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SEEDLING SHADE RESPONSES AND FUNCTIONAL ECOLOGY OF *HOPEA HELFEREI* AND *H. ODORATA*

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ABSTRACT

Hopea helferei and *H. odorata* grow in evergreen and semi-deciduous forests of Indochina, as far south as northern Malaya. *H. odorata* grows in dense forest on stream margins, and *H. helferei* on well-drained slopes. We examined developmental responses of seedlings to irradiance (3%, 12%, 40% and 100% of photosynthetically active radiation, PAR) and spectral quality (R:FR, red:far-red quantum ratios of 0.25 and 1.25). Besides observing growth, allocation, architecture and gas exchange characteristics we examined differences in leaf structure in particular detail. Seedlings of *H. odorata* grew most rapidly under shade and were the most plastic in response to light treatments. Irradiance and spectral quality influenced characters differently in both species, although leaf anatomical variables responded less and were not affected by R:FR. However, leaf structure (affecting mesophyll surface area), along with undersurface scales in *H. helferei*, help explain the lower maximum photosynthesis and growth rates of this more drought-tolerant taxon. The differences in the light-influenced seedling traits between these very closely related taxa suggest that such characters have evolved rapidly and are not likely to be phylogenetically conserved.

Key-words: seedling; shade response; irradiance; spectral quality

INTRODUCTION

Viewing the canopy across the Pasoh Forest Reserve in West Malaysia one encounters a tree flora of about 300 species (out of almost 3000 species for the entire peninsula). Perhaps 150 of those taxa reach the canopy, of which 75 may be dipterocarps. The basic challenge in the ecology and evolution of tropical plants, particularly in the Dipterocarpaceae (Ashton 1988), is to understand how so many species occupy what appears to be such a climatically uniform environment. Our working hypothesis is that these species occupy different niches, most likely determined by physical environmental factors. Light has been held to be the most important factor, although soils and aspect have also been invoked to explain species distributions. Seedling development, leading to establishment, is apparently a crucial stage in the life history of such trees. Two general approaches have been used to explain the functional ecologies of different species. One is a careful examination of distribution and performance in relation to natural environments in the forest (Whitmore 1996), and a second approach, the one we have adopted, is to create conditions simulating natural environments using shade houses and potted seedlings grown under these experimental conditions. In this article, we review recently completed research on the shade responses of two *Hopea* species, *H. helferei* and *H. odorata*, to understand the physiological and morphological bases for the different functional ecologies of these two closely related taxa. The basic growth responses of these two taxa were summarized in the previous Round Table Proceedings (Lee *et al.* 1996b), and in two articles (Lee *et al.* 1997, Lee *et al.* 2000). Here we use selected data to develop a comprehensive understanding of the differences in seedling development of the two taxa.

PLANTS STUDIED

This research was performed on seedlings of two species of *Hopea*, *H. helferei* (Dyer) Brandis and *H. odorata* Roxb., of the Dipterocarpaceae. They have the same geographical distributions, in Indo-China south to the northern Malayan Peninsula (Ashton 1982), but different site preferences (Smitinand *et al.* 1980). *H. helferei* grows in deep soils on exposed slopes in evergreen and semi-deciduous forests, and *H. odorata* primarily grows in dense

forest on the damp soils of river margins. Although *H. helferei* appears to be more drought tolerant, *H. odorata* is grown in Malaysia as an avenue and roadside tree and therefore must be somewhat tolerant of occasional water stress.

SHADE AND GROWTH CONDITIONS

Shade light under the forest canopy varies in quantity and spectral quality, and both factors may affect plant development. Reduced photosynthetically active radiation (400-700 nm, PAR) affects plants and plant development in shade is also influenced by the altered spectral quality of light passing through the layers of foliage. This passage intercepts red wavelengths (around 660 nm) and allows more of the far-red wavelengths (around 730 nm) to penetrate (Smith 1994). These wavelengths are particularly important because they alter the equilibria of the red and far-red forms of phytochrome, which also influence developmental responses at all levels of organization. Almost all research has combined shade conditions (reduced PAR) with the spectral quality of full sunlight (high R:FR) by using shade fabrics, and this is bound to underestimate the extent of developmental responses to shade light (Schmitt & Wulff 1993). The challenge in this research has been to separate the effects of light quantity and quality in assessing such effects, and the variation in PFD and R:FR has been analysed using a factorial experimental design.

