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Leaf water relations and anatomy of a tropical rainforest tree species vary with crown position

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Summary. Leaf water potentials, osmotic properties and structural characteristics were examined in the Australian tropical rainforest tree species, *Castanospermum australe*. These features were compared for individuals growing in the understorey and canopy of the undisturbed forest and in an open pasture from which the forest had been cleared. Leaf water potentials during the day declined to significantly lower values in the open-grown and canopy trees than in the understorey trees. During most of the day the open-grown tree experienced the lowest water potentials. These differences were paralleled by significant differences in tissue osmotic properties. The tissue osmotic potential at full hydration was lowest in the open-grown tree (−1.80 MPa), intermediate in the canopy trees (−1.38 MPa), and highest in the understorey trees (−0.80 MPa). As a result, the degree to which high and positive turgor pressures were maintained as water potentials declined was highest in the open-grown tree, intermediate in the canopy trees, and lowest in the understorey trees. The differences in tissue osmotic properties between individuals in the three crown positions were paralleled, in turn, by differences in leaf structual characteristics. Relative to leaves of the canopy and open-grown trees, leaves of the understorey trees had significantly larger epidermal cells with thinner cell walls, larger specific leaf areas and turgid weight: dry weight ratios, and a higher proportion of intercellular air space.

Key words: Water relations – *Castanospermum australe* – Tropical rainforest – Pressure-volume curves – Leaf anatomy

Lowland tropical rainforests are among the wettest terrestrial habitats on earth. Yet plants growing in these forests may be exposed to moderate tissue water deficits on a regular diurnal and seasonal basis (Robichaux et al. 1984). In addition, these plants may experience severe water deficits during years with lower-than-average rainfall. Information on the nature of these water deficits, their physiological effects on growth, and the mechanisms by which they are tolerated is important for understanding certain aspects of rainforest dynamics. These include the ability of a species to colonise open pastures from which the rainforest has been removed and to respond to the sudden change in environment when a gap is created in the canopy by the death or harvesting of a large dominant tree. This knowledge would have significance for reforestation in tropical rainforest regions and may also aid in predicting the long-term effects of human disturbance in these forests.

Many aspects of plant metabolism are deleteriously affected by water deficits, including cell growth, stomatal opening and photosynthetic carbon assimilation. As suggested by Hsiao et al. (1976) a reduction in turgor pressure may be the prime method of transduction of a moderate water deficit into a change in metabolism. Hence, mechanisms promoting turgor maintenance as tissue water content decreases may serve as important aids to plant growth and survival under conditions of low water availability (Begg and Turner 1976; Turner and Jones 1980). One mechanism promoting turgor maintenance in higher plants in the short term involves osmotic adjustment, the active accumulation of solutes resulting in a decrease in the tissue osmotic potential at full hydration (π_o) (Turner and Jones 1980; Morgan 1984). A lower value of π_o results in a higher value of tissue turgor pressure at full hydration. This increases, in turn, the value of turgor pressure at tissue water contents below full hydration.

Another mechanism which produces a lower value of π_o is the longer term morphological adaptation of foliage to environmental factors which result in smaller thicker walled cells, and hence a decrease in the relative volume of symplasmic water per cell (Cutler et al. 1977). The radiation level during leaf development is the environmental factor which has the greatest influence on leaf anatomy, producing classical 'sun' or 'shade' leaves (Nobel 1980).

This paper examines the nature of variation in leaf water potentials, osmotic properties and structural characteristics in *Castanospermum australe* Cunn. and C. Fraser ex Hook (Leguminosae), an important tree species in the lowland tropical rainforests of north-eastern Australia. These features are compared for individuals growing in the understorey and canopy of the undisturbed forest and in an open pasture from which the forest had been cleared. The degree to which variation in osmotic properties is paralleled by variation in leaf structural characteristics is examined.

