocotea aciphylla* (Nees & Mart. ex Nees) Mez (Lauraceae) from the mixed forest (oxisol soil) and *Eperua*

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*leucanta* Benth. (Caesalpinaceae) and *Micranda sprucei* (Müll.Arg.) R. E. Schultes (Euphorbiaceae) from the caatinga complex (podzol soil). *Eperua leucantha* is the dominant species of the bottom valley of the caatinga forest ('yaguacana' forest), and *M. sprucei* is the dominant species in the ecotone ('cunuri' forest) between the bottom valley and the sandy mounds, which is where the sclerophyllous forest is located ('bana'; Breimer 1985, Klinge 1978). In all species, top-canopy leaves are typical sun leaves that possess a dorsiventral anatomy with bundle-sheath extensions (heterobaric) and a chlorophyll *a* to *b* ratio of approximately 3:1 (Sobrado 2011). However *M. sprucei* from the caatinga slopes possesses the thickest leaves, lowest-density tissue, lowest palisade to spongy parenchyma ratio, highest sclerophylly index, highest carbon-to-nitrogen ratio, and most negative  $\delta^{15}\text{N}$  of all of the species in this study (Sobrado 2010). The characteristics of *M. sprucei* are comparable to those found in the sclerophyllous forest.

For all measurements, fully exposed top-canopy branches were selected from three trees per species that were tagged for the experiments during August 2011. Samples were collected by climbing each tree to reach the canopy top. Twenty leaf punches with a 3-cm diameter were collected from three twigs, respectively, at predawn in each tree species and their fresh weight was measured. Subsequently, the dry mass was determined after the leaves were oven-dried at 60 °C. The data were used to calculate the water content and expressed based upon the leaf area ( $W_a$ ) as well as fresh ( $W_f$ ) and dry mass ( $W_d$ ). The minimum tree water status was characterized by measuring the midday leaf water potential ( $\Psi$ , MPa) under rainy conditions and after six rainless days that occurred during August 2011.  $\Psi$  was measured with a pressure chamber (PMS, Model 1000, Corvallis, Oregon, USA) in two–three twig samples per tree species. Pressure–volume (P–V) curves were determined for two twigs from each tagged sample collected in the field at predawn for a total of six samples per species. Sampled twigs were allowed to dehydrate naturally on the laboratory bench and P–V curves were generated. During dehydration, the weight loss was determined on an analytical balance and  $\Psi$  was immediately measured using the pressure chamber. The reciprocal of  $\Psi$  ( $1/\Psi$ ) was plotted as a function of relative water content (RWC). These plots were used to calculate the osmotic potential at full ( $\Psi_{(100)}$ ) and zero ( $\Psi_{(0)}$ ) turgor, RWC at zero turgor ( $\text{RWC}_0$ ), and apoplastic (A) water content (Tyree & Hammel 1982). The volumetric elastic modulus ( $\varepsilon$ ) was calculated as the slope of the relationship of turgor pressure ( $\Psi_p$ ) above zero as a function of RWC. One-way analysis of variance (ANOVA) followed by the Holm–Sidak test was used to compare data across species if the data were normally distributed, and equal variance was measured. ANOVA-on-ranks test was used if data normality and/or equal variance tests failed,

