EFFECT OF LIGHT AND SOIL MOISTURE ON SEEDLING LEAF ANATOMY OF LOWLAND RAIN FOREST SPECIES

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Abstract

Seedlings of Dipterocarpus zeylanicus Thw., D. hispidus_Thw., Mesua ferrea L., and M. nagassarium (Burm. f.) Kosterm., were grown under light treatments, three of which simulated the radiation experienced by the forest understorey and forest edge, two of which simulated the centres of canopy openings of 200 and 400 m², and a control simulated full sunlight. For each light treatment the soil was either regularly watered to field capacity or kept at ⁻30% of field capacity. After two years measurements were made of stomatal frequency; of the thicknesses of the leaf-blades and of the upper epidermal, palisade mesophyll, and lower epidermal cell layers; and of cell dimensions. Significant differences were found between species and treatments. In general the measured dimensions increased with increase in light and decrease in soil moisture. Mesua ferrea had the thickest leaf blades followed by M. nagassarium, D. zeylanicus and D. hispidus, while D. zeylanicus had thicker epidermal and palisade mesophyll than M. ferrea; taken together, these findings suggest that M. ferrea has a much thicker mesophyll than the other species. In certain treatments, the two Dipterocarpus species had double rows of cells within both the palisade mesophyll and the lower epidermal layers; the frequency of this phenomenon increased with increasing light. It was not observed in Mesua, The greatest densities of stomata were found in D. hispidus, followed by M. ferrea, M. nagassarium, and D. zeylanicus. Differences in shade and drought tolerance between species are discussed.

Introduction

Within a forest there are different microenvironments due to disturbances of the canopy. The type, size and periodicity of disturbance affect the nature of the ground storey (Ashton, Gunatilleke & Gunatilleke, 1993). When disturbances create canopy openings in moist tropical forests they allow understorey vegetation to survive. Many tropical rain forest tree species seem to depend on canopy openings, or to benefit from them, for successful regeneration (Popma & Bongers, 1988). Depending upon the size of the opening, there will be changes in the temperature, relative humidity, amount and

quality of light, and availability of soil moisture can change. Seedlings in the ground storey must respond to these changes in order to survive and grow.

These microenvironmental changes affect the anatomy and morphology of seedling leaves (Wilson & Cooper, 1969). The ability of seedling leaves to change their anatomy and morphology in response to change in environment has been termed phenotypic plasticity (Young & Giese, 1990). For example, seedling leaves that are exposed to more light have physiological and anatomical adaptations similar to those of drought-avoiding plants, while shade leaves often resemble those of drought-intolerant species. Shade leaves are larger and thinner, while sun leaves are smaller and thicker (Transeau et al., 1940; Jackson, 1966; Vogel 1968; Nobel, 1977; Kramer, 1983; Keller et al., 1987).

Many studies have shown that leaf anatomy and morphology differ between tree species categorized on the one hand as pioneers and late-successional, or on the other hand as sun-loving and shade-tolerant (Carpenter & Smith, 1975; Fetcher et al., 1983; Vogel, 1968; Loach, 1967). Few studies have looked for different changes in leaf structure among tree species considered to belong to the same ecological grouping, eg, the same successional or the same light-tolerance status (Ashton & Berlyn, 1992). No studies have investigated changes within a given ecological grouping that has been grown under differing soil-moisture availability.

Our study examined the adaptation of seedling leaves of four species of canopy trees: *Dipterocarpus zeylanicus* Thw., *D .hispidus* Thw., *Mesua ferrea* L., and *M. nagassarium* (Burm.f.) Kosterm., that occur in the rain forest of southwest Sri Lanka, and that have all been characterized as late-successional and relatively shade tolerant. We tested the hypotheses that anatomical attributes of seedling leaves can be modified by growing them under different light intensities and soil moisture regimes such as are experienced in the forest.

Study site, species, and methods

Study site
The study was made at the field station of the Sinharaja Man and the Biosphere (MAB) Reserve in southwest Sri Lanka. The annual rainfall is about 5000 mm, and the mean monthly temperatures range from 25 to 27°C (Ashton, 1992). The topography is undulating between valley bottoms and ridge tops, with a mean elevation of about 600 m. The forest has been classified by De Rosayro (1942) and Ashton & Gunatilleke (1987) as a *Mesua-Shorea* type.

Species
The four species are endemic to southwest Sri Lanka. They are often dominant in the canopy stratum of late-successional forest. Both *D. zeylanicus* and *D. hispidus* are widespread below 300 m (Dassanayake, 1980; FAO, 1985). They are often gregarious on river banks and moist but well drained soils. *Mesua ferrea* occurs in or near streams,

but not on low-lying poorly drained land. *Mesua nagassarium* is generally restricted to ridge tops.

Experimental design

Six controlled-environment shelters were constructed. The quality of light was altered by using a mix of particular pigments in a varnish base that was sprayed onto a clear plastic film. Three light treatments exposed seedlings to uniform diffuse light that simulated the amount and quality of radiation in the forest understorey (FU), inside the forest edge (FE), and outside the forest edge (OE). The maximum photon photosynthetic fluxes (PPFD) for the treatments were respectively 50, 350 and 800 mmol $m^{-2}s^{-1}$). Two other light treatments exposed seedlings to amounts of direct radiation that were comparable to the centres of canopy openings of 100 m^2 (SO) and 400 m^2 (LO); these conditions were created by a series of parallel vertical slats, 1.5 m above the seedlings. While the sun rose from the east and set in the west the duration and number of sunlit periods were controlled by the slat orientation being north–south, at varying spacings. A control treatment exposed seedlings to full sun (FS), with a maximum PPFD of 2000 mmol $m^{-2}s^{-1}$).