Abbreviations. ψ_1 Leaf tissue water potential; ψ_{min}Lowest value of ψ_1 during the day ($\approx \psi$ noon); $\psi_{P=0}$ ψ_1 zero turgor; R Relative water content; P Tissue turgor pressure; π Tissue osmotic potential; π_o π at full hydration

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Materials and methods

Study site and microclimate

Castanospermum australe is a medium to tall tree growing up to 40 m in height and 1.2 m in diameter and is an important commercial species. It has a wide distribution along the east coast of Australia from northern New South Wales (lat. 29° S) to the Cape York Peninsula in northern Queensland (lat. 12° S). Mean annual rainfall varies from less than 1000 mm to 3800 mm (Boland et al. 1984).

The study was conducted in the Atherton Tablelands area of northern Queensland at latitude 17° S, elevation 720 m. Here *Castanospermum australe* occurs at all levels ranging from the upper canopy to regenerating saplings in the understorey. It also commonly grows as solitary individuals in cleared fields and pastures. These latter trees and the canopy trees are densely covered with thick, leathery leaves while the understorey saplings bear sparse paper-thin foliage. Seven trees were studied: one open-grown individual about 15 m tall, three canopy individuals about 40 m tall, and three understorey saplings 2–3 m tall. The canopy and understorey trees occurred within a 100 m radius while the open-grown tree was about 2 km away on the same well-drained, fertile basaltic soil.

The average annual rainfall at the site is 1400 mm with most of that occurring during the summer 'wet season' (January to March). The summer season preceding this study had been drier than usual with just over half of the average January to July rainfall being recorded. Cool cloudy conditions and morning drizzle prevailed during most of the study period in July and August 1984, although the two days on which the diurnal course of ψ_l was measured in the field were cloud-free.

Continuous meteorological data were recorded throughout the study in the canopy (35 m), in the understorey (2 m) and within a weather station and Stevenson screen in the open (Table 1). These data show a much greater total radiation load and a greater evaporative demand for the open-grown and canopy positions compared to the understorey. The Penman-Monteith potential daily transpiration for the open-grown, canopy and understorey positions, assuming a constant canopy conductance for all positions, was 1.6, 1.6 and 0.4 mm day^{-1} respectively.

Table 1. Summary of meteorological data from the study sites during July–August, 1984. Standard errors are given in parentheses

Parameter	Position		
	Open-grown (1.5 m)	Canopy (35 m)	Understorey (2 m)
Mean daily total photosynthetically active radiation (mol m^{-2})	30.9 (1.7)	30.9 (1.7)	1.6 (0.3)
Mean daily maximum photon flux density (μmol m^{-2} s^{-1})	1776 (33)	1776 (33)	211 (57)
Relative humidity			
Mean daily maximum (%)	98.3 (1.1)	99.7 (0.1)	95.5 (0.3)
Mean daily minimum (%)	54.8 (6.1)	61.5 (3.3)	70.8 (2.7)
h$<$70% RH	4.7	3.0	1.5
Temperature (°C)			
Mean daily maximum	20.9 (0.7)	22.2 (0.9)	19.7 (0.5)
Mean daily minimum	12.6 (0.8)	12.5 (0.6)	11.4 (0.5)

Field measurements

The leaves of *Castanospermum australe* are pinnately compound and about 30–40 cm long. Modified leaflets were used for determination of ψ_l since entire leaves were too big for the pressure chamber. About 1 cm of the base of the leaflet lamina was removed on each side of the midrib to produce a pseudo petiole.