Growth conditions for these experiments have been described in detail in Lee *et al.* (1997). Seedlings were grown in a series of replicated shade environments: (1) 40% solar PAR and 1.25 R:FR, HRR; (2) 12% PAR and 1.25 R:FR, MRR; (3) 12% PAR and 0.25 R:FR, MFR; (4) 3% PAR and 1.25 R:FR, LRR; and, (5) 3% PAR and 0.25 R:FR, LFR. Seedlings were also grown in direct sunlight at an adjacent site (SRR). The potted seedlings were placed randomly on a 9 by 9 grid within the shade houses, 0.4 m apart. The data were analysed using statistical methods described by Lee *et al.* 1997 and Lee *et al.* 2000. In addition to the standard analyses using 1-way ANOVA, the factorial design of #2-5 above made analyses using 2-way ANOVA possible and the construction of coefficients of determination (ratios of the sums of squares of the treatment variables, PFD and R:FR, over the total sums of squares) to determine the plasticity of responses to these two variables.

SHADE RESPONSES

Seedlings of both taxa were affected by the shade conditions. Growth rates were reduced at the lowest PFD, and somewhat suppressed in full sunlight (Table 1). Seedlings of *H. odorata* grew more rapidly under all conditions (Lee *et al.* 1997). Growth efficiency, accounting for the increment of dry mass per photons received, was also higher in *H. odorata* in all treatments (Table 1). Growth was reduced in conditions of low R:FR, by 56% in *H. helferei* and 12% in *H. odorata*. These treatment effects were significant in both cases (Table 3, by two way ANOVA). Differences in growth rates are partially explained by maximum photosynthesis rates; those of *H. odorata* were much higher except for the MRR treatment (Table 1). However, the reduced R:FR treatments did not affect rates of photosynthesis. These rates varied little in the factorial treatments of 3-12% full sunlight, but increased in 40% shade and full sunlight.

Reductions in growth rates under low R:FR conditions can be explained by the influence of spectral quality on the seedling architecture of both species. Low R:FR decreased allocation to leaves, branch production, and leaf area in both species (Table 1); a trend that occurred in all taxa in the broader investigation (Lee *et al.* 1996a). Thus, attempts to estimate growth rates of seedlings by using spectrally neutral shade fabrics will underestimate the influence of natural shade light, confirming the predictions of Schmitt and Wulff (1993).

Differences in maximum photosynthesis between the two taxa, and in different shade conditions, suggest that alterations in leaf structure could limit rates of gas exchange and carbon assimilation. We examined leaf structure in considerable detail (Lee *et al.* 2000, some

data in Table 2), yet these characters responded less to the treatment conditions than estimates of growth and seedling architecture, reflected in the reduced coefficients of determination for these characters (Table 3). Although leaf characters did not correlate with photosynthesis in a meaningful manner within the species, differences in these characters may at least partially explain the differences in photosynthesis between the two taxa. Higher photosynthesis in *H. odorata* may be explained partially by a greater internal surface for gas exchange, but not so much by stomatal density (even though those of *H. odorata* were larger). Perhaps the most important factor is the presence of a layer of scales on the lower surface of leaves of *H. helferei*.

Table 1. Influence of light treatments on growth, photosynthesis and plant architecture of the two *Hopea* species. Shared upper case letters indicate that the differences are not statistically significant. Treatments are highlighted and explained in the text.