Within each light treatment there were two watering treatments. The ever-moist soil condition (100% relative moisture content, RMC) received two litres of water per m^2 three times a week. The dry soil condition received two litres of water per m^2 whenever the RMC fell below 30%. In each regime the RMC was monitored by a battery-operated soil-moisture meter (Bouyoucos, 1953, 1972) that was attached to gypsum blocks buried in the soil.

For each species the seeds were a mix, collected from more than one parent trees. They were germinated on a nursery bed. In January 1993, when the seedlings were one month old, they were taken from the nursery and planted in the shelters, in forest topsoil mixed with an equal quantity of sand. Seedlings of each species were placed together in groups of nine. Four groups of seedlings, each group of a different species, comprised a block. There were four blocks for each light–soil moisture combination, so that altogether each species–light–soil moisture combination had four replicates.

Anatomical measurements

When the seedlings were two years old, samples were taken. For each light–soil moisture combination a leaf was taken from each of three different seedlings for each species. All the selected leaves were fully expanded, undamaged and with no signs of herbivory. Permanent microscope slides were prepared. For each leaf, a 0.5 × 1-cm section was taken from the middle portion of the lamina, across the midrib. These strips were immediately fixed in FAA (formalin, acetic acid and ethanol), dehydrated in ethanol, immersed in a xylene series, and then embedded in wax. A rotary microtome was used to cut sections 10 mm thick. They were stained with safranine and fast green and then mounted in Canada balsam (Berlyn & Miksche, 1976). Three slides were made from each strip.

On each slide, measurements were made of leaf-blade thickness and component dimensions (palisade cell height, palisade layer thickness, proportion of palisade cells double layered, upper and lower epidermal cell heights, and lower epidermal cell layer thickness). Six measurements were made on each slide for each parameter, avoiding the region of the midrib, by viewing through a Leitz light microscope with a 0.05×100 mm micrometer eye-piece and a 0.01×100 mm objective. Cell dimen-sion measurements were taken at 400× magnification and leaf thickness dimensions at 100×.

For *D. zeylanicus* and *M. nagassarium,* stomatal frequencies were observed from cellulose acetate positives of silicone-rubber impressions (Heichel, 1971). In *M. ferrea* the stomata are chambered, and *D. hispidus* has hairs on the leaf under-surface; peels were therefore taken for these species and boiled with alcohol in order to observe the stomata. Each impression or peel was taken midway between the base and tip on the upper and lower surfaces of a leaf. Five impressions or peels were used for each species and light–soil moisture combination. For each impression or peel, three different fields of view were sampled. The stomata were counted with an eye-piece graticule, at 400× magnification.

Data analysis
Data were analysed by ANOVA, using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS, see Ray, 1981). The analyses tested for differences between species, light treatments, soil moisture treatments and for interactions between species and treatments. Differences between the treatments and species were determined at the 5% significance level by Tukey's method.

Results

In all cases, in both text and tables, "significant(ly)" means at P < 0.05.

Stomatal frequency
In all four species stomata were found only on the lower surface; this finding corroborates many other studies of tree species (Metcalfe & Chalk, 1965) (Table 1, Figure 1). Stomatal frequency increased with increasing light intensity, and thus the greatest frequency was in leaves exposed to full sun. Differences between species were also apparent within the same light treatment. *Dipterocarpus hispidus* had a higher stomatal frequency than *D. zeylanicus.* The two species of *Mesua* had similar frequencies in some light treatments, but overall *M. ferrea* had more than *M. nagassarium.* Comparing all four species, *D. hispidus* had the greatest frequency, followed by *M. ferrea, M. nagassarium* and *D. zeylanicus.* In about three quarters of the comparisons of seedlings grown in ever-moist with those in dry soils, stomatal frequency was significantly different for the same species and the same light treatment.
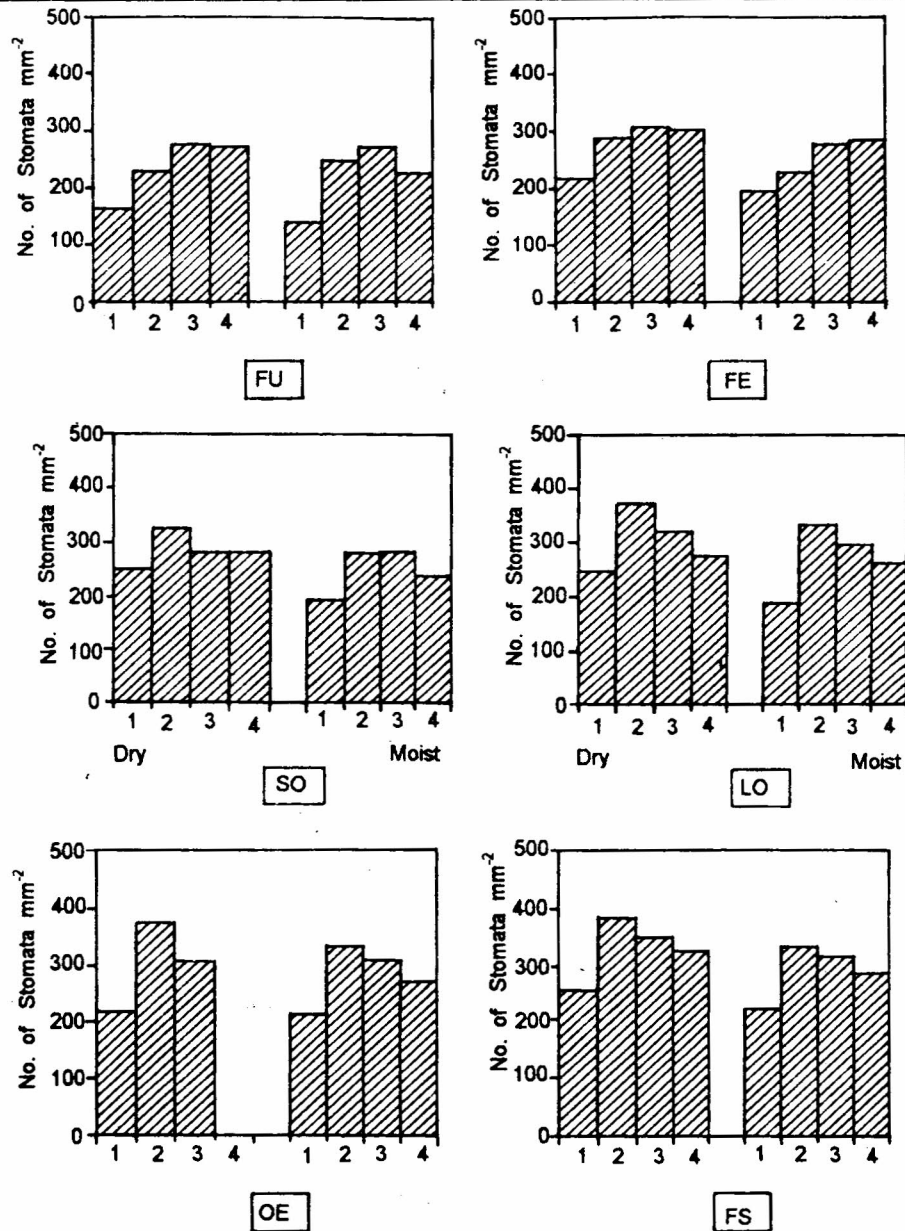
Figure 1: Stomatal frequency of leaves in (1) *D. zeylanicus,* (2) *D. hispidus,* (3) *M. ferrea,* and (4) *M. nagassarium,* under different light and soil-moisture treatments (FU = forest understorey; FE = inside forest edge; OE = outside forest edge; FS = full sun; SO = centre of small opening; LO = centre of large opening)

