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EFFECTS OF IRRADIANCE AND SPECTRAL QUALITY ON LEAF STRUCTURE AND FUNCTION IN SEEDLINGS OF TWO SOUTHEAST ASIAN *HOPEA* (DIPTEROCARPACEAE) SPECIES[1]

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We studied the development of leaf characters in two Southeast Asian dipterocarp forest trees under different photosynthetic photon flux densities (PFD) and spectral qualities (red to far-red, R:FR). The two species, *Hopea helferi* and *H. odorata*, are taxonomically closely related but differ in their ecological requirements; *H. helferi* is more drought tolerant and *H. odorata* more shade tolerant. Seedlings were grown in replicated shadehouse treatments of differing PFD and R:FR. We measured or calculated (1) leaf and tissue thicknesses; (2) mesophyll parenchyma, air space, and lignified tissue volumes; (3) mesophyll air volumes (V_{mes}/A_{surf}) and surfaces (A_{mes}/A_{surf}); (4) palisade cell length and width; (5) chlorophyll/cm^2 and *a*/*b*; (6) leaf absorption; and (7) attenuance/absorbance at 652 and 550 nm. These characters varied in response to light conditions in both taxa. Characters were predominantly affected by PFD, and R:FR slightly influenced many characters. Leaf characters of *H. odorata* were more plastic in response to treatment conditions. Characters were correlated with each other in a complex fashion. Variation in leaf anatomy is most likely a consequence of increasing leaf thickness in both taxa, which may increase mechanical strength and defense against herbivory in more exposed environments. Variation in leaf optical properties was most likely affected by pigment photo-bleaching in treatments of more intense PFD and was not correlated with A_{max}. The greater plasticity of leaf responses in *H. odorata* helps explain the acclimation over the range of light conditions encountered by this shade-tolerant taxon. The dense layer of scales on the leaf undersurface and other anatomical characters in *H. helferi* reduced gas exchange and growth in this drought-tolerant tree.

Key words: Dipterocarpaceae; *Hopea*; leaf structure; rain forest; red:far-red ratio; seedling; shade.

Producing new and structurally altered leaves is the primary means for plants to respond to changes in the radiation environment. Variation in leaf structure may affect plant function in at least three ways. First, leaf anatomy, particularly stomatal density and the extent and shape of mesophyll air spaces, affects resistance to gas exchange and may limit photosynthetic assimilation. Second, pigment content and distribution, influenced by anatomy, determine the efficiency of light capture by leaves and influence photosynthesis. Finally, leaf toughness may reduce a plant's susceptibility to herbivory, increase its longevity, and enhance the plant's carbon balance. Renewed interest in the contributions of leaf structure to photosynthesis and growth (Terashima, 1989; Smith et al., 1997) may help to solve the old mystery of the differences in growth rates among plants (Lambers and Poorter, 1992).

Here we examine the effects of light environments, differing both in photosynthetic photon flux density (400–700 nm, PFD) and in spectral quality (as documented by the red to far-red ratio of quanta, or R:FR; Smith, 1994) on leaf structure and function in seedlings of two rain forest trees native to Southeast Asia. We focus particularly on the responses of leaf anatomy and pigment content. The seedling growth, photosynthesis, and architecture have been described previously (Lee et al., 1997).

Leaf structure and function—Leaf anatomy may directly influence CO_2 uptake by its effects on diffusive resistances (Nobel, 1991). Earlier research in crop plants hoped to find such anatomical traits that could be manipulated to increase crop production (El-Sharkawy and Hesketh, 1965; Wilson and Cooper, 1967). These resistances begin at the boundary layer, influenced by leaf size and shape. Stomatal size and density play a crucial role (Wong, Cowan, and Farquhar, 1979). Although the role of mesophyll air space is less understood, its influence may also be substantial (Nobel, 1977; Parkhurst, 1994). The degree of mesophyll air space can be assessed as a percentage or an actual volume per unit area. The surface

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of the air space/cell contacts can be estimated as a ratio to the leaf surface (A_{mes}/A_{surf}) using stereological techniques (Parkhurst, 1982). Three anatomical variables altering A_{mes}/A_{surf} include (1) leaf thickness; (2) differentiation of palisade and spongy mesophyll; and (3) the degree of mesophyll air space (Nobel, 1991). Additional resistances, less amenable to analysis, include the mesophyll cell walls, cytosol, and chloroplasts.

Leaf anatomy affects the efficiency of light absorption in two ways. Light is absorbed by pigments, principally chlorophylls *a* and *b*, in the chloroplasts. Other pigments, including the carotenoid accessory pigments and flavonoids (as anthocyanins) also modify absorption. Leaf anatomy is important in the way in which it influences the distribution of chloroplasts in leaves (Lee et al., 1990; Vogelmann, 1994). Since chlorophyll is produced in plastids and not dispersed throughout the leaf, quanta may pass through them without being absorbed. This probability reduces the absorption by chlorophyll at the wavelengths it most effectively absorbs (such as at 650 nm) due to sieving or flattening effects (Duysens, 1956; Das et al., 1967). Additionally, cellular structures within the leaf (as cell walls and air spaces) scatter radiation in a complex fashion and make the internal light environment more uniform. This scattering increases the effective path length of radiation and increases the probability of absorption by chlorophyll at weakly absorbed wavelengths, as at 550 nm (Butler, 1964; Kirk and Goodchild, 1972).