Species/ Treatment	Dry mass (g) /1000 mol	Photosynth's mol CO ² m ⁻¹ s ⁻¹	Branch/Trunk internodes	% Root mass	Leaf area (cm ²)/ Stem length (cm ²)
<i>Hopea helferei</i>					
LFR	7.7 + 0.8A	2.90 + 0.14A	2.02 + 0.33A	19.9 + 0.7A	5.85 + 0.34A
LRR	17.7 + 2.1B	2.96 + 0.15A	2.78 + 0.40A	19.0 + 0.9A	9.57 + 0.58B
MFR	11.2 + 2.0A	2.69 + 0.21A	3.28 + 0.51AB	29.9 + 1.1BD	6.46 + 0.46AC
MRR	13.0 + 1.1B	2.97 + 0.20A	5.44 + 1.00AB	31.1 + 1.3BD	7.70 + 0.41AB
HRR	6.6 + 1.0A	3.44 + 0.32A	5.97 + 1.00B	37.7 + 2.3C	8.26 + 0.50BC
SRR	2.1 + 0.6A	4.81 + 0.69B	- + -	38.3 + 1.8CD	6.69 + 1.75AB
<i>Hopea odorata</i>					
LFR	22.8 + 1.8A	4.90 + 0.18 BC	3.12 + 0.14AB	20.2 + 0.9A	5.21 + 0.11A
LRR	25.8 + 3.4A	4.54 + 0.19B	4.01 + 0.34AB	18.5 + 0.6A	6.46 + 0.19BC
MFR	27.7 + 2.1A	4.34 + 0.28B	4.18 + 0.17B	29.3 + 1.5B	5.08 + 0.19AD
MRR	30.1 + 1.8A	2.70 + 0.20A	6.35 + 0.39C	37.8 + 2.3C	6.21 + 0.29BCD
HRR	7.6 + 0.7B	4.83 + 0.25BC	4.18 + 0.36B	48.8 + 1.5D	4.17 + 0.34A
SRR	2.7 + 0.6B	6.01 + 0.62C	2.88 + 0.42A	53.9 + 4.0D	4.28 + 0.97A

Table 2. Effects of light treatments on leaf characters of the two *Hopea* species. Symbols for the light treatments are given in the materials and methods section. Shared upper case letters indicate that the differences are not statistically significant. Treatments are highlighted and explained in the text.

Species/ Treatment	Leaf Thickness (M)	Air Surface A _{mes} /A _{surf}	Stomatal Density x 10 ³ cm ⁻²	% Lignified tissue	% Leaf allocation
<i>Hopea helferei</i>					
LFR	100.2 + 2.9A	11.3 + 0.5A	1.39 + 0.05A	8.1 + 1.2BC	45.9 + 0.9B
LRR	103.4 + 2.9A	12.0 + 0.5A	1.32 + 0.05A	10.0 + 1.3AC	49.1 + 0.7B
MFR	113.5 + 3.5B	13.0 + 0.5BD	1.48 + 0.10A	12.4 + 1.2BC	31.4 + 2.0AB
MRR	116.7 + 2.9B	13.6 + 0.5C	1.60 + 0.08A	15.4 + 1.4B	27.1 + 1.4AB
HRR	130.6 + 3.2C	14.2 + 0.5C	2.09 + 0.09B	11.4 + 1.3BC	21.2 + 1.4A
SRR	137.3 + 4.2D	12.6 + 0.7AD	2.40 + 0.08B	15.5 + 1.9B	20.9 + 1.5A
<i>Hopea odorata</i>					
LFR	110.4 + 2.9A	13.9 + 0.5AD	1.21 + 0.03A	2.9 + 1.4A	46.8 + 0.7C
LRR	107.9 + 3.2A	13.4 + 0.5A	1.16 + 0.03A	4.2 + 1.2AB	52.6 + 0.6D
MFR	119.9 + 2.9A	15.8 + 0.5BE	1.73 + 0.06B	5.7 + 1.3AB	30.4 + 1.1B
MRR	122.9 + 3.1B	15.0 + 0.5BD	1.67 + 0.05B	5.4 + 1.2AB	30.1 + 1.2B
HRR	152.4 + 3.0C	17.6 + 0.5C	2.69 + 0.06C	7.0 + 1.2C	18.6 + 1.1A
SRR	157.0 + 4.0C	17.8 + 0.6CE	2.49 + 0.13C	9.1 + 1.7C	17.8 + 2.4C

SPECIES DIFFERENCES

The shade responses and growth dynamics of these two species are consistent with what we know of their distributional ecology. Although seedlings of both taxa grew in shade conditions approaching that of the understorey of tropical forests, *H. odorata* grew more rapidly, which is consistent with its greater tolerance to shade. Both taxa were capable of a range of responses to varying shade conditions, in allocation to plant organs and in branching and crown shape. Both have a seedling architecture described by the model of Roux (Halle 1979), in which plagiotropic lateral branches are produced at intervals on the orthotropic main axis. However, in *H. helferei* the first and subsequent lateral branches dominate, giving the seedling a much broader crown; in *H. odorata* the main axis produces more height increment and a narrower crown. The latter growth pattern would be better poised to compete for limited light conditions in the forest understorey.

