VERTICAL PROFILES IN A BRUNEI RAIN FOREST: II. LEAF CHARACTERISTICS OF *DRYOBALANOPS LANCEOLATA*

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BARKER, M. G. & BOOTH, W. E. 1996. Vertical profiles in a Brunei rain forest: II. Leaf characteristics of *Dryobalanops lanceolata*. We investigated a range of morphological and ecophysiological characteristics of *Dryobalanops lanceolata* leaves occupying different vertical positions in a Brunei tropical rain forest. Characteristics were examined in understory leaves and in leaves at five heights within a tree canopy. Differences in all variables existed in the vertical profile as a whole, though low statistical power often prevented the discrimination between leaves at particular heights. Compared with understory leaves, those in the top canopy (31 m) had higher leaf mass area (LMA), and thickness of leaf, adaxial epidermis and palisade layers. Trends in the vertical profile were apparent for stomatal density and LMA (increasing with height) and driptip length (decreasing). Vertical differences in leaf nitrogen were probably due to high values in the mid-canopy. Height differences in net assimilation rate (A) during morning and mid-day were presumably the result of higher A in the upper canopy. Differences in stomatal conductance (g_s) within the profile probably resulted from higher mid-canopy g_s. We attributed vertical differences in leaf water potential (ψ) during the morning and mid-day to the higher ψ in understory leaves. We accepted the hypothesis that differences in leaf characteristics for a single species occur within the vertical profile, and are probably a function of microclimate and/or the plant's developmental stage. We rejected the hypothesis that distinct leaf characteristics exist at different height positions within the canopy, although vertical trends were apparent for some morphological and ecophysiological parameters.

Key words: Canopy - Dipterocarpaceae - leaf morphology - leaf ecophysiology - understory

BARKER, M. G. & BOOTH, W. E. 1996. Profil-profil menegak di dalam hutan hujan Brunei: II. Ciri-ciri daun *Dryobalanops lanceolata*. Kajian telah dibuat ke atas beberapa julat morfologi dan sifat-sifat ekofisiologi daun-daun *Dryobalanops lanceolata* yang berada pada kedudukan menegak yang berbeza di dalam hutan hujan tropika Brunei. Ciri-ciri telah dikaji pada daun-daun bawah dan daun-daun pada lima ketinggian di dalam kanopi pokok. Secara keseluruhannya perbezaan-perbezaan

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dalam semua pembolehubah wujud pada profil menegak walaupun kuasa statistik yang rendah sering menghalang perbezaan di antara daun-daun pada ketinggian tertentu. Daun-daun di kanopi atas (31 m) mempunyai keluasan jisim daun (LMA), ketebalan daun, epidemis adaksial dan lapisan palisad yang lebih berbanding dengan daun-daun bawah. Corak di dalam profil menegak ketara untuk densiti stomata dan LMA (bertambah dengan ketingginan) dan kepanjangan driptip (berkurangan). Perbezaan-perbezaan menegak dalam nitrogen daun mungkin disebabkan oleh nilai-nilai yang tinggi di dalam kanopi tengah. Perbezaan-perbezaan ketinggian dalam kadar asimilasi bersih (A) di waktu pagi dan tengahari mungkin disebabkan nilai A yang tinggi di dalam kanopi atasan. Perbezaan-perbezaan dalam konduktans stomata (g_s) di dalam profil mungkin hasilan daripada g_s kanopi tengah yang tinggi. Perbezaan menegak di dalam potensi air daun (ψ) di waktu pagi dan tengahari disebabkan nilai ψ yang tinggi pada daun-daun bawah. Kami menerima hipotesis bahawa perbezaan dalam ciri-ciri daun untuk spesies tunggal berlaku di dalam profil menegak dan berkemungkinan bertindak sebagai fungsi mikroklimat dan/atau peringkat perkembangan tumbuhan. Kami menolak hipotesis bahawa ciri-ciri nyata daun wujud pada kedudukan ketinggian yang berbeza di dalam kanopi walaupun corak menegak adalah jelas untuk setengah parameter-parameter morfologi dan ekofisiologi.

