

**THE GROWTH AND PHYSIOLOGY OF  
TROPICAL FOREST TREE SEEDLINGS  
IN RELATION TO LIGHT**

by

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for the degree of Doctor of Philosophy to the  
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**MEMORIAL**

**In memory of my parents,  
Fazal Karim and Sabura Karim**

**DEDICATION**

**To my brother Mohammed Bahadur and  
my wife Hoshne  
with  
recognition and love**

## **DECLARATION**

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was executed by myself, unless otherwise stated.

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## ABSTRACT

This work reports the results of experiments on the growth and physiology of tropical forest tree seedlings in relation to light. The experiments were carried out in a controlled environment simulating forest light conditions. The species studied were *Anthocephalus chinensis* (Lamk.) Rich. ex Walp., *Bischofia javanica* Blume, and *Hopea odorata* Roxb. from the moist forests of tropical Asia. The main aim of this study was to characterise the responses of seedlings in relation to (a) irradiance and nutrient supply, (b) shadelight quality, red to far-red (R:FR) ratio or low proportion of blue light, and (c) changing light availability.

The species displayed differential growth responses when their seedlings were grown at different levels of irradiance and nutrient supply. Growth of gap species *Bischofia* was more plastic than that of the climax species *Hopea*. The growth of the former was substantially restricted when the nutrient supply was low at the higher irradiances.

Pioneer species *Anthocephalus* responded to a low R:FR ratio by a large increase in stem extension growth with concomitant increase in allocation of dry matter to stem at the expense of leaf development. The effect of R:FR ratio on extension growth was independent of a low proportion of blue light. Very small amounts of blue light in the shadelight restricted leaf expansion in *Anthocephalus* and *Bischofia*, and increased specific stem length in the former. The climax species *Hopea* was relatively unresponsive to the R:FR ratios or the different proportions of blue light in the shadelight.

High irradiance brought about photoinhibition of photosynthesis in the fully developed low-light leaves of *Bischofia*. This photoinhibition was mostly caused by the inactivation of photosystem II, and followed by chlorophyll bleaching. Recovery of the photoinhibited leaves was substantial. This recovery was manifested by a range of changes, from the leaf movement to structural changes in the leaf and synthesis of chlorophylls.

Low irradiance resulted in a reorganisation of photosynthetic machinery in the fully developed high-light leaves of *Bischofia*. Consequently, there was a substantial decrease in chlorophyll a to b ratio, which was very close to the value for the low-

light control leaf. Light saturated photosynthetic capacity was decreased by about 40%.

On reciprocal transfer of *Bischofia* seedlings between the high and the low light regimes, relative growth rates were changed according to the availability of light through morphological and physiological adjustments. The transferred seedlings remained different from those maintained as controls because of the carry-over effects of the previous environment. Photosynthetic light response curves, leaf chlorophylls and leaf anatomy were examined to explore the causes of differential growth responses of these seedlings.

The results are discussed in the context of regeneration and management of tropical forests.

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# CHAPTER 1

## Introduction and Aims of the Study

### 1.1 Need for a scientific management of regeneration

Natural forests in the tropics have declined in extent steadily over several hundred years, and in recent years, the rate of disappearance of forests has increased sharply. Currently, this rate is in between  $7 \times 10^6$  and  $10 \times 10^6$  ha per year (review by Palo 1987; Whitehouse 1991). The main cause of this destruction is expanding population pressure and conversion of forest land to agriculture. The growing industrial demand has also greatly accelerated the rate of exploitation. Very little effort has been made to restock the felled areas. Some countries practice techniques of enrichment planting within natural forests, but these have had only a limited success. Clearly, the surviving natural forests will no way fill the wood requirements of the region alone. To furnish adequate timber, plantation forestry is needed too.

Plantation forestry in the tropics consists of monocultural plantation with a few species, the exotics being in higher proportions. Extensive areas have been planted with species whose silviculture and management are well understood. Currently, the planting effort has been increased (Sedjo 1987). By 1985, the estimated total area of plantations is about  $27.5 \times 10^6$  ha, which is only 3.1% of the total moist forests of the tropics (Fontaine 1986). These plantations would produce  $400 \times 10^6$  m<sup>3</sup> of wood per year assuming a conservative average yield of 15 m<sup>3</sup> per ha per year. This amount of wood equals only 10% of the world's estimated requirement in 1995 (Evans 1982). Though it is expected that to the year 2000, the plantation area will expand to about  $50 \times 10^6$  ha at the rate of more than  $1.5 \times 10^6$  ha per year (Evans 1982), it will not substantially relieve the pressure to exploit the dwindling reserves of natural forests because of less emphasis on planting of indigenous hardwoods. The main constraint acting against the planting of relatively slow-growing indigenous tree species has been the necessity for long-term investment. Moreover, autecological characteristics of many commercially valuable species are unknown.