In all tables:

FU = forest understorey; FE = inside forest edge; OE = outside forest edge; FS = full sun; SO = centre of small opening; LO = centre of large opening.

Table 1 : Stomatal frequency

Data are the mean number per mm^2 from different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. The capital letters before the figures denote differ-ences between species in each light treatment. Species with the same letter are not significantly different.

		Dry	Moist
FU			
D. zeylanicus	B	162.2 (1.3) a	140.0 (7.8) b
D. hispidus	A	226.7 (16.7) b	247.4 (12.7) a
M. ferrea	A	275.6 (9.2) a	271.8 (9.0) a
M. nagassarium	A	270.4 (14.8) a	225.9 (13.0) b
FE			
D. zeylanicus	B	212.6 (8.7) a	191.1 (6.4) a
D. hispidus	A	282.2 (21.8) a	224.4 (8.01) b
M. ferrea	A	300.7 (7.1) a	271.8 (4.5) b
M. nagassarium	A	296.30 (20.5) a	241.5 (9.5) b
OE			
D. zeylanicus	C	213.3 (14.3) a	207.4 (7.8) a
D. hispidus	A	369.6 (8.2) a	328.2 (5.8) b
M. ferrea	B	303.0 (4.9) a	304.4 (5.6) a
M. nagassarium		NA	266.7 (14.8)
FS			
D. zeylanicus	C	250.4 (11.2) a	215.6 (16.8) b
D. hispidus	A	380.7 (8.2) a	331.8 (22.1) b
M. ferrea	AB	345.2 (8.5) a	314.1 (7.1) b
M. nagassarium	B	322.2 (3.4) a	283.7 (5.8) b
SO			
D. zeylanicus	B	244.4 (11.8) a	190.4 (12.1) b
D. hispidus	A	318.5 (17.3) a	274.1 (20.8) b
M. ferrea	A	276.3 (3.7) a	274.8 (10.7) a
M. nagassarium	AB	274.8 (15.7) a	236.3 (13.5) a
LO			
D. zeylanicus	D	245.9 (10.0) a	191.1 (9.0) b
D. hispidus	A	375.6 (8.4) a	332.6 (7.3) b
M. ferrea	B	320.0 (7.7) a	297.8 (10.2) b
M. nagassarium	C	275.6 (1.3) a	264.4 (7.2) a

Leaf-blade thickness

Leaf-blade thickness increased with increase in light (Table 2, Fig.2), and for all species the thickest leaf blade was observed in the full-sun treatment. In most cases, differences between species were significant within the same light treatment. In *Dipterocarpus* the thicker leaf blades were observed in *D. zeylanicus*. In *Mesua*, the thickness was not significantly different between the two species within the same light treatment, excepting LO and SO, in which *M. ferrea* had the thicker blade. *Mesua* species had thicker leaf blades than *Dipterocarpus* species; when comparing all species together, the order was *M. ferrea, M. nagassarium, D. zeylanicus,* and *D. hispidus.*

In most instances significant differences in leaf-blade thickness could not be observed between ever-moist and dry soil treatments. The main exception was the understorey light intensity treatment (FU), in which *D. zeylanicus, M. ferrea* and *M. nagassariam* showed differences. These species had thicker blades when grown in the ever-moist soil treatment than when grown in the dry soil.