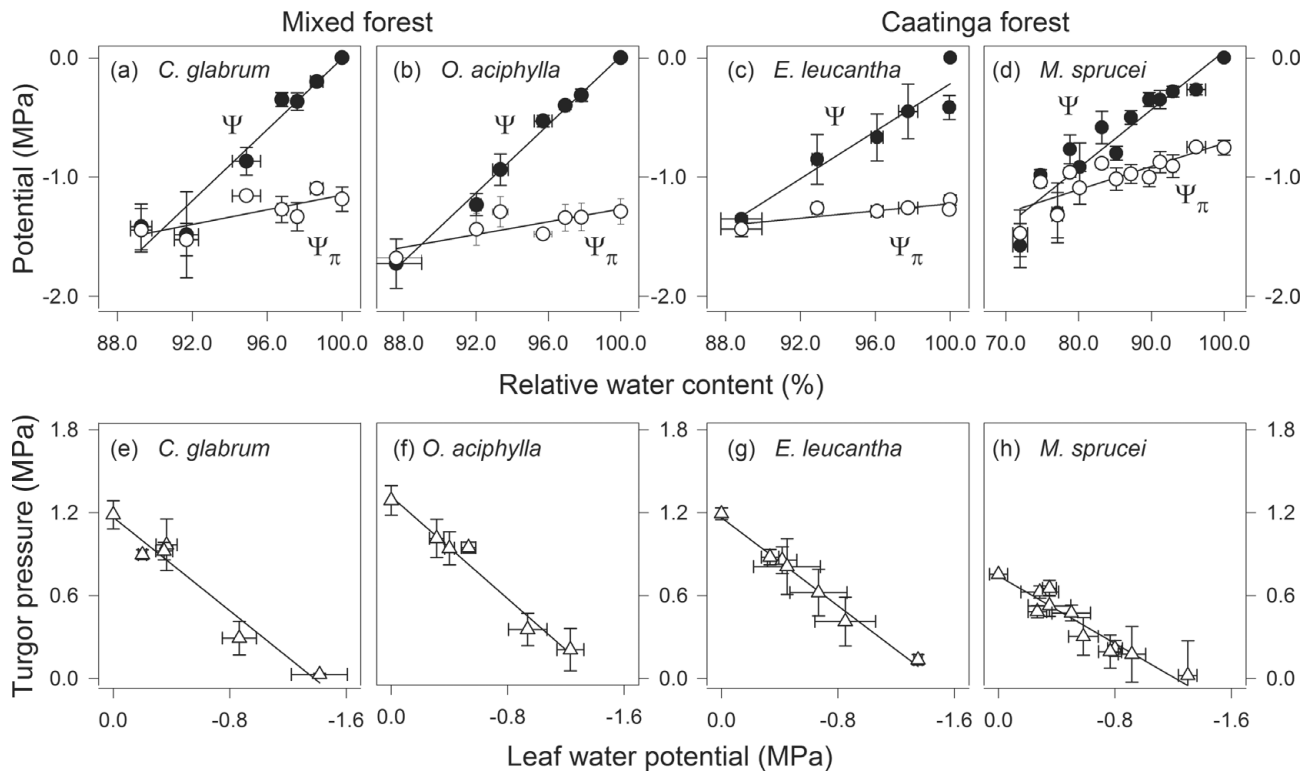
followed by a Tukey test. The significance level was set at  $P < 0.05$ . All analyses were performed using SigmaStats 3.1 software for Windows (Systat Software, Inc, Chicago, Illinois, USA).

The leaf  $W_a$  was significantly higher in the thicker leaves of *M. sprucei* compared with *C. glabrum*, *O. aciphylla* and *E. leucantha* (Table 1; Sobrado 2010). Moreover,  $W_f$  and  $W_d$  were species specific and not differentially associated with forest types (Table 1). Values of  $W_f$  were comparable to those of the species from the open sclerophyllous forest ( $0.58 \pm 0.02 \text{ g g}^{-1}$ ; Sobrado 2008). On rainy days, the minimum  $\Psi$  averaged  $-0.98 \pm 0.05$  MPa, which was significantly higher than that found during rainless days ( $-1.2 \pm 0.05$  MPa) (Table 1). The osmotic potential at full ( $\Psi_{\pi(100)}$ ) and zero ( $\Psi_{\pi(0)}$ ) turgor was not statistically different in *C. glabrum*, *O. aciphylla* and *E. leucantha* and averaged  $-1.19 \pm 0.04$  and  $-1.38 \pm 0.06$  MPa, respectively (Table 1, Figure 1a–c). However, the values of these parameters for *M. sprucei* were significantly higher ( $-0.75 \pm 0.06$  and  $-1.12 \pm 0.12$  MPa, respectively; Table 1, Figure 1d). Therefore, *M. sprucei* develops lower leaf turgor ( $\Psi_p$ ) and loses it at the highest  $\Psi$  compared with the other species (Figure 1e–h). Conversely, *M. sprucei* had the highest elasticity ( $< \varepsilon$ ; Table 1), which resulted in the highest leaf dehydration among the species (Figure 1a–d), while still maintaining  $\Psi$  within the range of positive turgor (Figure 1e–h). Thus, high elastic cells of *M. sprucei* allow for the partial compensation of unfavourable high  $\Psi_{\pi}$ . Turgor maintenance as  $\Psi$  declines can be achieved through low  $\Psi_{\pi}$  and high cell elasticity. However, variation in  $\Psi$  across species for turgor loss arises predominantly from differences in  $\Psi_{\pi}$  rather than from differences in  $\varepsilon$  (Lenz *et al.* 2006). The apoplastic water content (A) was comparable across species and averaged  $27.7 \pm 1.84\%$  (Table 1). Therefore, values of  $\Psi_{\pi(100)}$  and  $\Psi_{\pi(0)}$  were higher (less negative) in all of the species than values found in the open sclerophyllous forest ( $-1.48 \pm 0.05$  and  $-1.92 \pm 0.05$  MPa, respectively) (Sobrado 2009a). These results implied that dominant species in the mixed forest as well as in the valley and slope of the caatinga maintain turgor pressure at considerably higher  $\Psi$  than those located on top of the sandy mounds. Importantly, the values of  $\varepsilon$  and A found in these species overlapped with those found in the open forests on the top of the sandy domes except in *M. sprucei* with the lowest  $\varepsilon$ . In the open sclerophyllous forests, the range of  $\varepsilon$  and A are 6–16 MPa and 18–43%, respectively (Sobrado 2009a).