The contribution of leaf structure to sieving and path-lengthening effects can be assessed by comparing the ratio of absorbance of chlorophyll in vivo to that in vitro, the attenuance to absorbance ratio (Ruhle and Wild, 1979; Terashima and Saeki, 1983; Lee et al., 1990). Such a ratio should indicate sieving effects at strong absorbance wavelengths (<1.00 at 652 nm) and path-lengthening effects at weakly absorbed wavelengths (>1.00 at 550 or 700 nm). These ratios will depend upon the chlorophyll concentration and the distribution of chloroplasts. Differentiation of mesophyll tissue into palisade and spongy cell types should increase the sieving effects, along with the shape of the palisade cells. Long and narrow cells increase this effect (Lee et al., 1990). Because of the difference between the refractive indices of air and cell components (1.40–1.45), the extent and distribution of air spaces strongly influence path-lengthening effects. Still other anatomical features, as pubescence and surface waxes, influence absorption (Ehleringer, Björkman and Mooney, 1976; McClendon, 1984). Pubescence or scales on the abaxial surface may actually increase absorption (Eller and Willi, 1981).

Shade responses—Shadelight under forest canopy varies in quantity and spectral quality, and both factors may affect leaf development, structure, and function. Reduced PFD affects leaf development in the classical ways (size and thickness) described in numerous studies (Chabot and Chabot, 1977; Dengler, 1980; Björkman, 1981; Jurick, Chabot, and Chabot, 1982). Since such research has combined shade conditions (reduced PFD) with the spectral quality of full sunlight (high R:FR), it is bound to underestimate the extent of anatomical responses to shadelight (Schmitt and Wulff, 1993). Reduced R:FR, affecting phytochrome equilibria in plant tissues (Smith, 1994), may influence leaf anatomy, although there are few such studies in response to R:FR. The challenge in this research has been to separate the effects of quantity and quality in assessing such effects. Ashton and Berlyn (1992, 1994) simultaneously altered PFD and RFR in shade experiments and demonstrated significant changes in leaf anatomy with physiological significance. Chazdon and Kaufman (1993) encountered such parallel changes in their choice of natural light environments, as did Araus and Hogan (1994).

The approach described here combines the variation in PFD and R:FR in a factorial experimental design with simultaneous leaf measurements of (1) photosynthesis and transpiration; (2) quantitative anatomy allowing estimates of A_{mes}/A_{surf}; (3) pigment composition and leaf absorption permitting estimates of attenuance/absorbance; and (4) stomatal density and other anatomical differences.

Plants studied—We examined seedling leaves of two species of *Hopea*, *H. helferi* (Dyer) Brandis and *H. odorata* Roxb., of the family Dipterocarpaceae. These are closely related taxa, both placed within subsection *Hopea* by Ashton (1982), and extremely similar based on restriction fragment length polymorphism analysis of chloroplast genes (Tsumura et al., 1996). They have the same geographical distributions, Indo-China south to the northern Malayan Peninsula, but different site preferences (Lee et al., 1997). *Hopea helferi* grows on exposed slopes in evergreen and semideciduous forests, and *H. odorata* grows in more protected river margins.

The purpose of this research was to answer three interrelated questions. First, how do variations in light intensity (PFD) and spectral quality (R:FR) affect the development of leaf anatomical characters? Second, how might these anatomical differences explain the differences in photosynthesis and growth among treatments within each species and between the two taxa? Third, how do the developmental responses in leaf anatomy help explain the differences in the physiology and the ecological distributions of *Hopea helferi* and *H. odorata*?

MATERIALS AND METHODS

Seeds for germination and growth trials were obtained from tree populations of *H. helferi* and *H. odorata* in the arboretum at the Forest Research Institute of Malaysia (FRIM), near Kuala Lumpur. Seedlings were grown in a series of replicated shade environments: (1) 40% solar PFD and 1.25 R:FR, HRR; (2) 12% PFD and 1.25 R:FR, MRR; (3) 12% PFD and 0.25 R:FR, MFR; (4) 3% PFD and 1.25 R:FR, LRR; and (5) 3% PFD and 0.25 R:FR, LFR. We also grew seedlings in direct sunlight at an adjacent site (SRR). The potted seedlings were placed randomly on a 9×9 grid within the shadehouses, 0.4 m apart. These methods and conditions were described in detail by Lee et al. (1996, 1997).

We selected tissue from the most recently matured leaf on each plant for all analyses, the same leaf and area used for gas exchange measurements (Lee et al., 1997). We chose areas midway between the blade margin and midrib, keeping them on moist paper toweling in low light and processing them in the laboratory within 2 h.

We measured diffuse reflectance, diffuse transmittance, and then calculated absorption, with the integrating sphere attachment to a Li-1800 spectroradiometer (LI-COR Inc., Lincoln, Nebraska; Lee and Graham,