Introduction

The rain forest environment is spatially and temporally heterogeneous, both horizontally and vertically. Several previous studies have focused on horizontal variation in the rain forest environment, especially in comparisons of tree seedlings in understory and gaps (e.g. Popma & Bongers 1991, Ashton & Berlyn 1992, Chazdon & Kaufmann 1993, Newell *et al.* 1993). Very little attention has been directed to the characteristics of rain forest leaves in the vertical plane, although several studies have shown vertical gradients in microclimate (Lemon *et al.* 1970, Aoki *et al.* 1978, Broadmeadow *et al.* 1992, Smith *et al.* 1992, Barker 1996). Few studies have focused on tree leaf characteristics in the vertical profile (but see Doley *et al.* 1987, Oberbauer *et al.* 1987). The paucity of such studies is probably due mainly to difficulties of access (e.g. Barker & Sutton in press).

Sub-canopy plants, particularly juvenile forms of canopy and emergent trees, are exposed to a wide range of micro-environments during their development from the seedling stage (Oberbauer & Strain 1986). Physiological adjustments are required as the tree encounters different micro-environments in the vertical forest profile. The processes involved are not yet adequately understood (Bazzaz 1984). At any one stage of development, physiological adaptations of leaves also occur within the tree itself, in response to variation in microclimate (Marek *et al.* 1989). It is likely that continuous differences, rather than a simple "sun"/"shade" dichotomy, occur in within-canopy leaf characteristics (Hollinger 1989).

There are other temporal changes requiring adaptive or acclimation responses which result from the dynamic nature of forest structure. Canopy and emergent species may reach their mature form only after experiencing a succession of gap openings and canopy closure during their development. Evidence suggests that the frequency of exposure of an individual tree to canopy opening increases with tree height (Hubbell & Foster 1986) implying a high degree of physiological flexibility (Pearcy 1987).

Comparisons of leaf gas exchange in understory and canopy of tropical rain forests have been reported in relatively few previous studies (Pearcy 1987). Some laboratory-based gas exchange studies have been conducted using fresh leaves sampled from the upper forest strata (e.g. Koyama 1978, 1981, Wanner & Soerohaldoko 1979). Though laboratory measurements have been found to agree well with those in the field (Ellsworth & Reich 1993), there remains an important need to make measurements *in situ*, i.e. in the context of the micro-habitat in which the leaf developed. Comparatively few studies have been conducted to compare characteristics of canopy and understory leaves, or leaves within the canopy itself (Mooney *et al.* 1984, Oberbauer & Strain 1986, Koch *et al.* 1994). In addition, interest is increasing in scaling leaf-level processes to those of the tree crown or the forest canopy (Dolman *et al.* 1991, Ellsworth & Reich 1993).

The present study was conducted against this background. In particular, we ask: Do ecophysiological and morphological leaf characteristics differ in vertical profile involving (a) understory and canopy leaves of a single tree species and, more particularly, (b) within the crown of a canopy tree? Results are interpreted in terms of leaf functioning and in also relationship with vertical microclimate, investigated in a companion study (Barker 1996).

Methods

Study site and plant material

The study was carried out within the Bati Apoi Forest Reserve (488 km^2), a lowland mixed dipterocarp forest in the Temburong District of Brunei (4° 31' N, 115° 08' E). The annual mean of rainfall in the area is approximately 4000 mm. The study plants were located on a small, level spur on a steep valley side facing west, approximately 200 m east of the Belalong Field Studies Center (61 m a.s.l.). Base rock at the study area is shale, with a thin covering of clay.

Both the main study tree and the seedlings used in the study were *Dryobalanops lanceolata* Burck., a locally-common canopy tree species of the Dipterocarpaceae. In Brunei, *D. lanceolata* is confined to heavy clay soils (Ashton 1964). The study focused on a single canopy tree (hereafter referred to as the 'tree'), 32.5 m high, with lowest branches at 19 m. Diameter at breast height (DBH) of the tree was 36 cm and therefore a canopy tree (i.e. DBH *c.* > 30 cm) using the definition given by Manokaran and Kochummen (1994). The crown had a juvenile oblong-cylindric monopodial architecture; adjacent *D. lanceolata* trees were sympodial at *c.* > 60 cm DBH (pers. obs.). The study tree had a canopy *c.*14 m deep, consisting of five distinct layers of branches, and was shaded to some extent by a single layer of loose upper canopy of an adjacent tree, *c.*10 m above. The canopy above was open to the west (i.e. facing away from the hillside) and partially closed to the east. Access to the canopy was by ropes supported by the overhead canopy tree. A supplementary study was conducted with 30 to 100 cm-tall arbitrarily-selected *D. lanceolata* seedlings, adjacent to the main tree. All leaves used in the study were young and fully expanded and we assumed, therefore, that leaf characteristics resulted from their position in the vertical profile and were not due to ontogeny.