The stems of the latter species are also much less robust [about half the stem mass/length (Lee *et al.* 1997)] and probably more susceptible to mechanical damage.

The important differences between the species may be related more to moisture than to light availability. The reduced growth rates of *H. helferei* are probably the result of modifications in leaf structure that reduce not only carbon dioxide assimilation but also moisture loss. The leaves are mechanically tougher with a greater proportion of lignified tissue in the mesophyll (Table 2), smaller stomata, smaller palisade and epidermal cells (Lee *et al.* 2000), and a dense scale layer on the lower surface. The drought tolerance of *H. odorata* may be mainly due to its ability to allocate more biomass to root production under higher insolation. It is also more plastic in its responses to shade conditions, as seen in the larger coefficients of determination (Table 3). Shadehouse experiments have the advantage of reducing the variables that influence seedling growth. Other factors not included in this investigation, such as drought tolerance or insect and pathogen resistance, may be more important than responses to shade conditions for these two taxa.

THE EVOLUTION OF SHADE RESPONSES IN HOPEA

These two species have distinctly different patterns of response to reduced PFD and altered R:FR. These differences are remarkable given their taxonomic relationship. The two taxa are very closely related and Ashton (1982) placed them both in the subsection *Hopea*. RFLP analysis of chloroplast genes of those and other taxa (Tsumura *et al.* 1996) give further evidence of their close relationship. Thus, these results are evidence that genes controlling responses to both light intensity and spectral quality are capable of very rapid evolutionary change, and not likely to be phylogenetically conserved. Comparisons of leaf structure and function between the two taxa help to explain their different functional ecologies (Smith *et al.* 1997) and suggest that differences in intrinsic growth rates among taxa may often be the result of leaf structural constraints on gas exchange (Lambers & Poorter 1992).

Table 3. Coefficients of determination of anatomical, optical and physiological leaf characters of *Hopea helferei* and *H. odorata* seedlings. Total plasticity is seen in the addition of effects of PFD, 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: * <0.05, ns = not significant.

	PFD	R:FR	Interactions
<i>Hopea helferei</i>			
Growth, mass/mol	0.143*	0.011ns	0.040*
Photosynthesis	0.001ns	0.003ns	0.001ns
Leaf area/stem length	0.186*	0.012ns	0.046*
Branch/trunk internodes	0.031*	0.051*	0.007
% Roots	0.257*	0.000ns	0.005ns
% Leaves	0.287*	0.001ns	0.010ns
Stomatal density	0.267*	0.019ns	0.002ns
Leaf Thickness	0.267*	0.019ns	0.002ns
% Lignified Tissue	0.089*	0.068*	0.000ns
Ames/Asurf	0.225*	0.027ns	0.000ns
Mean + Standard error	0.115 + 0.039	0.019 + 0.007	
<i>Hopea odorata</i>			
Growth, mass/mol	0.008ns	0.024*	0.000ns
Photosynthesis	0.131*	0.091*	0.037*
Leaf area/stem length	0.101*	0.003ns	0.002ns
Branch/trunk internodes	0.150*	0.174*	0.020*
% Roots	0.255*	0.006ns	0.029*
% Leaves	0.205*	0.001ns	0.003ns
Stomatal density	0.079*	0.001ns	0.000ns
Leaf Thickness	0.293*	0.070*	0.003ns
% Lignified Tissue	0.075*	0.003ns	0.020ns
Ames/Asurf	0.187*	0.011ns	0.000ns
Mean + Standard error	0.148 + 0.028	0.039 + 0.018	

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