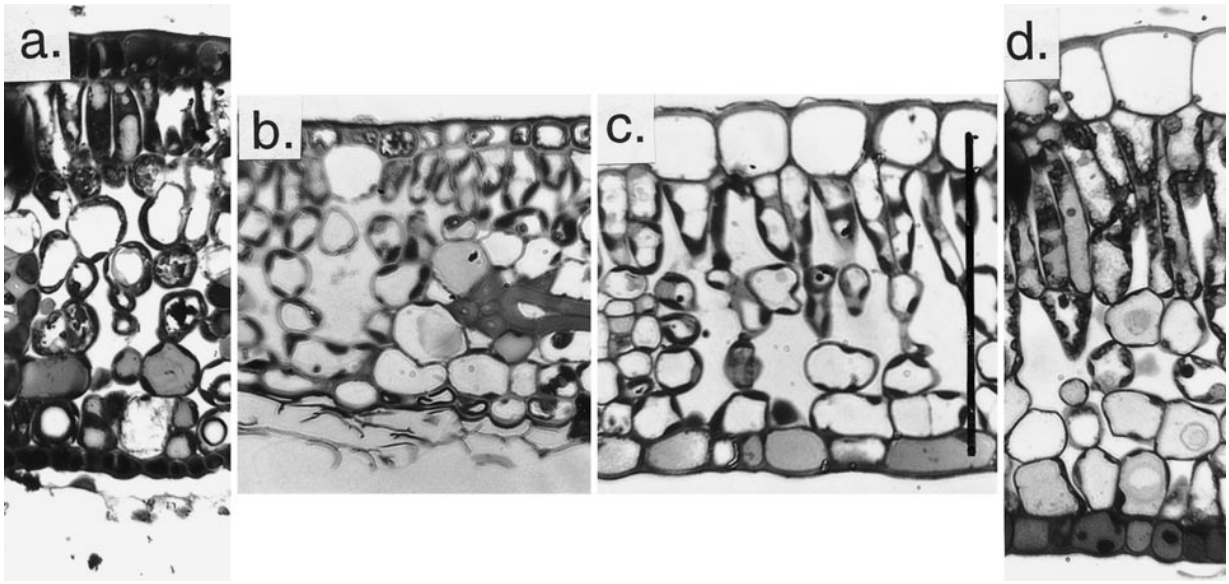

Fig. 1. Leaf transverse sections of *Hopea* seedling treatments. Treatment abbreviations are described in Materials and Methods and Fig. 2. (a) SRR, *H. helferi.* (b) LFR, *H. helferi.* (c) LFR, *H. odorata.* (d) SRR, *H. odorata.* Vertical bar = 100 μm.

1987). We used a solar spectrum at FRIM as the reference for calculations.

We measured chlorophyll *a* and *b* concentrations from 1 cm diameter samples obtained with a cork borer, with the n,n-dimethyl formamide technique of Moran (1982). Attenuance/absorbance ratios (att/abs) were calculated using log-transformed absorption values at 550, 652, and 800 nm (Lee et al., 1990). The extinction coefficient of $\epsilon = 34.5$ L/mg at 652 nm, equal for chlorophylls *a* and *b,* was obtained from Arnon (1949), and ϵ calculated from an 80% acetone leaf extract as a ratio at 550 nm, of 4.82 L/mg, using a Shimadzu spectrophotometer (Shimadzu Instruments, Tokyo, Japan).

For the anatomical analysis 1-mm^2 samples were fixed in 0.50 Karnovsky's fixative (Karnovsky, 1965) for 24 h at 25°C and transferred to 70% aqueous ethanol. Specimens were then dehydrated in an ethanol series and embedded in Spurr resin (Ladd, Burlington, Vermont, USA). Sections were cut at 1.5 μm with a Reichert-Jung Supercut 2050 Microtome (Reichert-Jung, Heidelberg, Germany) with diamond histoknife, stained with 1% aqueous toluidine blue, and mounted on glass slides. An image analysis system (Agvision, Decagon Instruments, Pullman, Washington, USA) attached to a standard light microscope facilitated quantitative anatomical measurements. Magnification at 400× produced a field with sections 200 μm across. We measured (1) leaf section area; (2) mesophyll area; (3) lengths and widths of the three largest palisade cells; (4) total mesophyll air space and perimeter; and (5) distance of upper epidermis across the section. We then calculated the mean leaf and mesophyll thicknesses. Palisade cell volumes were estimated by assuming a cylindrical shape for these cells, with hemispherical ends. Epidermal cell volumes were estimated from their thickness and the numbers of cells observed per field. We used stereological techniques to estimate areas of mesophyll-airspace contacts in relation to adaxial leaf surface (A_{mes}/A_{surf}) and mesophyll air space volume in cubic millimeters per cubic centimeter of leaf surface (Parkhurst, 1982, and personal communication; Buisson and Lee, 1993). Given the deviation of cell shapes from uniformity, we used a shape factor of 1.20 for calculations (Thain, 1983).

We randomly located replicates of each treatment on the roofs of two adjacent buildings, and we analyzed results as a stratified random block design using the General Linear Models Procedure of ANOVA (SAS, 1985). We used individual pots, randomly located within shadehouses, as the statistical unit. Initial data were checked for normality and rank transformed when necessary. We used the Fisher's least significant difference test, with a significance threshold of $P < 0.05$, for post hoc comparisons. Relative influences of PFD and R:FR on the development of different traits were estimated from the two-way ANOVA of rank-transformed data from the factorial design of the low- and medium-irradiance treatments (LRR, LFR, MRR, and MFR). These influences were visualized by calculating their coefficients of determination (Sokal and Rohlf, 1981), dividing sums of squares from the two-way ANOVA by the total sums of squares. We also calculated Pearson product correlations among many characters, again using rank-transformed data.

RESULTS

Treatments significantly affected leaf morphology, anatomy (Fig. 1), pigment composition, and optical properties. The primary effects were attributable to intensity (PFD), although spectral quality (R:FR) influenced some characters (Table 1).

Leaf anatomy—Seedlings developed thicker leaves under higher PFD, and R:FR did not affect this character (Figs. 1, 2). Thickness was correlated with greater leaf mass/area (Lee et al., 1997) and palisade length (Table 2). Although leaves of *H. odorata* were only slightly thicker than *H. helferi*, the contributions of tissue layers varied; palisade cells were longer in the former and the spongy mesophyll layer was thicker in the latter. The distribution of cell types within the mesophyll layer did not differ much among light treatments within each species (Fig. 3), but *H. helferi* produced significantly more lignified tissue than *H. odorata*. Volumes of palisade and upper epidermal cells were increased by higher PFD, and were significantly greater in *H. odorata* (Table 3). Palisade cell shape, particularly maximum width, was influenced by light conditions. Low R:FR moderately decreased palisade cell width in both species. Leaf thickness also increased with the amount of mesophyll air volume per leaf area (V_{mes}/A_{surf}; Tables 2, 3). These spaces were also strongly associated with increased mesophyll-air

TABLE 1. Coefficients of determination of anatomical, optical and physiological leaf characters of *Hopea helferi* and *H. odorata* seedlings. Total plasticity is seen in the addition of effects of intensity (PFD), spectral quality (R:FR) and interactions. Asterisks indicate the significance of treatment differences between the two species by three-way ANOVA and for treatments by two-way ANOVA: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns = not significant.