Leaflets were harvested for ψ_l measurements from the seven study trees at approximately 2 h intervals from dawn until dusk on 28 July 1984. On the next day, two of the canopy and two of the understorey trees were sampled more frequently throughout the day, with 15 min intervals during the period of most rapid change in ψ_l. The canopy trees were sampled with a shot gun while the open-grown tree was sampled with extendable pruning clippers. Leaves were taken from the mid crown of each tree and consistently from the northeast side of the crown. Leaves were wrapped in plastic bags immediately after harvesting. About 15 s elapsed between removing the leaft from the plastic bag and inserting a modified leaflet into the pressure chamber (Model 1000 PMS Instrument Co., Corvallis, Oregon). The chamber lid was modified to enable rapid petiole insertion and external adjustment of the tension on a soft rubber seal. Balancing pressure endpoints were determined with the aid of a binocular microscope. The pressure chamber measured the hydrostatic pressure of the tissue apoplasm, which was assumed to approximate closely the water potential of the tissue symplasm (ψ_l) (Passioura 1980).

Laboratory measurements

The relationship between ψ_l and R was determined for 4 to 12 leaflets from each tree. Leaves were harvested from each tree shortly after dawn before any significant water deficits had developed, and placed in a wet plastic bag. They were recut under water in the laboratory and rehydrated with the cut petiole in a container of distilled water for two hours. After their saturated weights were measured, the leaflets were allowed to dry under ambient conditions on the laboratory bench. Periodically, the weight and ψ_l of each leaflet were measured. The weight was measured immediately before and immediately after each ψ_l determination. The average of these two weight measurements was used in the subsequent calculation of R. The measurements were repeated for 4 to 7 h until it was clear that the points had diverged from a linear relationship between ψ_l^{-1} and R below zero turgor. The leaflets were then oven-dried at 80° C for 24 h to obtain their dry weights. All weights were measured with 1 mg resolution (Model 1518 MP8 digital balance, Sartorius Instruments Ltd, Göttingen, West Germany).

The relationship between P and R was calculated from the relationship between ψ_l^{-1} and R according to the procedures outlined by Tyree and Jarvis (1982) and Robichaux (1984). The relationship between ψ_l^{-1} and R was plotted for each leaflet and the data were rejected if the correlation coefficient for the linear region was less than 0.99 (two leaflets out of 48). Analyses of variance were conducted on the values of $\psi_{P=0}$ and π_0 for each crown position. The diurnal course of P was calculated for each tree from the relationship between P and ψ_l and the diurnal course of ψ_l.

Cuticular phytoglyphs (Carr et al. 1971) were prepared for leaflets from each tree using hydrogen peroxide and acetic acid to separate the cuticle and Sudan II as a stain (O'Brien and McCully 1981). Photographic enlargements at 400 × magnification were used to measure the surface areas and relative proportions of cell walls of the epidermal cells. An ultramicrotome (Ultrotome III, L.K.B., Bromma, Sweden) was used to prepare 2 µm-thick, transverse sections of the mid-portions of mature leaflets that had been embedded in L.K.B. historesin. The periodic acid/Schiff's reagent was used as a stain (O'Brien and McCully 1981).

The surface areas (A) of 20–30 randomly selected leaflets from each tree were measured (Electroplan electronic leaf area planimeter, Paton Industries Pty Ltd, Adelaide, South Australia). The oven-dry weights (W_d) of the leaflets were measured as above and specific leaf area (A/W_d) was calculated.

Results

The mean diurnal courses of ψ_l in trees in the three crown positions on 28 July 1984 are shown in Fig. 1. The patterns were similar on the following day. In the understorey trees, ψ_l declined to a minimal value at midday, then increased gradually throughout the afternoon. In the canopy and open-grown trees ψ_l declined very rapidly as soon as sunlight fell directly on the leaves, opening the stomata and raising the vapour pressure deficit. ψ_l remained near $\psi_{\min}$ for 2–6 h, followed by a slightly less rapid recovery in the late afternoon. The maximal rate of change in ψ_l occurred on the second morning, when one canopy tree experienced a decline of 1.5 MPa in 80 min (data not shown). Except near dawn and dusk, ψ_l in the canopy and open-grown trees was much lower than in the understorey trees. $\psi_{\min}$ was 1.07 and 0.75 MPa lower in the open-grown and canopy leaves, respectively, than in the understorey leaves. During most of the day, the open-grown tree experienced the lowest ψ_l.