The approach taken in this study has been to treat individual leaves within the tree as independent replicates, consistent with most comparable studies (e.g. Doley *et al.* 1987, Oberbauer *et al.* 1987, Pearcy 1987, Marek *et al.* 1989, Roy & Salager 1992, Koch *et al.* 1994, Zotz & Winter 1994). If an individual plant (i.e. the tree) is the normal unit of replication, then justification is needed for the apparent pseudoreplication in this study. Leaves within a tree canopy are known to be highly heterogeneous (see Harper 1989, Marek *et al.* 1989), and substantial physiological differences can occur between them (see Holbrook & Lund 1995). Such heterogeneity is likely in this study, even within layers of similar-aged leaves, since microclimate was spatially and temporally variable at any given height in the canopy (Barker 1996). It has been argued that branches within a tree are autonomous, at least in terms of water relations (Sprugel *et al.* 1991). We are not aware of a comparable postulate for leaves. Though canopy leaves affect the microclimate of each other (see Caldwell *et al.* 1986), for the purposes of this study we assume that each leaf is autonomous with respect to its microhabitat and can therefore be considered to be independent replicates.

Leaf morphology and foliar nitrogen content

Twenty young, fully expanded leaves were arbitrarily selected from each of the five height layers within the tree (n = 20 for morphology and leaf nitrogen determinations). Leaves were sampled from most main branches in each layer to ensure that, within each layer, every aspect of the tree which bore leaves was included. The two youngest, fully expanded leaves were removed from each seedling (samples bulked, n = 10 for morphology and leaf nitrogen determinations). Leaves from the tree and the seedling group were collected on two days (1700-1800 h) and placed immediately into humidified sealable plastic bags and taken to the laboratory for leaf area determinations. Driptips of leaves were measured from the mid-point of inflection on the leaf outline (Lightbody 1985). Leaves were then air dried in open sun during the next two days. Leaf mass per area (LMA, dry weight area^{-1}) was calculated for each leaf. The dried leaves were subsequently analysed for nitrogen, using an indophenol blue reaction following digestion (Golterman *et al.* 1978). Results were calculated both as mmol g^{-1} leaf dry weight, and mmol m^{-1} leaf area.

Before drying, a sub-set of six leaves was arbitrarily taken from the seedlings and from each height layer group from the tree, and stomatal impressions were made. Five randomly selected fields were then examined under a microscope. Counts of stomata for each leaf were bulked, and the stomatal density was expressed per unit area (mm^2) (n = 6, for tree and seedlings).

Three additional young, fully expanded leaves were sampled from the top canopy layer (31 m) of the tree and from understory (0 m) seedlings. Three longitudinal sections were prepared from the central lamina area of each leaf, and microscopically examined under a 40 × objective. For each section, three arbitrarily-chosen positions were used for measurements of total leaf thickness, adaxial epidermal thickness, and depth of the palisade mesophyll layer (sections bulked; n = 3 leaves for tree and seedlings).

Gas exchange and leaf water potential

Gas exchange was measured for net CO_2 assimilation rate (A) and stomatal conductance (g_s) using a portable infra red gas analyser (LCA3; Analytical Development Company, Hoddesdon, UK), calibrated against a standard CO_2 source. Measurements within the tree canopy were made on 10 tagged leaves at each of the five 'height layers' of the tree in sequence, beginning with the highest layer (i.e. 31 m). In each case, the youngest fully expanded leaves (based on their branch position, coloration, size) were used, and the original leaf orientation was maintained during measurements. Sampling was conducted during two days: 19 March = 0800-1600 h, 23 March = 0900-1500 h. The sequence of sampling was the same each day. Duplicate measurements for each leaf replicate were averaged both within and between days for each height and time group (see below) (n = 10).

Gas exchange measurements were also conducted on the 10 understory seedlings on a single day (21 March); there were seven measurement periods, each approximately 30 min, distributed during the day (0800-1700 h). Two tagged leaves were used on each plant, measuring leaves in the same sequence; results for each leaf pair were bulked for each of three time groups (n = 10). Data for each sampling day were grouped into three 3-h time intervals, to allow broad comparisons between morning (0800-1100 h), mid-day (1100-1400 h) and afternoon (1400-1700 h) for the main tree and the seedlings. Median values were calculated for each 3-h time interval and each height layer .