Leaf characters	PFD	R:FR	Interactions
Hopea helferi			
Leaf thickness	0.267***	0.019ns	0.002ns
Palisade length	0.227***	0.039ns	0.002ns
Palisade width	0.007ns	0.038ns	0.071*
% lignified tissue	0.089*	0.068*	0.000ns
A_{mes}/A_{surf}	0.225***	0.027ns	0.000ns
Mesophyll air volume	0.037ns	0.034ns	0.000ns
% absorption	0.108**	0.028ns	0.001ns
Att/abs_{550}	0.127**	0.000ns	0.017ns
Att/abs_{652}	0.106*	0.006ns	0.011ns
Chlorophyll/cm^2	0.204***	0.016ns	0.000ns
Chlorophyll *a*/*b*	0.000ns	0.012ns	0.007ns
Mean ± 1 SE	0.127 ± 0.028	0.026 ± 0.006	
Hopea odorata			
Leaf thickness	0.293***	0.070**	0.003ns
Palisade length	0.334***	0.001ns	0.010ns
Palisade width	0.420***	0.000ns	0.013ns
% lignified tissue	0.075*	0.003ns	0.020ns
A_{mes}/A_{surf}	0.187***	0.014ns	0.000ns
Mesophyll air volume	0.041ns	0.001ns	0.000ns
% absorption	90.492***	0.047*	0.059**
Att/abs_{550}	0.164***	0.000ns	0.000ns
Att/abs_{652}	0.392***	0.039*	0.059*
Chlorophyll/cm^2	0.563***	0.063**	0.059**
Chlorophyll *a*/*b*	0.240***	0.174***	0.038*
Mean ± 1 SE	0.270 ± 0.054	0.039 ± 0.016	

space contact surface (A_{mes}/A_{surf}) in both species, although the treatment effects at low and medium PFD were not large.

Pigment content—Chlorophyll concentrations were comparable in both species and were reduced from growth at higher PFD (Table 4). Chlorophyll *a*/*b* ratios were generally reduced from growth at higher PFD. The low chlorophyll *a*/*b* for *H. odorata* in the MRR treatment was associated with the low maximum photosynthesis (A_{max}) previously described for these plants (Lee et al., 1997).

Leaf optics—Leaf absorptances were comparable between the two taxa, but greater in *H. helferi* from plants grown at higher PFD (Table 2). Most treatment effects were due to PFD (Table 4), and *H. odorata* was more variable for this character. Attenuance/absorbance (att/abs) generally increased at 652 nm, indicating a reduction in the sieving effect, and generally increased at 550 nm, indicating an increase due to light scattering (Table 4). Again, these ratios were only substantially affected by PFD, particularly at the highest irradiances. Att/abs were greater in *H. helferi* for low-light treatments at 652 nm, and for all treatments at 550 nm, indicating generally reduced sieve and higher scatter effects in the leaves of this species.

Interactions between PFD and R:FR were generally of little importance in explaining variation in leaf characters; coefficients of determination for the interactions were small and not significant (Table 1). The most likely contribution to interaction effects would be the effect of spectrally altered light reflected from adjacent plants in the LRR and MRR treatments (Ballaré et al., 1993; Lee et al., 1997). Reflected light is not a significant developmental factor under these experimental conditions, particularly since reduced R:FR generally did not affect the development of leaf characters.

DISCUSSION

In general, these shade treatments influenced leaf anatomical characters much less than they did variations in growth, allocation, and seedling architecture (Lee et al., 1997). Also, variations of characters within species were smaller than those between the two taxa. These results are helpful in answering questions raised in the introduction.

PFD vs. R:FR—Spectral quality had negligible importance in the development of leaf anatomical characters. Reduced R:FR did not affect the contribution of tissue layers to total leaf thickness (Fig. 2), nor tissue composition in the mesophyll (Fig. 3). These results are inconsistent with the small literature reporting significant R:FR effects on palisade cell shape, contributing to thinner leaves, as in tropical vines (Lee, 1988) and papaya (Buisson and Lee, 1993). However, the life history characteristics of the latter plants predict greater plasticity of light responses because of the heterogeneity of habitats encountered by the vines and the early-successional niche of papaya (Paz and Vázquez-Yanes, 1998). Most of the coefficients of determination for the R:FR effects of both species were very small, although characters from *H. odorata* varied more than *H. helferi* (Table 1).

Determinants of leaf function—Although not proof of any causal relationship, correlations among characters suggest the possibility of functional relationships between them, and lack of significant correlations would suggest that such relationships are extremely unlikely. Many of the leaf characters were significantly correlated with each other in a complex manner (Table 2). Some of the correlations make sense in light of the measurements. For instance, leaf thickness (LFT), mesophyll air volume (MAV), and mesophyll/leaf surface area (A_{mes}/A_{surf}, AMA) were positively correlated. As leaf thickness increased, influenced by PFD, the other two characters were also bound to increase. Leaf absorption should be heavily dependent on chlorophyll concentration, and the two factors were highly correlated in both species.

Other correlations may be due to parallel, but functionally unrelated, effects of the light treatments. Total plant growth rate for both species (Lee et al., 1997) was strongly correlated with many leaf characters (Table 3). These correlations are primarily the consequence of (1) the increase in leaf thickness and (2) the reduction in chlorophyll concentration with intensity of light treatments.