The differences in the diurnal values of ψ_l experienced by trees in the three crown positions were accompanied by significant differences in their tissue osmotic properties (Table 2). Values of π_0 were highest in the understorey trees, intermediate in the canopy trees and lowest in the open-grown tree. These differences had a marked effect, in turn, on the degree to which high turgor pressures were maintained as ψ_l declined. For any value of ψ_l, values of P were lowest in the understorey trees, intermediate in the canopy trees and highest in the open-grown tree (Fig. 2). In addition, the values of ψ_l at which P reached zero differed markedly between individuals in the three crown positions (Fig. 2, Table 2).

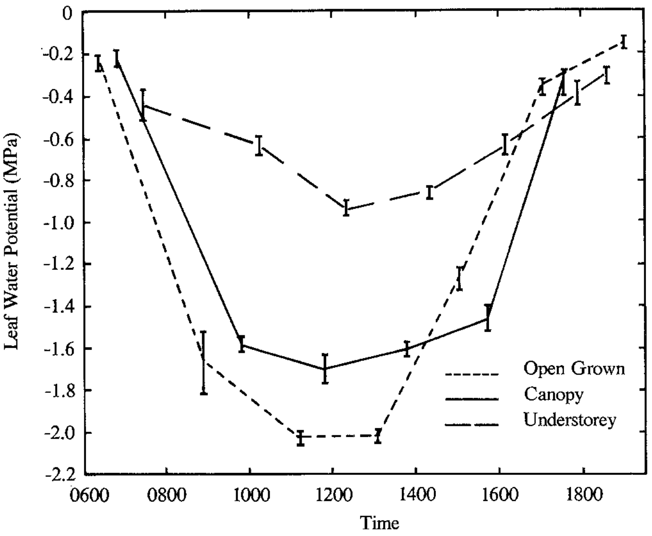

Fig. 1. Mean diurnal courses of leaf tissue water potential on 28 July 1984 in one open-grown tree, three canopy trees and three understorey trees of *Castanospermum australe*. Vertical bars represent one standard error

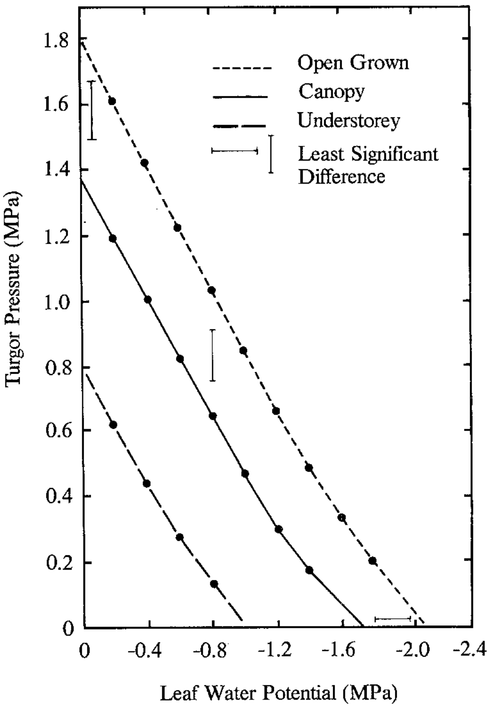

Fig. 2. The mean relationship between tissue turgor pressure and tissue water potential for one open-grown tree (12 samples), three canopy trees (16 samples) and three understorey trees (18 samples) of *Castanospermum australe*, from pressure-volume curves of leaves collected at dawn

The differences in tissue osmotic properties between individuals from the three crown positions were also associated with differences in leaflet structure (Table 3). The understorey low-light leaflets were thinner, with more than twice the specific leaf area, and had much larger, thinner-walled epidermal cells than leaflets from the more exposed positions. Figure 3 shows transverse sections of representative leaflets from the three crown positions. Relative to the open-grown and canopy leaflets, understorey leaflets have a larger proportion of intercellular air space, shorter palisade mesophyll cells, fewer layers of palisade mesophyll cells, reduced frequency of spongy mesophyll cells and reduced density of lateral veins.