Component dimensions

The different light and soil-moisture treatments showed significant differences between epidermal cell thicknesses. These differences were apparent for both upper (Table 3, Fig. 3) and lower (Table 4) epidermal cells. Both upper and lower epidermal cell height were greatest in FS treatment (2000 mmol m^{-2}s^{-1}).

Of the two dipterocarps, *D. zeylanicus* had the greater upper epidermal cell thickness. In *Mesua* both species were usually similar within the same light treatment. The dipterocarps had significantly thicker upper epidermal cells than the *Mesua* species.

In most cases, lower epidermal cell thickness was significantly different between species for any given light treatment. *D. zeylanicus* had the greatest, followed by *D. hispidus, M. ferrea* and *M. nagassarium. Mesua ferrea* had a papillated lower epidermal cell layer with sunken stomata. In almost all comparisons for any species between ever-moist and dry soils differences in lower epidermal cell thicknesses were not significant. The same was also generally the case for comparisons of upper epidermal cell thickness between ever-moist and dry soil treatments. Where it was not the case, the dry soil treatment usually showed greater cell dimensions than the ever-moist soil treatment.

In the higher light treatments both dipterocarps had double rows of lower epidermal cells, while *Mesua* did not. This feature could be observed to a decreasing extent, in the order FS>LO>OE>SO. *D. zeylanicus* had a thicker epidermal cell layer than *D. hispidus* for each light treatment. With few exceptions, when grown in the same light treatment the lower epidermal layer thicknesses of the dipterocarps did not change significantly, between ever-moist and dry soil treatments.
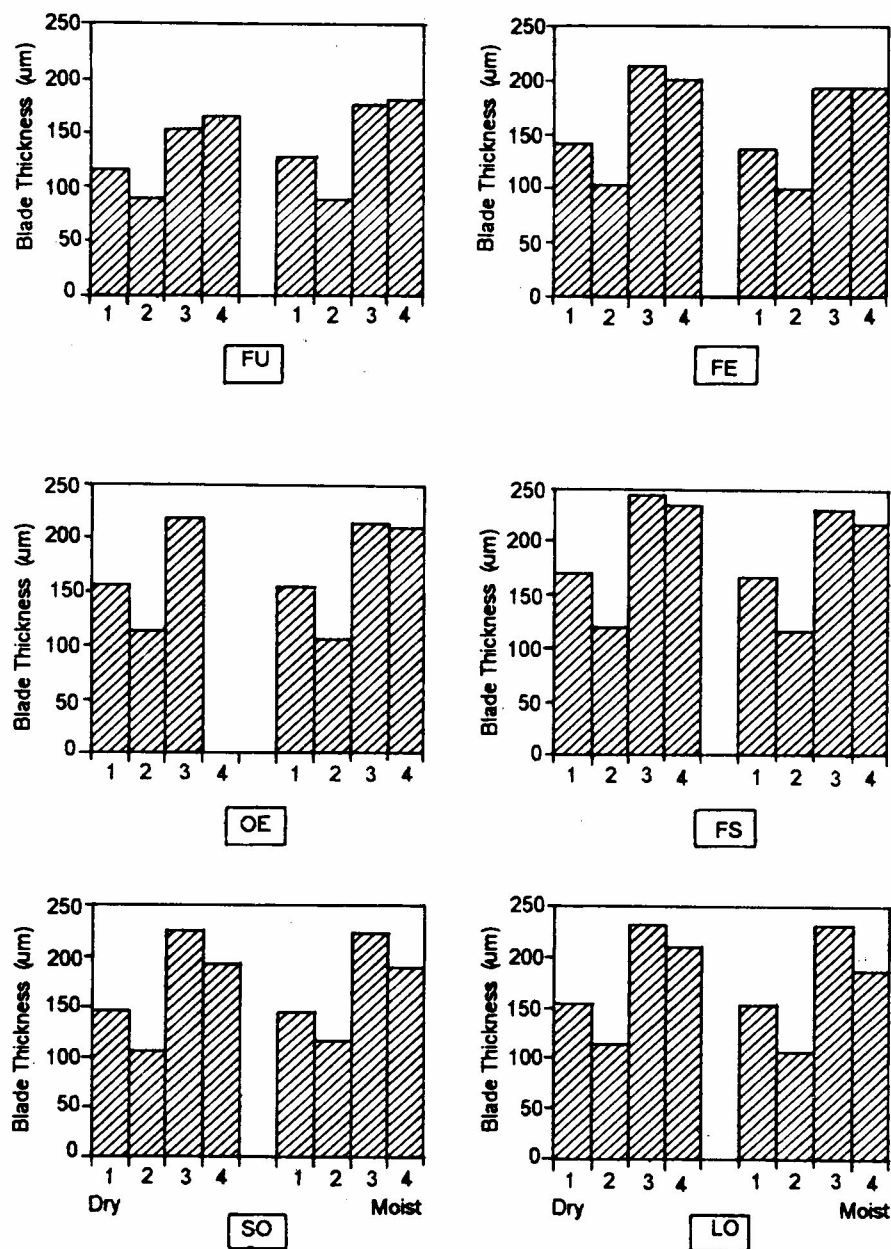
Figure 2 : Leaf-blade thickness of (1) *D. zeylanicus,* **(2)** *D. hispidus,* **(3)** *M. ferrea,* **and (4)** *M. nagassarium,* **under different light and soil-moisture treatments (as in Fig. 1)**

Table 2: Leaf-blade thickness

Data are means (in μm) from different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. The capital letters before the figures denote differences between species in each light treatment. Species with the same letter are not significantly different.