The classical pattern of response and adaptation to higher light intensity is a thicker leaf with longer palisade cells (Björkman, 1981). Strauss-Debenedetti and Berlyn (1994) measured such changes among taxa of different

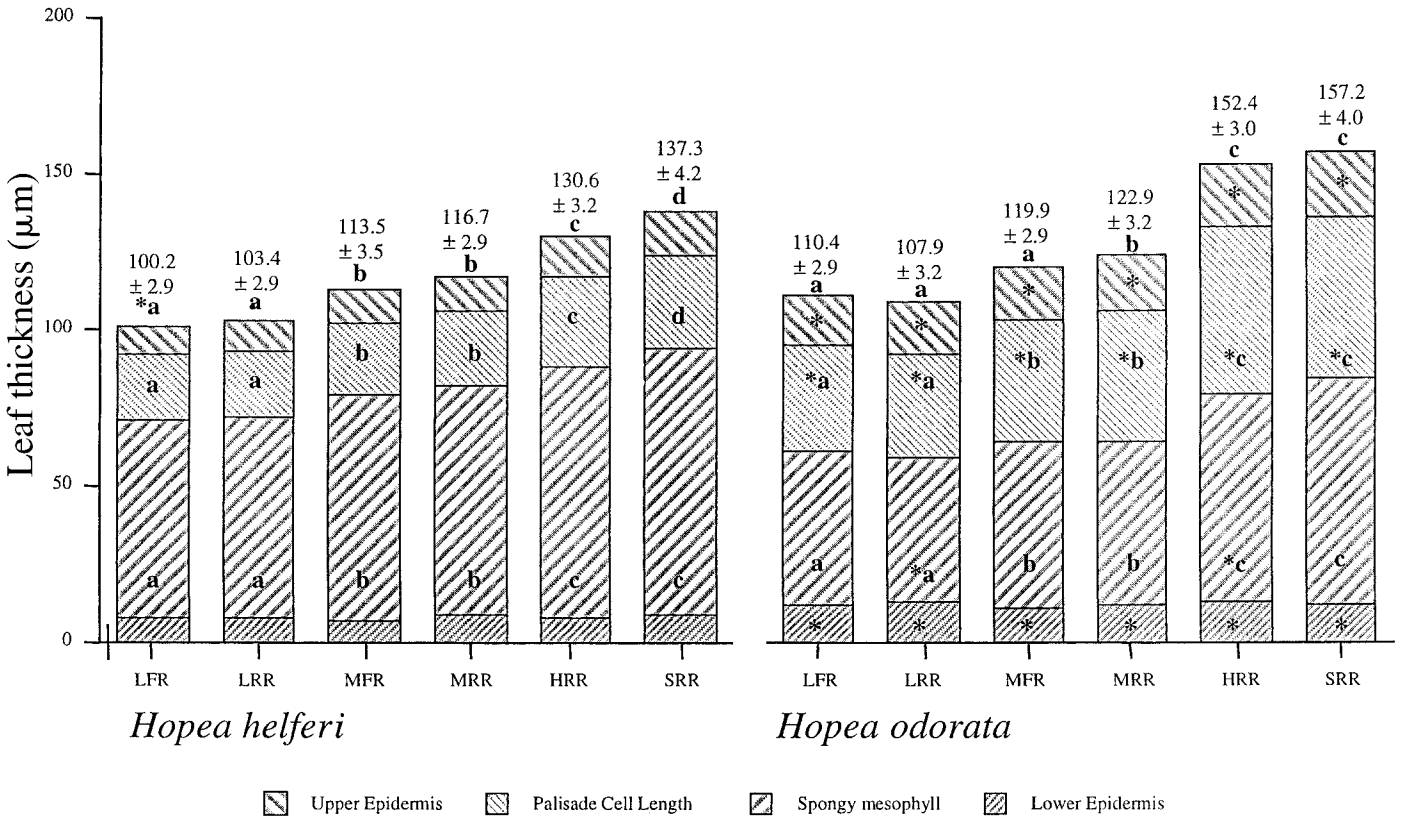

Fig. 2. Leaf thicknesses, including tissue layers, among shade treatments of *Hopea helferi* and *H. odorata*. Top values are the mean leaf thicknesses ± 1 SE. Shared letters indicate layers are not statistically different. Asterisks (*) in *H. odorata* indicate that treatments between the two taxa were statistically different. Treatment abbreviations: (1) SRR = 100% solar PFD and 1.25 R:FR; (2) HRR = 40% solar PFD and 1.25 R:FR; (3) MRR = 12% PFD and 1.25 R:FR; (4) MFR = 12% PFD and 0.25 R:FR; (5) LRR = 3% PFD and 1.25 R:FR; and (6) LFR = 3% PFD and 0.25 R:FR.

TABLE 2. Ranked correlation matrix of significant shade responses. Responses are defined more fully in Tables 1 and 2, and in Materials and Methods. Values in **boldface** are statistically significant, * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.005$, ns = not significant.

Variable (symbol)	MDA	MMO	PHO	%AB	M/S	MTH	CHL	A/A	LFA
Hopea helferi									
Mass/day (MDA)	—	0.199 ns	0.100 ns	**−0.625** ***	**0.445** ***	**0.581** ***	**−0.656** ***	**0.477** ***	**0.836** ***
Mass/mol (MMO)	0.159 ns	—	−0.170 ns	0.054 ns	0.096 ns	−0.060 ns	**0.202** *	−0.118 ns	**0.598** ***
Photosynthesis (PHO)	**0.346** **	**−0.487** ***	—	−0.106 ns	**−0.216** *	0.138 ns	**−0.202** *	−0.076 ns	0.118 ns
% absorption (%AB)	**−0.651** ***	**0.367** ***	**0.205** *	—	**−0.216** *	**−0.287** **	**0.818** ***	**−0.588** ***	**0.383** ***
A_{mes}/A_{surf} (M/S)	**0.436** ***	**−0.342** ***	0.009 ns	0.131 ns	—	**0.573** ***	**−0.295** **	**0.237** *	**0.382** ***
Mesophyll (MTH)	**0.609** ***	0.159 ns	0.051 ns	**−0.695** ***	**0.722** ***	—	**−0.447** ***	**0.275** *	**0.434** ***
Chlorophyll/cm^2 (CHL)	**−0.692** ***	**0.343** ***	0.164 ns	**0.914** ***	**−0.498** ***	**−0.734** ***	—	**−0.884** ***	**−0.365** ***
Att/abs$_{652}$ (A/A)	**0.585** ***	−0.208 ns	**−0.183** ns	**−0.725** ***	**0.396** ***	**0.577** ***	**−0.878** ***	—	0.179 ns
Leaf area (LFA)	**0.589** ***	**0.761** ***	**−0.574** ***	−0.018 ns	0.032 ns	−0.062 ns	−0.070 ns	0.169 ns	—
Hopea odorata									