Leaf water potential measurements were made using a portable pressure chamber (PMS, Corvalis, Oregon, USA). There were six 1.5-h measurement periods, between 0900-1800 h on the same day (20 March). During each measurement session, three young, fully expanded leaves were used from each of the five height layers within the study tree. Sampling began in each case with the top height layer and progressed sequentially to the understory. Three randomly-selected seedlings growing in the understory within a radius of *c.* 5 m from study tree were also sampled. Different groups of seedlings were used during each measurement period to allow repeated destructive sampling of similar-aged leaves. All readings were taken within 60 s of excision. Data were grouped into three 3-h time intervals: morning (0900-1200 h), mid-day (1200-1500 h) and afternoon (1500-1800 h), each interval incorporating, (with some exceptions) readings from six plants or (for each height group) leaves (n = 5-7).

Data analysis

Non-parametric statistics were performed on all data due either to heteroscedasticity (confirmed by Bartlett's test) or non-normal distribution. Outliers (in LMA, A, and g_s data) were identified by Dixon's test (at $p = 0.05$) and excluded from analysis. Since data were non-parametric, medians were determined rather than means, except for PFD data. Data for each parameter were compared between height groups and time interval groups by ANOVA. Where results were significantly different ($p \leq 0.05$), post-hoc pairwise tests were performed using a

non-parametric Tukey-type multiple comparison ($p \leq 0.01$; Zar 1984). For leaf anatomy dimensions, a Mann-Whitney U test was used.

Results

Results for ANOVAs were statistically significant ($p < 0.05$) for all parameters and measurement periods, with the single exception of net assimilation during the afternoon (1400-1700) period. Except for the variable leaf mass area (LMA), pairwise Tukey comparisons did not give significant differences using this procedure. Such failure to detect differences was probably due to the relative lack of power of pairwise comparisons and constitutes a Type II error (see Zar 1984).

Leaf morphology and foliar nitrogen content

Within-profile differences (all $p \leq 0.001$) occurred for leaf area, driptip length, stomatal density, LMA and foliar nitrogen content (Figure 1). Although pairwise differences could not be detected, results for understory leaf characteristics were apparently different from those of the tree for driptip length, LMA and foliar nitrogen expressed on a per area basis. This was confirmed for LMA (Tukey, $p < 0.01$). Within-canopy height-related trends were not strong for any parameter, though general progressive changes were apparent for median values of stomatal density and LMA (both increasing with height), and driptip length (decreasing). In the case of driptip length, within-canopy trends disappeared when these data were expressed as a proportion of total leaf length (data not shown). Stomatal density was not related to leaf area (data not shown). Leaf nitrogen content, expressed either on a mass or area basis, showed a trend of higher values in the mid-canopy (25, 28 m).

Leaf dimensions were greater in the canopy compared with understory leaves for all variables (Mann-Whitney U, $p \leq 0.05$) (Figure 1). Total leaf thickness was *c.* 1.2 greater in canopy compared with understory leaves. Adaxial epidermis and palisade mesophyll were respectively 1.4 and 1.6 greater in canopy leaves.

Gas exchange and leaf water potential

Net assimilation rates (A) generally corresponded with photon flux density (PFD) within each height group in the vertical profile, especially in the morning and mid-day periods (Figure 2). Within-profile differences in A were generally evident during the morning ($p < 0.05$) and mid-day periods ($p < 0.001$) and within-canopy height-related trends were apparent as a progressive change in median values during these times. There was a general shift to higher A values from morning to mid-day. The increase in PFD values during the afternoon was accompanied by greater variability in A in the canopy leaves. Highest A values were in the upper canopy (31 m) leaves during morning (median = 1.2 μmol m^{-2} s^{-1}) and mid-day (median = 2.1 μmol m^{-2} s^{-1}), and in mid-canopy leaves (21 m) during the afternoon period (median = 2.2 μmol m^{-2} s^{-1}).