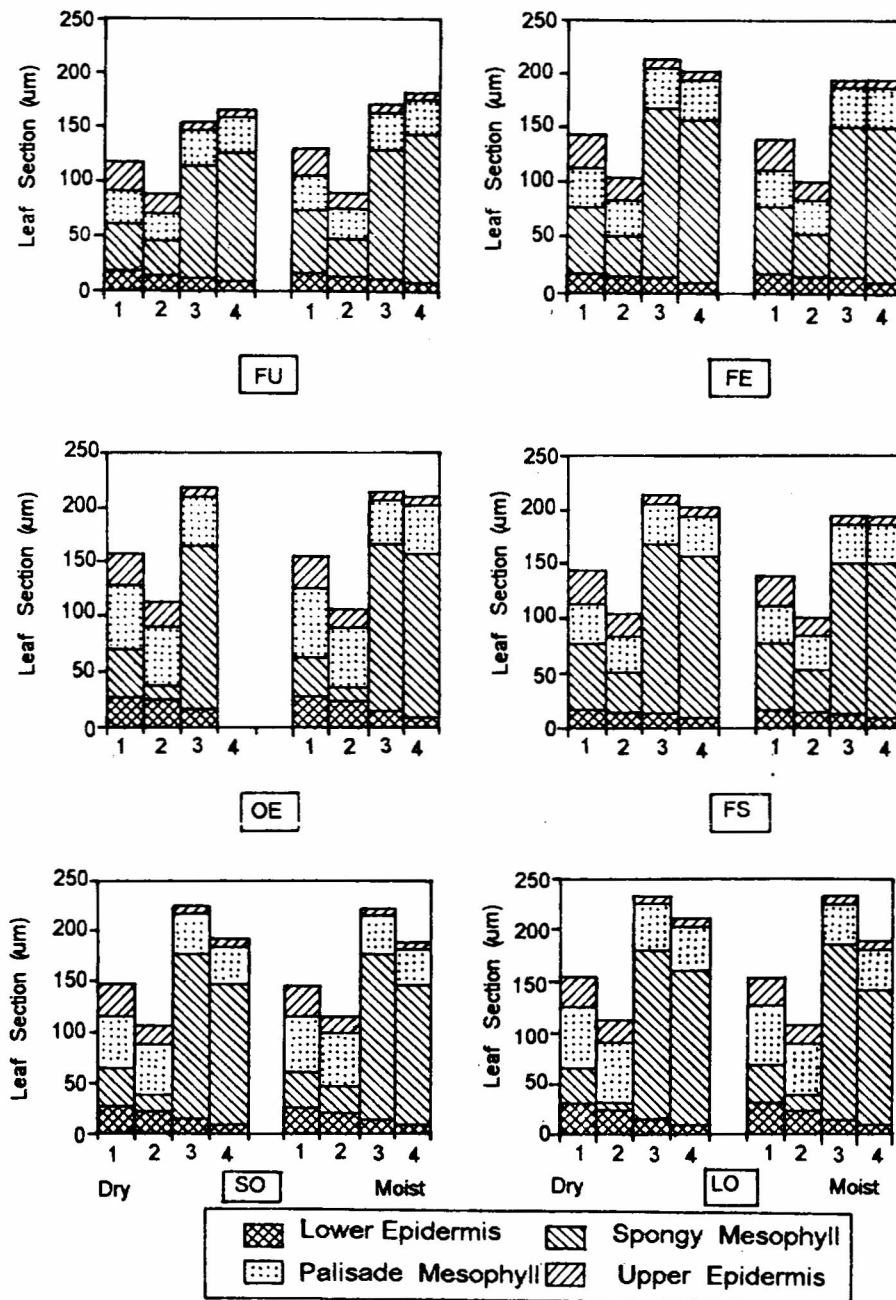|		Dry	Moist
FU			
D. zeylanicus	B	113.9 (2.1) b	126.1 (3.4) a
D. hispidus	C	85.3 (5.0) a	85.6 (1.6) a
M. ferrea	A	151.1 (0.9) b	173.9 (3.9) a
M. nagassarium	A	162.6 (3.4) b	179.4 (0.7) a
FE			
D. zeylanicus	B	138.7 (2.8) a	133.5 (0.9) a
D. hispidus	C	99.2 (2.4) a	96.1 (1.2) a
M. ferrea	A	209.2 (2.2) a	189.2 (2.0) b
M. nagassarium	A	197.8 (4.2) a	189.3 (4.0) a
OE			
D. zeylanicus	B	152.9 (2.5) a	150.7 (1.5) a
D. hispidus	C	109.1 (6.5) a	102.7 (2.7) a
M. ferrea	A	215.0 (3.8) a	210.3 (2.0) a
M. nagassarium		NA	205.8 (2.2)
FS			
D. zeylanicus	B	163.9 (2.8) a	161.1 (2.5) a
D. hispidus	C	114.9 (2.6) a	112.1 (4.8) a
M. ferrea	A	238.4 (10.7) a	224.6 (2.1) b
M. nagassarium	A	228.9 (2.9) a	209.9 (1.6) b
SO			
D. zeylanicus	C	143.5 (5.9) a	141.9 (4.7) a
D. hispidus	D	102.4 (0.7) a	112.5 (3.9) a
M. ferrea	A	220.9 (3.1) a	218.2 (6.4) a
M. nagassarium	B	187.7 (1.5) a	184.4 (2.6) a
LO			
D. zeylanicus	C	151.0 (6.7) a	150.5 (4.9) a
D. hispidus	D	109.6 (2.9) a	103.9 (1.5) a
M. ferrea	A	228.2 (0.8) a	229.1 (1.4) a
M. nagassarium	B	206.8 (8.4) a	185.2 (4.2) b

Figure 3 : Stylized leaf cross-sections of (1) *D. zeylanicus,* (2) *D. hispidus,* (3) *M. ferrea,* and (4) *M. nagassarium,* under different light and soil moisture treatments (as in Fig. 1)

Table 3 : Upper epidermal cell thickness

Data are means (in μm) from different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. The capital letters before the figures denote differ-ences between species in each light treatment. Species with the same letter are not significantly different.