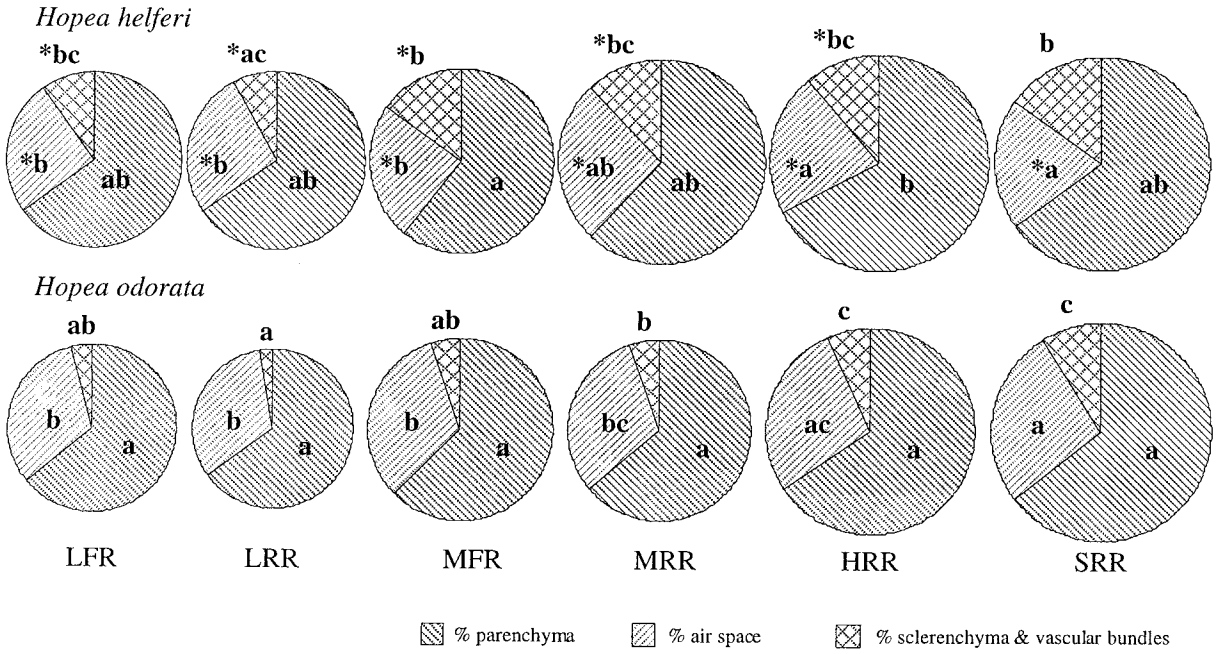

Fig. 3. Differences in cell composition of leaf mesophyll tissue among shade treatments and between the two taxa. Asterisks (*) above *Hopea helferi* indicate that comparable treatments between the two taxa were statistically different. Shared letters indicate that cell/air space composition was not significantly different between treatments. The size of each pie reflects the thickness of the mesophyll layer, and treatment abbreviations are described in Fig. 2.

successional status in the Moraceae. There was no clear relationship between successional status and photosynthetic plasticity, and leaf anatomy did not vary with physiological traits in a clear manner. Chazdon and Kaufman (1993) found a correlation between mesophyll thickness and photosynthesis in a gap species of *Piper*. Ashton and Berlyn (1992) observed a complex pattern of anatomical and physiological relationships in four closely related *Shorea* species (Dipterocarpaceae) in Sri Lanka. Leaf structure, function, and ecology were generally correlated in individual taxa.

Photosynthesis (A_{max}; Lee et al., 1997) was poorly correlated with leaf characters in both *Hopea* species. The strongest correlation was with conductance, which was measured at the same instance in each leaf. The lack of significant correlation of photosynthesis with A_{mes}/A_{surf} and mesophyll air volume in both species suggests that variations in these characters do not significantly alter CO_2 uptake in these species. A_{mes}/A_{surf} was correlated with A_{max} in *Plectranthus parviflorus*, and in several other high-light species (Nobel, Zaragoza and Smith, 1975; Nobel, 1977; Longstreth, Hartsock, and Nobel, 1980). However, Araus et al. (1986) did not observe such differences in more shade-tolerant species, and mesophyll conductance and leaf thickness were negatively correlated in several taxa (Syvertson et al., 1995). Theoretical

TABLE 3. Effects of light treatments on leaf anatomical measurements and calculations in the two *Hopea* species. Symbols for the light treatments are given in the Materials and Methods section. Shared uppercase letters indicate that the differences are not statistically significant. Asterisks indicate that differences between corresponding shade treatments between the two taxa are statistically significant. Treatment abbreviations are described in Fig. 2.