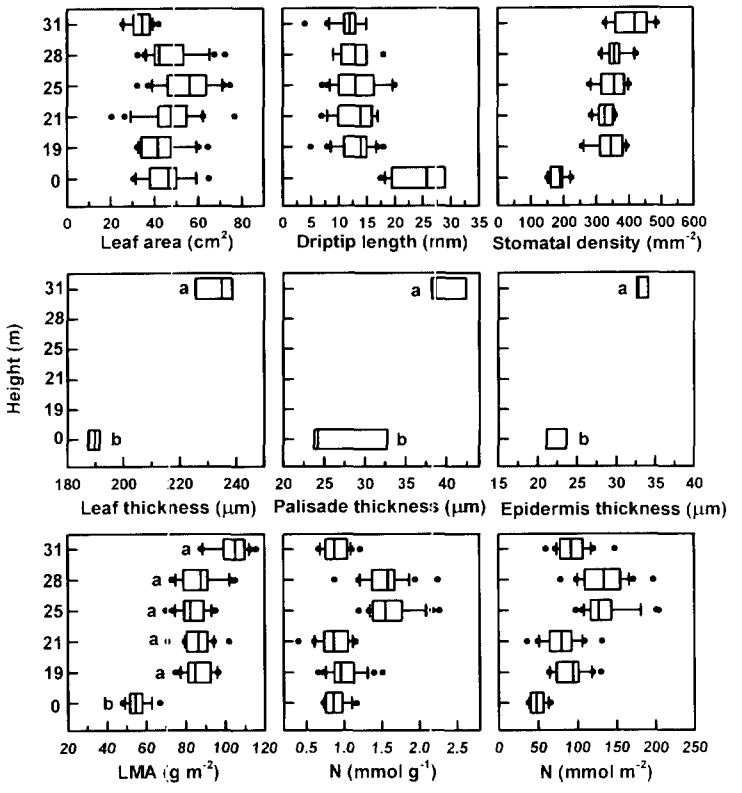

Figure 1. Morphological characteristics of *D. lanceolata* leaves at different heights. Box plots for each height group show a median within the box, which comprises 25th to 75th percentiles. 'Whiskers' on each box incorporate 10th and 90th percentiles, whilst symbols show 5th and 95th percentiles. Results are presented for leaf area, driptip length (n = 20 for tree, n = 10 for seedlings) and stomatal density (n = 6) (upper panel) and for leaf mass area (LMA), foliar nitrogen (N) expressed on the basis of leaf mass and leaf area (n = 20 for tree, n = 10 for seedlings) (lower panel). For LMA, comparisons between height groups were made using non-parametric Tukey-type multiple comparison; medians which are not statistically different at $p < 0.01$ share the same letter. Leaf anatomy dimensions (leaf, adaxial epidermis and palisade layer thickness) are shown (n = 3) for 0 m and 31 m heights (center panel). Comparisons were made by a Mann-Whitney U test. Statistically significant differences at $p < 0.05$ are shown by different letters.

Vertical differences in stomatal conductance (g_s) occurred during morning, mid-day and afternoon periods ($p < 0.001$, $p = 0.007$, $p < 0.001$ respectively) (Figure 3). Variability was high in the mid-canopy (25, 28 m). Highest g_s tended to be highest in the upper canopy during the morning (> 25 m) and mid-day (> 21 m). There was an overall reduction in g_s during the afternoon. Within-profile differences occurred in leaf water potential values (ψ) during morning, mid-day and afternoon periods ($p = 0.025$, $p = 0.008$, $p = 0.025$ respectively) (Figure 3). Understory leaves had the highest (i.e. least negative) ψ during the morning and

mid-day sessions (medians = -0.21 MPa, -0.25 MPa, respectively), with a slight decline by the afternoon (median = -0.5 MPa). The lowest median ψ was -0.83 MPa, at 28 m, during both mid-day and afternoon periods. There were no clear within-canopy vertical trends for leaf water potential.

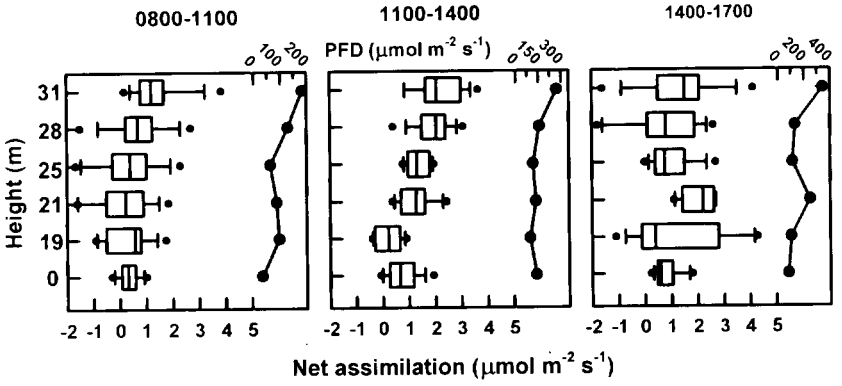

Figure 2. Net assimilation rate in *D. lanceolata* leaves at different heights (box plots). Results (n = 10) are for periods: morning (0800-1100 h), mid-day, (1100-1400 h), afternoon (1400-1700 h). See Figure 1 caption for a description of the symbols used in the box plots. Line graphs show mean photon flux density (PFD), measured simultaneously with *A*; SE for most PFD values are less the diameters of the symbols.