Table 2. Tissue osmotic potential at full hydration (π_o) and water potential at zero turgor ($\psi_{P=0}$) in one open-grown, three canopy and three understorey trees of *Castanospermum australe*. Standard errors of '*n*' samples are given in parentheses

	π_o (MPa)	$\psi_{P=0}$ (MPa)	n
Open-grown	−1.80 (0.06)	−2.13 (0.07)	12
Canopy	−1.38 (0.04)	−1.73 (0.05)	16
Understorey	−0.80 (0.04)	−1.01 (0.05)	18
Least significant difference ($P<0.01$)	0.17	0.22	

Table 3. Epidermal cell dimensions and specific leaf area in open-grown, canopy and understorey trees of *Castanospermum australe*. Standard errors are given in parentheses

	Average surface area of epidermal cells (μm^2)	Average proportion of epidermal cells as cell walls (%)	Specific leaf area ($m^2\ kg^{-1}$)
Open-grown	23.8 (0.8)	23.9 (0.7)	9.3 (0.2)
Canopy	16.3 (0.3)	22.7 (0.3)	9.6 (0.2)
Understorey	45.5 (1.4)	10.4 (0.3)	24.1 (0.2)
Least significant difference ($P<0.01$)	4.7	1.2	0.8

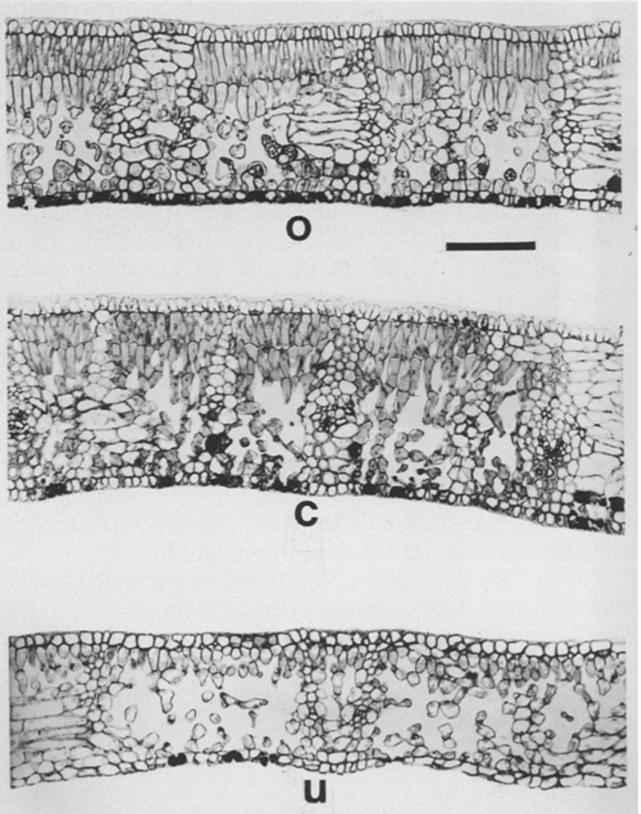

Fig. 3. Transverse sections (2 μm thick) of leaflets from open-grown (*O*), canopy (*C*) and understorey (*U*) trees of *Castanospermum australe*. Bar = 0.1 mm

Discussion

The leaves of *Castanospermum australe* had substantially different water relations in the contrasting environments studied. Leaves of canopy trees in undisturbed forest and trees in open pasture experienced a larger diurnal amplitude of ψ_l than saplings in the forest understorey. Similar results were obtained by Oberbauer (1983) for canopy and understorey individuals of *Pentaclethra macroloba* growing in a lowland tropical rainforest in Costa Rica, where midday values of ψ_l were 0.4–0.5 MPa lower in the canopy trees than in the understorey individuals.