Species treatment	Palisade width (μm)	Air surface A_{mes}/A_{surf}	Air volume (mm^3/cm^2)	Cell volumes (μm^3)	
				Upper epidermis	Palisade cell
Hopea helferi					
LFR	9.5 ± 0.2*A	11.3 ± 0.5*A	2.13 ± 0.12*A	840 ± 166*ADE	1304 ± 160*A
LRR	9.5 ± 0.2*A	12.0 ± 0.5*A	2.28 ± 0.12AC	922 ± 157*A	1336 ± 160*A
MFR	9.1 ± 0.3*A	13.0 ± 0.5*BD	2.31 ± 0.15*AC	1054 ± 183*BD	1331 ± 194*A
MRR	10.2 ± 0.2*BC	13.6 ± 0.5*C	2.53 ± 0.12*BC	1024 ± 161*CD	1761 ± 160*B
HRR	10.4 ± 0.2*B	14.2 ± 0.5*C	2.50 ± 0.14*B	1154 ± 165*D	2191 ± 175*C
SRR	9.4 ± 0.3*AC	12.6 ± 0.7*AD	2.33 ± 0.18*AB	1129 ± 242*A	1887 ± 233*BC
Hopea odorata					
LFR	10.8 ± 0.2B	13.9 ± 0.5AD	2.59 ± 0.12AD	4524 ± 157AD	2801 ± 160A
LRR	11.2 ± 0.2B	13.4 ± 0.5A	2.57 ± 0.13A	4843 ± 177A	3002 ± 175A
MFR	10.2 ± 0.2A	15.8 ± 0.5BE	2.96 ± 0.12BCD	4192 ± 157D	2903 ± 160A
MRR	10.9 ± 0.2B	15.0 ± 0.5BD	2.84 ± 0.13ABE	4867 ± 171A	3586 ± 170B
HRR	11.1 ± 0.2B	17.6 ± 0.5C	3.31 ± 0.13C	5080 ± 157AC	4894 ± 165C
SRR	11.3 ± 0.3B	17.8 ± 0.6CE	3.29 ± 0.17CE	6118 ± 216C	4984 ± 221C

TABLE 4. Influence of light treatments on leaf optical properties and chlorophyll content and composition. Shared uppercase letters indicate that the differences are not statistically significant. Asterisks indicate that differences between corresponding shade treatments between the two taxa are statistically significant. Treatment abbreviations are described in Fig. 2.

Species treatment	Absorptance (%)	Attenuance/ absorbance$_{652\ nm}$	Attenuance/ absorbance$_{550\ nm}$	Chlorophyll	
				(mg/cm^2)	*a*/*b*
Hopea helferi					
LFR	0.882 ± 0.006D	0.82 ± 0.04*A	2.96 ± 0.13*AD	41.17 ± 1.28B	2.60 ± 0.08*C
LRR	0.868 ± 0.006DE	0.81 ± 0.04*A	2.86 ± 0.12*A	39.94 ± 1.21*B	2.47 ± 0.08*CD
MFR	0.858 ± 0.007CBE	0.88 ± 0.04AC	3.25 ± 0.14*BD	35.59 ± 1.36B	2.55 ± 0.08*CD
MRR	0.853 ± 0.006*CBE	0.98 ± 0.04BC	3.53 ± 0.13*B	30.98 ± 1.25AB	2.51 ± 0.08*BD
HRR	0.816 ± 0.006*A	1.02 ± 0.04B	3.62 ± 0.13*B	22.53 ± 1.25A	2.22 ± 0.08*A
SRR	0.843 ± 0.009*B	1.13 ± 0.05B	4.30 ± 0.17*C	19.65 ± 1.81A	3.16 ± 0.10*C
Hopea odorata					
LFR	0.877 ± 0.007D	0.72 ± 0.04A	2.66 ± 0.13AE	44.53 ± 0.87C	2.16 ± 0.08C
LRR	0.879 ± 0.007D	0.71 ± 0.04A	2.66 ± 0.14A	45.33 ± 0.94C	2.06 ± 0.08C
MFR	0.854 ± 0.006C	0.80 ± 0.04B	2.84 ± 0.13BEF	36.43 ± 0.92B	2.02 ± 0.08C
MRR	0.797 ± 0.007B	0.89 ± 0.04C	2.81 ± 0.14C	27.64 ± 0.97A	1.56 ± 0.09B
HRR	0.774 ± 0.007A	0.94 ± 0.04C	3.12 ± 0.14CDF	23.29 ± 0.95A	1.31 ± 0.08A
SRR	0.787 ± 0.009AB	0.93 ± 0.06C	3.00 ± 0.20D	23.52 ± 1.27A	1.98 ± 0.11C

calculations by Parkhurst (1994) suggest that mesophyll limitations on gas diffusion for leaves may be only a few percent, much less important than stomatal conductance (Wong, Cowan, and Farquhar, 1979; von Cammerer and Farquhar, 1981; Sharkey, 1985). However, the results of Niinemets, Kull, and Tenhunen (1998) on a suite of temperate woody species suggested that anatomical/morphological leaf traits were more important than biochemical ones in explaining differences in photosynthetic yield. Clearly, we need more research in this area (Smith et al., 1997).

Optical properties were also not correlated with photosynthesis. The variables affecting att/abs$_{652}$ include chloroplast distribution, mesophyll cell size and shape, and pigment concentration. Positive correlations with leaf thickness and associated characters indicate that the more elaborate mesophyll structure in thicker leaves reduces sieving effects. However, anatomical influences are weak compared to the strong negative correlation with chlorophyll concentrations in both species. Att/abs$_{550}$ was significantly correlated with leaf thickness and internal anatomical characters, suggesting their contribution to path-lengthening effects. However, the strong negative correlation with chlorophyll content is similar to the ratio at 652 nm. Given the weak correlation of these characters with maximum photosynthesis (Table 2), it is more likely that the correlations are the consequences of variation of other traits, particularly leaf thickness and chlorophyll concentration, than directly due to light treatments.