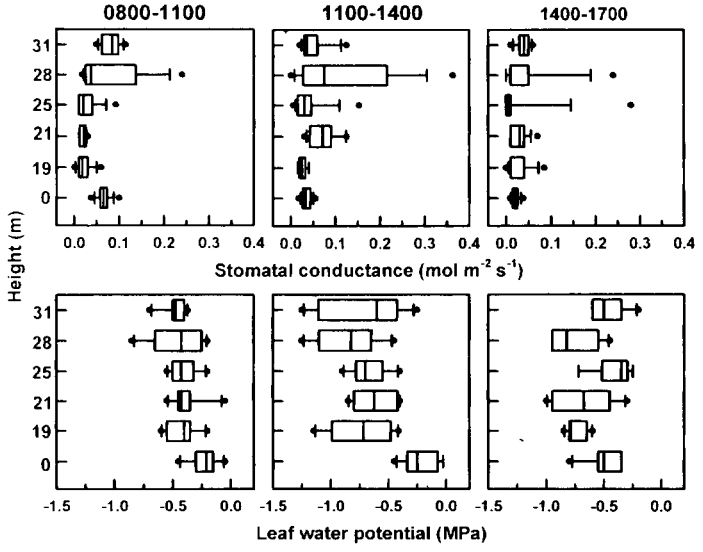

Figure 3. Stomatal conductance (g_s) and leaf water potential (ψ) in *D. lanceolata* leaves at different heights. Results for g_s (n = 10; upper panel) and ψ (n = 5 - 7; lower panel) are for the periods: morning (0800-1100 h), mid-day, (1100-1400 h), afternoon (1400-1700 h). See Figure 1 caption for a description of the symbols used in the box plots.

Discussion

Contrasting structure and physiology have been observed for canopy and understory leaves (Mooney *et al.* 1984), and also for leaves within the canopy itself (Koyama 1978, Oberbauer & Strain 1986). In this study, within-profile differences existed for all measured leaf morphological characteristics. Leaf mass per area (LMA) and anatomical dimensions were different between canopy and understory leaves. Within-profile differences also occurred for ecophysiological characteristics, though any vertical trends for these were apparently dependent on time of day. Vertical trends in leaf structure and ecophysiology were not consistently apparent throughout the tree canopy; consequently, we found no clear evidence for a 'multi-layered' deployment of canopy leaves with different ecophysiological characteristics (see Givnish 1984). We interpret height-related trends below for each variable mainly in terms of likely positional effects related to microclimate. Another explanation, particularly for comparisons between understory (i.e. seedling) and canopy (i.e. mature tree) leaves, is that leaf characteristics are influenced by the developmental stage of the plant. However, the two interpretations are not mutually exclusive and, furthermore, leaf characteristics associated with seedlings, saplings and mature trees may be a consequence of the microclimate conditions which they generally experience.

LMA was broadly associated with height, consistent with other studies of vertical forest profiles (Yoda 1978, Chiariello 1984, Hollinger 1989, Ellsworth & Reich 1993). Leaves in the top canopy, especially, had higher LMA, and were more xeromorphic in character. In addition to possibly reducing excessive water loss, higher tissue density in upper canopy leaves might reduce herbivory which may be important since they have relatively high leaf nitrogen content which would increase their nutritive value. Differences in leaf area occurred within the profile as a whole, but height-related trends were not apparent. Although some studies report decreases in leaf area with increases in height (e.g. Wanner & Soerohaldoko 1979), vertical trends in leaf size are often more complex and are poorly understood (see Chiariello 1984).

Leaf N showed significant within-profile differences, expressed on both an area or mass basis. However, there was no clear height-related trend within the crown itself, consistent with one temperate study (Ellsworth & Reich 1993) but in contrast to another (Hollinger 1989), despite the substantial light gradient within the canopy (Barker 1996). We do not believe that N was limiting at the study site, as leaf N : P ratios (M. Barker unpubl. data) were in the range 17.7 - 31.0 and leaf N:K ratios were 5.3-11.4, similar to those of Medina and Cuevas (1989) for a nutrient-rich Amazonian forest. The reason for maximum leaf N values being in the mid-canopy leaves is not clear. It may be because there was a greater proportion of younger leaves (see Hirose & Werger 1987a), for instance due to more rapid leaf turnover, or because they had a higher photosynthetic capacity (e.g. Hollinger 1989), even though decreasing leaf N is predicted to decline with depth in the canopy (Hirose & Werger 1987b).