		Dry	Moist
FU b			
D. zeylanicus	A	25.6 (1.3) a	25.3 (0.4) a
D. hispidus	B	18.0 (0.4) a	14.6 (0.7) b
M. ferrea	C	7.3 (0.3) a	7.5 (0.7) a
M. nagassarium	C	6.4 (0.1) b	6.8 (0.1) a
FE			
D. zeylanicus	A	30.0 (0.5) a	26.9 (0.4) b
D. hispidus	B	20.5 (0.4) a	16.0 (0.4) b
M. ferrea	C	8.1 (0.1) a	7.2 (0.1) a
M. nagassarium	C	8.6 (0.3) a	7.8 (0.2) a
OE			
D. zeylanicus	A	29.3 (0.6) a	30.0 (1.2) a
D. hispidus	B	22.8 (1.3) a	16.6 (0.3) b
M. ferrea	C	8.4 (0.3) a	7.6 (0.3) a
M. nagassarium	C	NA	7.7 (0.1)
FS			
D. zeylanicus	A	33.2 (1.3) a	32.2 (0.9) a
D. hispidus	B	22.9 (1.3) a	17.9 (0.5) b
M. ferrea	C	9.8 (0.0) a	8.2 (0.1) b
M. nagassarium	C	8.3 (0.1) a	8.4 (0.1) a
SO			
D. zeylanicus	A	30.9 (1.2) a	29.4 (3.3) a
D. hispidus	B	18.4 (1.3) a	16.7 (0.6) a
M. ferrea	C	7.9 (0.1) a	7.3 (0.2) a
M. nagassarium	C	8.2 (0.3) a	7.0 (0.4) a
LO	A	28.7 (0.6) a	26.7 (0.3) a
D. zeylanicus	B	22.5 (0.7) a	18.3 (0.5) b
D. hispidus	C	7.9 (0.2) a	8.1 (0.2) a
M. ferrea	C	8.0 (0.2) a	8.1 (0.2) a
M. nagassarium			

Table 4 : Lower epidermal cell thickness

Data are means (in µm) for different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. The capital letters before the figures denote differ-ences between species in each light treatment. Species with the same letter are not significantly different.

		Dry	Moist
FU			
D. zeylanicus	A	15.4 (0.6) a	13.4 (0.3) b
D. hispidus	B	11.2 (0.2) a	10.7 (0.3) a
M. ferrea	C	8.6 (0.3) a	9.1 (0.1) a
M. nagassarium	D	5.9 (0.9) a	5.3 (0.1) a
FE			
D. zeylanicus	A	13.8 (0.6) a	13.4 (0.1) a
D. hispidus	B	11.2 (0.3) a	10.8 (0.3) a
M. ferrea	C	9.8 (0.4) a	10.0 (0.3) a
M. nagassarium	D	6.0 (0.0) a	5.6 (0.3) a
OE			
D. zeylanicus	A	14.5 (0.9) a	12.8 (0.3) a
D. hispidus	B	12.8 (0.3) a	11.8 (0.5) a
M. ferrea	B	11.9 (0.2) a	10.7 (0.3) b
M. nagassarium		NA	5.4 (0.1)
FS			
D. zeylanicus	A	17.9 (0.9) a	15.6 (0.6) b
D. hispidus	B	12.8 (0.3) a	11.8 (0.5) a
M. ferrea	B	12.6 (0.5) a	11.9 (0.3) a
M. nagassarium	C	6.3 (0.2) a	6.3 (0.1) a
SO			
D. zeylanicus	A	14.3 (0.7) a	13.6 (0.3) a
D. hispidus	B	10.8 (0.4) a	11.5 (0.7) a
M. ferrea	B	11.7 (0.1) a	10.9 (0.6) a
M. nagassarium	C	5.6 (0.5) a	5.3 (0.2) a
LO			
D. zeylanicus	A	15.5 (0.7) a	15.4 (0.2) a
D. hispidus	B	12.4 (0.1) a	12.6 (0.2) a
M. ferrea	C	11.9 (0.2) a	11.3 (0.4) a
M. nagassarium	D	5.7 (0.1) a	6.1 (0.3) a

Table 5 : Lower epidermal cell layer thickness of the two *Dipterocarpus* species
Data are means (in μm) from different leaves with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment; species with the same letter are not significantly different. Similarly, the capital A and B before the figures denote differences between species in each light treatment.

			Dry	Moist
OE				
D. zeylanicus	*D.*	A	23.4 (0.6) a	24.2 (0.6) a
hispidus		B	21.3 (0.3) a	20.3 (0.5) a
FS				
D. zeylanicus	*D.*	A	29.4 (1.0) a	26.2 (0.6) a
hispidus		B	23.1 (0.6) a	19.6 (0.2) b
SO				
D. zeylanicus	*D.*	A	24.9 (0.8) a	22.9 (0.2) a
hispidus		B	18.3 (0.3) a	17.3 (0.4) a
LO				
D. zeylanicus	*D.*	A	27.1 (0.6) a	28.2 (0.9) a
hispidus		B	21.0 (0.1) a	20.7 (0.6) a

Table 6 shows palisade cell heights. Since the palisade cell structure largely depends upon the intensity of light (Simon et al., 1990), it was not surprising that the palisade cell height increased with increasing light intensity and varied between treatments as follows: FS>LO>OE>SO>FE>FU. Some significant differences were also apparent between species within a given light treatment. For palisade layer thickness there was only one significant difference (in treatment OE) between species in the same light treatment; the results for the dipterocarps are shown in Table 7. The *Mesua* species had greater palisade cell height than the dipterocarps. Where there were significant differences, *M. ferrea* had the greatest cell height, followed by *M. nagassarium*, *D. zeylanicus*, and *D. hispidus*. There were only a few significant differences in cell layer thickness between the different soil-moisture treatments.