Gas exchange was weakly correlated with growth and developmental characters. Stomatal conductance (Lee et al., 1997) was weakly correlated with stomatal density in *H. helferi,* and stomatal density with photosynthesis for *H. odorata,* partly due to the large variances in the conductance data. Maximum photosynthesis was positively correlated with seedling growth rates (mass/day), but only significantly so for *H. odorata,* with its greater responses. Leaf characters were more significantly correlated among leaf characters for *H. odorata* than for *H. helferi,* consistent with the greater response of the former leaf characters to the shade treatments (Tables 1, 2). Photosynthesis was negatively correlated with growth efficiency (mass per mole, MMO) because of the reduced growth efficiency at the highest light treatments (Lee et al., 1997). Photosynthesis was correlated with growth per day in *H. odorata,* but not significantly so in *H. helferi*. Photosynthesis measurements, no matter how carefully measured, are instantaneous, and may not correlate as well as other characters, as the more lengthy measurements of growth.

Species differences—Leaf morphology and anatomy between the two species differ in several important ways that help explain their functional ecology. First, *H. odorata* produced larger palisade cells in all treatments. Cell size and intercellular spaces contributed to the greater A_{mes}/A_{surf} of this species. Smaller leaf cells (both palisade and upper epidermal cells documented for *H. helferi*) have been associated with drought tolerance in many plants (Larcher, 1995).

Second, leaf stomatal density was greater in *H. odorata,* except at the lowest light treatments. Stomatal aperture lengths were also greater in *H. odorata* [15.5 ± 0.5 nm (N = 10) for all treatments compared to 12.4 ± 0.4 nm (N = 10) for *H. helferi*], with no differences between treatments. This contributed to the greater stomatal conductances measured in *H. odorata* in all but the MRR treatment (Lee et al., 1997), and should promote higher rates of carbon assimilation. Evidence for the ecological advantages of these anatomical differences would be their correlations with water use efficiency (WUE). Unfortunately, the larger variances of transpiration measurements made those of the ratios even larger, and none of the treatments were significantly different. The WUE of *H. odorata* ($3.84 \pm 1.03 \times 10^{-3}$) was not significantly less than that of *H. helferi* ($5.10 \pm 1.03 \times 10^{-3}$) seedlings grown at 40% of sunlight. This variance also decreased the likelihood of any significant correlations.

Third, treatments of *H. helferi* had similar or greater PFD absorption capacity despite slightly reduced chlorophyll concentrations. Although lower in *H. odorata,* chlorophyll *a*/*b* ratios varied little within species except at very high light levels, indicating no significant changes in photosystem stoichiometry due to the shade treatments (Chow, Melis, and Anderson, 1990). Sieving effects (mirrored by greater attenuance/absorbance$_{652\ nm}$) were larger

in *H. odorata,* and path-lengthening effects were smaller (smaller attenuance/absorbance$_{550\ nm}$). A single factor that may contribute to these optical characters is the leaf undersurface scales in *H. helferi,* which would backscatter light back into the mesophyll (Eller and Willi, 1981). Such backscatter would increase total absorptance, reduce sieving effects, and increase path-lengthening effects.

Fourth, leaves of *H. helferi* produced more lignified cells (sclerenchyma and vasculature) in the mesophyll—generally vertically all across this tissue layer. Finally, leaves of *H. helferi* produce undersurface scales (Fig. 1; Ashton, 1982). Such scales should reduce stomatal conductance, A_{max}, and presumably limit growth rates as well. Both the lignification and scales help explain the greater mass per area of the leaves of *H. helferi*.

The greater plasticity of response of most leaf characters may provide *H. odorata* with a greater capacity of growth responses in different environments. These anatomical differences have clear implications for function and help explain the greater drought tolerance of *H. helferi.* They may also contribute to greater leaf toughness and durability, which may reduce rates of herbivory in these plants. Both produce terpenoid compounds that may be more important deterrents than these structures. All of these differences are remarkable for their physiological consequences in two species so closely related (Tsumura et al., 1996), suggesting that such characters, controlled by light conditions, evolved quite rapidly.

Conclusions—Seedling leaves of *Hopea helferi* and *H. odorata* developed differences in anatomy, optical properties, and physiology in the shade conditions of this research. Photon flux density was by far the most important variable in influencing leaf characters in both taxa, although spectral quality affected palisade cell widths in both taxa. Within each species, variation in characters of leaf anatomy and optics was not well correlated with physiological performance, suggesting that they are primarily the consequence of other characters affected by the light treatments. Increasing leaf thickness, affected by higher light exposure in both taxa, influenced internal mesophyll architecture. Yet, leaf thickness and toughness may be more important in increasing mechanical strength, reducing herbivory, and increasing drought tolerance. Reductions in chlorophyll concentrations per unit area influenced optical properties, but are most likely the consequence of photo-bleaching in the higher light treatments (HRR and SRR). Plant architecture and allocation (both influencing leaf display) appear more important in explaining growth rates within the two taxa than leaf anatomy and optics.

Seedling leaves of *Hopea helferi* and *H. odorata* differed in several important ways, and those of the latter were more plastic in response to the range of light conditions. This species grows primarily in dense forest along streams and appears more shade tolerant (Lee et al., 1997). Secondly, leaves of *H. helferi* produced a dense layer of undersurface scales in all treatments. Since other anatomical differences (including leaf thickness and stomatal density) were small between the two species, this layer of scales most likely contributes to the reduced stomatal conductance and reduced maximum photosynthesis rates. Despite the lack of correlation between maximum photosynthesis (A_{max}; Lee et al., 1997) and seedling growth rates within each species, the significantly reduced A_{max} and dry mass increments in *H. helferi* are most likely due to the presence of these scales. This species grows primarily on well-drained soils on slopes in evergreen forest. The significant advantage for this species is probably the greater economy of water use in an environment where seedlings are frequently subject to drought.

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