In conclusion, the top-canopy leaves of the species assessed in this study experienced a very mild decrease in leaf  $\Psi$  ( $\sim -1.1$  to  $-1.5$  MPa) during the rainless period. Furthermore, the analysis of tissue water relations showed that these species were unable to develop very low  $\Psi$  within the range of turgor maintenance.

**Table 1.** Leaf water content based on surface area ( $W_a$ ), fresh weight ( $W_f$ ) and dry mass ( $W_d$ ), midday leaf water potential ( $\Psi$ ) during rainy (RY) and rainless (RL) days, as well as the following pressure-volume parameters: osmotic potential at full ( $\Psi$ ) and zero turgor ( $\Psi_\pi$ ), relative water content at zero turgor ( $RWC_0$ ), apoplastic water content (A) and elastic modulus ( $\epsilon$ ). All measurements were taken in *Caryocar glabrum* and *Ocotea aciphylla* from the mixed forest and *Eperua leucantha* and *Micranda sprucei* from the Amazon caatinga. Each value is the mean  $\pm$  SE of measurements taken from three trees per species. For each parameter, the mean followed by a letter represent statistical differences at  $P < 0.05$ .

Parameter	Mixed forest		Caatinga forest	
	<i>C. glabrum</i>	<i>O. aciphylla</i>	<i>E. leucantha</i>	<i>M. sprucei</i>
$W_a$ (g m <sup>-2</sup> )	157 $\pm$ 3b	173 $\pm$ 8b	174 $\pm$ 10b	228 $\pm$ 9a
$W_f$ (g g <sup>-1</sup> )	0.66 $\pm$ 0.01ab	0.58 $\pm$ 0.01b	0.68 $\pm$ 0.01a	0.62 $\pm$ 0.01b
$W_d$ (g g <sup>-1</sup> )	1.91 $\pm$ 0.05ab	1.41 $\pm$ 0.06c	2.14 $\pm$ 0.14a	1.65 $\pm$ 0.06bc
RY $\Psi$ (MPa)	-0.75 $\pm$ 0.03a	-1.25 $\pm$ 0.03c	-0.97 $\pm$ 0.03b	-0.97 $\pm$ 0.03b
RL $\Psi$ (MPa)	-1.13 $\pm$ 0.02ab	-1.48 $\pm$ 0.04c	-1.23 $\pm$ 0.02b	-1.07 $\pm$ 0.04a
$\Psi_{\pi(100)}$ (MPa)	-1.09 $\pm$ 0.02b	-1.29 $\pm$ 0.11b	-1.19 $\pm$ 0.04b	-0.75 $\pm$ 0.06a
$\Psi_{\pi(0)}$ (MPa)	-1.28 $\pm$ 0.02ab	-1.50 $\pm$ 0.16b	-1.36 $\pm$ 0.1b	-1.12 $\pm$ 0.12a
RWC <sub>0</sub> (%)	89 $\pm$ 1a	89 $\pm$ 2a	90 $\pm$ 4a	78 $\pm$ 2b
A (%)	28 $\pm$ 1	27 $\pm$ 1	24 $\pm$ 3	33 $\pm$ 6
$\epsilon$ (MPa)	11 $\pm$ 1b	14 $\pm$ 1a	8 $\pm$ 3b	4 $\pm$ 1c