While the *Mesua* species did not show this feature, the dipterocarps were observed to have several palisade cells stacked up on each other. The occurrence of this phenomenon increased with increasing light intensity; this explains why for both species the thickest cell layers were in the FS treatment. This double stacking phenomenon did not occur as a continuous layer. The proportion of double layering was

therefore also recorded (Table 8); it increased with increasing light. In all treatments *D. zeylanicus* had a significantly higher proportion than *D. hispidus*.

Table 6 : Palisade cell depth

Data are means (in μm) from different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. The capital letters before the figures denote differences between species in each light treatment. Species with the same letter are not significantly different.

		Dry	Moist
FU			
D. zeylanicus	A	31.0 (0.9) a	31.1 (0.9) a
D. hispidus	B	26.0 (1.6) a	28.6 (0.6) a
M. ferrea	A	33.1 (0.6) a	34.9 (1.3) a
M. nagassarium	A	34.0 (1.0) a	31.8 (0.7) a
FE			
D. zeylanicus	B	35.6 (0.5) a	33.8 (0.3) b
D. hispidus	C	32.1 (1.4) a	31.4 (0.2) a
M. ferrea	A	38.4 (0.7) a	37.3 (0.8) a
M. nagassarium	AB	37.1 (0.7) a	36.2 (0.5) a
OE			
D. zeylanicus	B	35.8 (1.2) a	39.2 (1.2) a
D. hispidus	C	31.8 (0.7) a	33.3 (1.1) a
M. ferrea	A	45.6 (0.2) a	39.9 (1.3) b
M. nagassarium	A	NA	44.9 (1.3)
FS			
D. zeylanicus	B	40.8 (0.8) a	42.7 (1.0) a
D. hispidus	BC	37.6 (1.6) a	38.4 (2.4) a
M. ferrea	AB	46.7 (2.2) a	46.2 (1.8) a
M. nagassarium	A	51.8 (0.5) a	43.2 (0.0) b
SO			
D. zeylanicus	C	30.9 (1.2) a	32.5 (1.5) a
D. hispidus	C	31.5 (0.4) a	32.7 (0.3) a
M. ferrea	A	40.3 (0.2) a	38.1 (0.4) b
M. nagassarium	B	36.0 (0.9) a	34.2 (0.1) a
LO			
D. zeylanicus	AB	37.8 (2.3) a	38.3 (1.1) a
D. hispidus	B	36.7 (1.6) a	33.9 (0.6) a
M. ferrea	A	44.5 (1.4) a	39.5 (0.4) b
M. nagassarium	A	42.1 (1.2) a	38.2 (0.5) b

Table 7 : Palisade cell layer thickness of the two *Dipterocarpus* species
Data are means (in μm) from different leaves with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. Similarly, the capital A and B before the figures denote differences between species in each light treatment. Species with the same letter are not significantly different.

			Dry	Moist
OE				
D. zeylanicus	*D.*	A	57.5 (1.6) a	61.9 (0.9) a
hispidus		B	52.8 (1.0) a	54.2 (1.5) a
FS		A	63.6 (0.8) a	56.3 (5.3) a
D. zeylanicus	*D.*	A	57.9 (1.3) a	60.6 (2.3) a
hispidus				
SO		A	52.2 (1.4) a	55.6 (1.7) a
D. zeylanicus	*D.*	A	49.2 (0.5) a	52.7 (1.0) b
hispidus				
LO		A	59.7 (1.8) a	57.9 (0.6) a
D. zeylanicus	*D.*	A	58.6 (2.3) a	50.2 (0.3) b
hispidus				

Table 8 : Proportion of layering of palisade cells for the two *Dipterocarpus* species
Data are means from different leaves, with standard errors in parentheses. A lower case a after one figure and b after the other denotes a significant difference between the soil-moisture treatments in the same species and light treatment. Similarly, the capital A and B before the figures denote differences between species in each light treatment. Species with the same letter are not significantly different.

			Dry	Moist
OE				
D. zeylanicus	*D.*	A	0.33 (0.01) a	0.31 (0.02) b
hispidus		A	0.30 (0.03) a	0.28 (0.01) b
FS				
D. zeylanicus	*D.*	A	0.40 (0.01) a	0.38 (0.01) a
hispidus		B	0.35 (0.02) a	0.34 (0.01) a
SO				
D. zeylanicus	*D.*	A	0.27 (0.02) a	0.26 (0.01) a
hispidus		B	0.22 (0.01) a	0.24 (0.02) a
LO				
D. zeylanicus	*D.*	A	0.30 (0.01) a	0.32 (0.02) a
hispidus		B	0.29 (0.01) a	0.27 (0.01) a

Discussion

In combination, an array of anatomical characteristics can partly determine the morphological light and drought tolerance of a species (Ashton & Berlyn, 1992). The effect of light on the leaf morphology of woody angiosperms is well-known. Several investigators have reported that anatomical changes in the leaves, such as stomatal frequency, leaf-blade thickness, and other cell component dimensions, vary with exposure to light (Cutter, 1978; Simon et al., 1990). For example, stomatal frequency increases with increasing light intensity. This variation in stomatal frequency in differing light environments has been attributed to change in leaf size. Greater light intensities promote reduction in leaf size in order to prevent desiccation (Ciha & Brun, 1975). However this is not true for all species. For *Shorea*, section *Doona*, at least two of the species show little change in leaf size between light environments (Ashton, 1995). Stomatal frequency can be used to gauge drought tolerance of species with similar morphology, (Ashton & Berlyn, 1992). For example, because *Dipterocarpus hispidus* has greater stomatal frequency than *D. zeylanicus* it could be deduced that the former is less tolerant. Similarly *M. ferrea*, with a greater stomatal frequency, could be less drought tolerant than *M. nagassrium*.