Driptip length was different within the profile and, though not confirmed by pairwise comparisons, appeared to be systematically height-related. Decreases in driptip length with increasing height is consistent with other tropical rain forest studies (Williamson 1981, Williamson *et al.* 1983, Rollet 1990). Various explanations for this height-related phenomenon have been offered, mostly in terms of a mechanism for drying the adaxial leaf surface more quickly to reduce ion-leaching, the growth of epiphyllae, reflection of PFD (see Lightbody 1985), and the need to support a 'heavier' leaf (Dean & Smith 1978). Driptips could also limit a reduction of leaf temperature, which might otherwise cause a lowering of transpiration rate and hence reduced soil nutrient uptake (Dean & Smith 1978). Because of their higher heat load, leaves higher in the canopy dry more quickly and hence have a reduced need for driptips.

Differences in stomatal density occurred within the profile; the apparent relationship of increasing stomatal density with height observed in this study is well-established (Oberbauer & Strain 1986) and may correspond with the higher frequency of stomata in 'sun' compared with 'shade' leaves (Fetcher *et al.* 1983, Ashton & Berlyn 1992). Given that no clear correlation existed between leaf area and stomatal density, our data are probably not simply an artifact of leaf size (see Shields 1950). Observations revealed no apparent stomatal size variations with height, hence increased stomatal frequency would be expected to result directly in an enhanced capacity for stomatal conductance in canopy leaves.

Anatomical dimensions were greater in top canopy compared with understory leaves. Canopy leaves in the upper forest profile have been shown to be thicker (by *c.*1.7 ×) than understory leaves in another species (Oberbauer & Strain 1986, Oberbauer *et al.* 1987). Such characteristics of canopy leaves are equivalent to those observed in 'sun' leaves in high light environments for other tropical rain forest species (Oberbauer *et al.* 1987, Ashton & Berlyn 1992, Chazdon & Kaufmann 1993), and are essentially xeromorphic. Smaller cell size in canopy leaves, qualitatively observed in this study, could function to decrease osmotic potential at full hydration, allowing greater resistance to desiccation (Myers *et al.* 1987, Oberbauer *et al.* 1987).

Vertical differences in net assimilation rate (A) during morning and mid-day periods ($p < 0.05$, $p < 0.001$) may have been largely due to corresponding gradients in PFD, which influenced A between heights and (for morning and mid-day) measurement periods. The afternoon was characterised by increased light penetration into the lower canopy (Figure 2, and Barker 1996) and higher lower-canopy assimilation rates. The relatively direct effect of spatial or temporal changes of PFD on A suggest that leaves were not photosynthetically saturated. Maximum assimilation rate (A_{max}) values for tropical rain forest canopy leaves have been reported in the range 1.4 - 15 μmol m^{-2} s^{-1} (Holbrook & Lund 1995). In this study, highest median A was 2.2 μmol m^{-2} s^{-1} and, at least in seedlings, A_{max} for *D. lanceolata* is *c.* 4.4 μmol m^{-2} s^{-1}, reached at a PFD of *c.* 390 μmol m^{-2} s^{-1} (M. Barker, unpubl. data); this saturating PFD was only exceeded for < 4 % of all gas exchange measurements, mostly in the upper canopy in this study. It therefore seems likely that prevailing cloudy or hazy conditions prevented expected higher rates

of assimilation in leaves exposed to high PFD. There is thus still some uncertainty regarding potential height-related differences in photosynthetic capacity, particularly between understory and canopy leaves. Previous comparable studies have shown canopy and understory leaves to have little or no difference in A_{max} (Oberbauer & Strain 1986, Doley *et al.* 1987), though Hollinger (1989) obtained a within-canopy gradient of increasing A_{max} with height.