As well as differences in ψ_l there were major differences in π_o and $\psi_{P=0}$ between the three crown positions. The open-grown and canopy values of π_o correspond with values reported for mature or fully expanded leaves of many deciduous tree species (Cheung et al. 1975; Tyree et al. 1978; Roberts et al. 1980; Parker et al. 1982; Bahari et al. 1985) and several sclerophyllous evergreen tree species (Ladiges 1975; Roberts et al. 1980). Values of π_o as high as those found in the understorey foliage have only been reported in broadleaved tree species for immature or expanding foliage (Tyree et al. 1978; Parker et al. 1982).

With respect to differences in tissue osmotic properties between canopy and understorey trees Oberbauer (1983) reported similar results for *Pentaclethra macroloba*, with π_o values being 0.29–0.34 MPa lower in canopy individuals than understorey individuals. Also, Walter (1971) reported that the osmotic potentials of canopy tree species in a wet tropical forest in East Africa were significantly lower than those of understorey herbaceous species, which suggests that the former had greater turgor-maintenance capacities. The latter results must be interpreted cautiously, however, since π values were not reported for full hydration. Thus, it is possible that the lower osmotic potentials in the canopy species merely reflected a greater degree of tissue desiccation (Turner and Jones 1980).

The capacity of leaves to maintain positive values of P as ψ_l decreased would appear to differ markedly between open-grown, canopy and understorey individuals as a result of the significant differences in tissue osmotic properties. However, the effectiveness of these differences in maintaining P depends not only on the maximal P achievable but also on the plant's ability to actively lower its osmotic potential during the day. With the exception of a study on apple trees reported by Davies and Lakso (1979) there is little evidence of active diurnal osmotic adjustment in tree species (Rada et al. 1985).

The presence of diurnal changes in π_o was not investigated here, but if there was no significant diurnal osmotic adjustment, the data in Figs. 1 and 2 could be combined to estimate the diurnal values of P experienced by trees in the three crown positions. In contrast to the diurnal course of ψ_l, the diurnal course of P would be remarkably similar in the three crown positions, particularly during the middle of the day when ψ_l was lowest.

The leaf structural differences between the three crown positions probably reflect the influence of differing radiation regimes more than differing water stress regimes. The differences are characteristic of those found in foliage grown under high versus low light levels (Nobel et al. 1975; Doley 1978). While reduced cell size often accompanies water stress, the environmental variable having the greatest influence on leaf anatomy is radiation level during leaf development (Nobel 1980) and it is this factor which showed the largest difference between the open-grown/canopy positions and the understorey (Table 1). However, the differences in leaf structure may well affect their turgor maintenance capacities.

In *Castanospermum australe*, the differences in tissue osmotic properties between individuals in the three crown positions may have resulted both from differences in cell size and from differences in amounts of solutes. The differences in epidermal cell dimensions suggest that cell sizes in leaves of canopy and open-grown trees were significantly smaller than in leaves of understorey trees. A reduction in average cell size, and hence in the relative volume of symplasmic water per cell, may result in a decrease in π_0 even though the number of moles of solutes per cell may remain constant (Cutler et al. 1977; Cutler and Rains 1978). These smaller cell sizes may have contributed in part to the lower π_0 values of the canopy and open-grown trees. However, differences in solute concentrations probably accounted for much for the difference in π_0, particularly in the open-grown tree. For example, the 0.42 MPa difference in π_0 between the canopy and open-grown trees did not appear to be accompanied by a smaller cell size in the latter individual.

The results of this study, together with those of Oberbauer (1983), thus suggest that lowland tropical rainforest tree species growing in contrasting environments, particularly with respect to radiation, exhibit markedly different tissue osmotic properties which may have significant consequences in terms of turgor maintenance at low water potentials. In the case of *Castanospermum australe*, this mechanism may contribute to its ability to colonize open pastures from which the rainforest has been cleared. This ability may have significance, for reforestation efforts in tropical rainforest regions.

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