**Figure 1.** Leaf water potential ( $\Psi$ ) and osmotic potential ( $\Psi_\pi$ ) as a function of relative water content (a–d) as well as leaf turgor pressure ( $\Psi_p$ ) as a function of leaf water potential (e–h) in two species of the mixed forest (*Caryocar glabrum* and *Ocotea aciphylla*) and two species of the Amazon caatinga forest (*Eperua leucantha* and *Micranda sprucei*). Plots were derived from six pressure–volume (P–V) curves for each species. Linear regressions are shown with the following equations:  $\Psi = 0.15 \text{ RWC} - 14.9$  ( $r = 0.97$ ;  $P < 0.001$ ),  $\Psi_\pi = 0.03 \text{ RWC} - 4.25$  ( $r = 0.77$ ;  $P < 0.05$ ) and  $\Psi_p = 0.85 \Psi + 1.17$  ( $r = 0.98$ ;  $P < 0.001$ ) for *C. glabrum*,  $\Psi = 0.14 \text{ RWC} - 14.3$  ( $r = 0.99$ ;  $P < 0.001$ ),  $\Psi_\pi = 0.03 \text{ RWC} - 3.96$  ( $r = 0.80$ ;  $P < 0.05$ ) and  $\Psi_p = 0.93 \Psi + 1.32$  ( $r = 0.99$ ;  $P < 0.001$ ) for *O. aciphylla*,  $\Psi = 0.09 \text{ RWC} - 10.2$  ( $r = 0.95$ ;  $P < 0.01$ ),  $\Psi_\pi = 0.02 \text{ RWC} - 2.75$  ( $r = 0.83$ ;  $P < 0.05$ ) and  $\Psi_p = 0.79 \Psi + 1.16$  ( $r = 0.99$ ;  $P < 0.001$ ) for *E. leucantha* and  $\Psi = 0.05 \text{ RWC} - 4.84$  ( $r = 0.94$ ;  $P < 0.001$ ),  $\Psi_\pi = 0.02 \text{ RWC} - 2.66$  ( $r = 0.82$ ;  $P < 0.001$ ) and  $\Psi_p = 0.61 \Psi + 0.76$  ( $r = 0.96$ ;  $P < 0.001$ ) for *M. sprucei*.

Thus, drought resistance does not seem to be a highly selected trait in these particular habitats that have relatively advantageous topographical and hydrological conditions. Intriguingly, *M. sprucei* from the caatinga slopes, which possesses sclerophyllous leaves, was particularly susceptible to a loss in turgor pressure at high leaf  $\Psi$  ( $\sim 1.1$  MPa) compared with the other species. Indeed, the mechanical constitution of sclerophyll leaves confers resistance to stress and provides protection from herbivory to enhance leaf longevity and long-term carbon gain (Read *et al.* 2006, Turner 1994). However, previous findings do not support the drought tolerance of sclerophyllous species (Read *et al.* 2006, Salleo *et al.* 1997). Thus, sclerophyll species, such as *M. sprucei* and those in the open sclerophyllous forests at the mounds of the caatinga, seemed to have been selected due to the low fertility of both sites, as previously stated (Sobrado 2009b, 2010). Nevertheless, the open sclerophyllous forests (sandy domes) suffer unpredictable drought spells as well, which would favour the establishment of species that are able to tolerate both nutrient and water deficiencies. Therefore, these species have a lower minimum leaf  $\Psi$  ( $\sim 1.5$  MPa), tolerate a lower water status, and maintain turgor pressure ( $\Psi \sim 2$  MPa; Sobrado 2009a). Conversely, *M. sprucei*, with sclerophyllous leaves, is confined to the slopes of the caatinga (ecotone), but is never found in the open sandy mounds, which is possibly due to its relatively high drought sensitivity. Therefore, this region, with a high rainfall pattern, possesses well-defined habitats that have contrasting nutrient and water availabilities, which seems to influence the local distribution of species.

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