The amount of light can also have a direct influence on blade thickness (Simon et al., 1990). For the study species this may be due to increases in mesophyll and palisade tissue in the brighter light treatments. All spongy mesophyll, palisade and epidermal cells were densely packed in the high light treatments, which reduced air space within the leaf. This phenomenon can increase leaf-blade thickness and also promote greater water-use efficiency. Leaf-blade thickness can also be used as a gauge for determining both light and drought tolerance of similar species (Ashton & Berlyn, 1992). *Dipterocarpus zeylanicus*, which has a thicker leaf blade than *D. hispidus* could be considered more light and drought tolerant. Similarly, although in most comparisons no difference could be found between the *Mesua species*, for the SO and LO treatments, *M. ferrea* had a thicker leaf blade, suggesting that it is the more light and drought tolerant of the two.

Anatomical differences between species are also apparent for epidermal cell dimensions and epidermal layer thicknesses; the upper epidermal layer is the one most exposed to direct radiation. Upper epidermal cell dimensions can also indicate light tolerance (Ashton & Berlyn, 1992). In this study *D. zeylanicus* was found to have upper epidermal cells of greater height than those of *D. hispidus*. *Mesua ferrea* had lower epidermal cells that were papillated and with sunken stomata – a characteristic that would make it more drought tolerant than *M. nagassarium*. Both *Dipterocarpus* species had double rows of cells in the lower epidermal layer; *Mesua* did not show this feature. It should promote greater water-use efficiency.

Both the palisade cell height and layer thickness increased with increase in exposure to light in all four species. Increased palisade cell layers can reduce the spaces in the leaf by their dense packing as compared with that of the irregularly shaped spongy mesophyll cells. This dense packing in high light environments can promote greater

capture of light radiation and greater protection from desiccation. The greater cell height in *D. zeylanicus* indicates that it is more light demanding than *D. hispidus*. Stacking of palisade cells upon each other was seen only in *Dipterocarpus*. This phenomenon was observed only in the more intensive light treatments and its occurrence declined in the order FS>OE>LO>SO. It was greater in *D. zeylanicus* than in *D. hispidus*, which suggests that the former could be more efficient in capturing light.

Available soil moisture is also important in determining leaf anatomy and morphology (Young & Giese, 1990). In this study stomatal frequency was greater in the dry-soil treatment in only a few instances, most notably in *D. zeylanicus*. In these dry conditions leaves exhibited more xerophytic characteristics, that are similar to those of leaves exposed to large amounts of desiccating radiation. When there are more stomata the distances between them are less and the amount of water transpired is greater; these factors increase the humidity of the leaf surface. The moisture gradient between the leaf and its surrounding environment is therefore reduced and there is a net reduction in water loss. In this experiment, in the dry-soil treatment, water was added to bring the soil to full capacity whenevet the moisture content reached less than 30%. We speculate that under these conditions, except in the case of *D. zeylanicus*, the species were enabled to withstand droughty conditions by controlling the stomatal apertures.

In most cases blade thickness, upper and lower epidermal layer thicknesses, and palisade cell height did not change significantly with the availability of soil moisture.

In conclusion, this study demonstrated that the seedlings of species classified as late-successional canopy trees have morphological and anatomical leaf characteristics that change with light and with soil-moisture availability. The greatest differences in dimensions were observed between light treatments rather than soil-moisture treatments. Of the two dipterocarps, *D. zeylanicus*, which had thicker leaf blades and greater height for the upper and lower epidermal and palisade cell layers, could be regarded as more light tolerant than *D. hispidus*. Its low stomatal frequency and greater differences under the two soil-moisture treatments also suggest that *D. zeylanicus* is probably the more drought tolerant of the two. In *Mesua* most of the anatomical and morphological dimensions differed between the two species within the direct sun treatments (SO, LO). In these treatments the thicker leaf blade and greater palisade cell height of *M. ferrea* would suggest that it is more light tolerant than *M. nagassarium*, whereas the lower stomatal frequency in *M. nagassarium* would perhaps make it the more drought tolerant of the two species.

Comparing genera, the greater blade thickness and palisade cell height of the *Mesua* species prompts speculation that they are more light demanding than the dipterocarps. As regards stomatal frequency *D. hispidus* had the most, and *D. zeylanicus* the least, while the Mesua species had an intermediate count – which would make *D. zeylanicus* the most drought tolerant. However, some anatomical features are genus specific. Examples such as double layering of palisade and lower epidermal cells, the shape of palisade cells, and stomatal size, make intergeneric comparisons difficult. Also, for

example, *M. ferrea* has sunken stomata with an external air-chamber, and *D. hispidus* has hairs on the leaf surface. These facts can confuse even comparisons within genera, and it is important to take them into account when speculating on species differences in light and drought tolerance based on leaf morphology.

Acknowledgements

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