The lack of strong within-canopy differences in photosynthetic characteristics leads us to conclude that A is likely to be the product of several factors, not all of which may be directly height-related. Leaves at different heights probably have the capacity to acclimate photosynthetic and other responses to the conditions prevailing at a given location in the vertical forest profile (Pearcy 1987, Marek *et al.* 1989, Holbrook & Lund 1995). Also, height-related differences in short-term, dynamic responses will not necessarily be apparent if there is no corresponding microclimate gradient during, or shortly before, the time of sampling. A in canopy leaves is also likely to be affected by a combination of physiological factors, including stomatal conductance (g_s) (see Oberbauer & Strain 1986, Roy & Salager 1992), leaf temperature (Koch *et al.* 1994) or respiration rates within the crown (Marek *et al.* 1989). However, in this study no clear relationships between A and g_s (Figures 2 & 3) or leaf temperature (data not shown) were apparent.

Vertical differences in g_s during each measurement period ($p < 0.01$) may reflect broad (unconfirmed) differences between upper- and lower-profile values. Upper-canopy g_s values were highly variable, possibly as a consequence of the dynamic behavior of relative humidity and vapor pressure deficit (VPD) in this part of the profile (Barker 1996). The range of g_s values reported here is in agreement with those measured in other tropical rain forest canopy studies (e.g. Pearcy 1987, Doley *et al.* 1988, Dolman *et al.* 1991, Roy & Salager 1992). The highest values of g_s were in the upper canopy (> 25 m), consistent with previous reports for tropical rain forest trees (Oberbauer *et al.* 1987, Dolman *et al.* 1988, Roberts *et al.* 1990), and also with the high stomatal densities measured there. Increases in stomatal density with height in the canopy may allow correspondingly greater transpiration rates in the upper canopy, where VPD is generally at a maximum (Barker 1996). Previous work has shown a steady diurnal decline in g_s following peak morning (*c.* 0900 h) values (Roberts *et al.* 1990, Dolman *et al.* 1991, Roy & Salager 1992). However, no such consistent diurnal patterns were apparent in our results.

Height-related statistically-significant differences in leaf water potential (ψ) during morning and mid-day were almost certainly due to a contrasting behavior of understory compared with canopy leaves. Understory median ψ in the morning (-0.21 MPa) and mid-day (-0.25 MPa) was between 25 to 53 % of that of the canopy. This is consistent with other studies in which canopy leaves have developed a larger diurnal amplitude in ψ compared with understory leaves (Myers *et al.* 1987, Oberbauer *et al.* 1987, Bazzaz 1991, Roy & Salager 1992). Median ψ values were lower (i.e. more negative) throughout the profile at mid-day compared with the morning and (except for leaves from 25 m and 31 m) still more in the afternoon. The apparent afternoon recovery of leaves at 25 m and 31 m following a mid-day low value is a type of behavior observed in canopy leaves of other species (Myers

et al. 1987, Oberbauer *et al.* 1987, Bazzaz 1991, Roy & Salager 1992). In our study, height effects within the canopy itself were not apparent, possibly because ψ is not simply a function of height above the ground, but also influenced by unequal transpiration rates and hydraulic resistances within the crown (Ginter-Whitehouse *et al.* 1983). Furthermore, within-crown differences in ψ may be less apparent when there is sufficient soil water available (Gallego *et al.* 1994), which we believe was the case in this study since soil moisture was $\approx$ 35 % w/w. The understory/canopy difference in ψ may reflect corresponding differences in tissue osmotic properties (Myers *et al.* 1987, Oberbauer *et al.* 1987), for which anatomical features provide indirect evidence.

Conclusion

Distinct morphological differences existed within the vertical profile, probably representing an integrated response to the leaf's microclimatic environment. Vertical differences also occurred in leaf ecophysiological characteristics, which were subject to temporal changes. We accept the hypothesis that vertical differences in morphological and ecophysiological differences occur within leaves of the same species from different vertical positions. We attribute this effect to vertical differences in microclimate and/or developmental differences in leaves. Contrasting characteristics were apparent between understory and canopy leaves for leaf water potential, stomatal conductance, foliar nitrogen (on a per area basis), anatomical dimensions and leaf mass area, though differences were only supported by statistical tests for the last two variables. The existence of contrasting understory and canopy leaf characteristics suggests the possibility of transitional stages at intermediate positions in the vertical profile, for example in the sapling layer. We reject the hypothesis that leaf characteristics are related to height within the crown of the canopy tree. However, trends were apparent in which variables were influenced by height positively (stomatal density, leaf mass area) or negatively (driptip length) or in a more complex way (foliar N). Stronger within-canopy trends may be non-existent, or possibly confounded by a lack of simple and stable vertical microclimate gradients within the canopy. Also, leaf ecophysiological characteristics may not respond immediately to fluctuations in microclimate, indicating the need for prolonged measurements over a range of microclimate conditions.

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