It is expected that with the increase in plantation area, the pressure on the surviving natural forests will be lessened. But the large monocultural plantations themselves represent a substantial loss of diversity. The loss of diversity may increase the risks of

attack by pests and pathogens. Evidence of pest outbreak is common in tropical plantations (see Pannell 1989). Diversity in tropical moist forests is nearly always a necessary condition for the production of quality timber of indigenous species. The indigenous hardwoods are rarely successfully grown except as part of the forest ecosystem (Pannell 1989).

Gaps created in the forests by logging are recolonised as in the undisturbed forests. This recolonisation process involves a great diversity of interactions among and between animals and plants, and results in new forests with different sets of species dominating at different stages of succession. Each species may have quite different ecological requirements. The success of any species depends on its growth rate, physiological flexibility that facilitates its competition for light and nutrient requirements relative to competitors, and nutrient availability at the site. However, the filling of gaps by economically desirable species is not ensured. This natural regeneration process can, of course, be directed to increase population of any particular species. This can be achieved by the protection of valuable species and the elimination of unwanted ones in order to facilitate establishment and growth of propagules coming from nearby desirable mother trees. Alternatively, where the regeneration of commercial species are scarce or absent, the seedlings of the desired species can be planted in the gap at the right time and at the right age. The competitors may be systematically eliminated by weeding and thinning. However, to make such a management system successful, much needs to be known about the ecology and specific requirements of the desirable species in order to provide the correct treatment at the right time.

Recently, Gómez-Pompa and Burley (1991) have reviewed all the general methods of regeneration practised in the tropical forests and their relative success. There are several examples of success of regeneration following disturbance, both logging and clear-cutting. Nair (1991) has pointed out that regeneration, if at all successful, is due to the accidental coincidence of favourable conditions and is not something planned scientifically. Failure of natural regeneration is also common in the tropical forests (Nair 1991; review by Gómez-Pompa and Burley 1991). The causes of these failures are multiple. The lack of knowledge on the requirements of individual species has, however, been considered as one of the main causes of failure. Sometimes, the absence of regeneration in the form of seedlings or saplings limits natural restocking, and even when seedlings are present, drastic changes in light and moisture conditions consequent to canopy opening adversely affect their establishment and growth (Nair

1991). The acclimation potential of a given species in response to these changes in the environment may, therefore, be important for its survival and growth.

For the maintenance of ecological diversity and sustained timber yields, management of secondary forests is required. Plantation forestry is also needed for the production of adequate timber. Whichever form of forestry is employed, a good understanding of ecophysiological differences between constituent species is the prerequisite of the successful management of regeneration in the tropical forests.

## 1.2 Review of Literature

### 1.2.1 Plant responses to shadelight

Sunlight is a broad band of radiation, spanning the ultraviolet, visible and near infrared parts of the electromagnetic spectrum. For plants growing in forest understorey habitats, there are quantitative and qualitative changes in the available energy (Holmes 1981; Chazdon and Fetcher 1984; Lee 1987). First, light passing through vegetation is attenuated in photosynthetic photon fluence rate (400-700 nm), and hence the quantity of photosynthetically active radiation is drastically reduced. Second, a marked reduction in the quantity of radiation in the blue part of the spectrum. The third change is the strong depletion of the red waveband and relatively weak attenuation of the far-red waveband. As a result of these changes in the spectrum, the forest shadelight is characterised by low photosynthetic photon flux density, a low proportion of blue, and low red to far-red ratio (R:FR). This ratio is known to be especially important in photomorphogenesis (review by Smith 1986), the red band being defined as 660 nm, the far-red as 730.

The R:FR ratio is approximately 1.10-1.25 under full sun and as little as 0.10 under forest canopies (Chazdon and Fetcher 1984; Lee 1987). These two wavelengths affect the ratios of the Pfr and Pr forms of phytochrome (review by Smith 1986). Although phytochrome responds to all radiation between approximately 350 nm and 750 nm, it is in the red and far-red wavebands that the quantum effectiveness is maximum (Holmes 1981). A low R:FR ratio can influence plant development in many ways through its effects on the photoequilibrium and biological activities of phytochrome (Smith 1982; Morgan *et al.* 1983; Corré 1983a; Kwesiga and Grace 1986; Lee 1988; Warrington *et al.* 1989). Studies on herbaceous plants show that species from open

habitats are more responsive to R:FR ratios than the species which typically grow in vegetational shadelight (review by Smith 1986). A low R:FR ratio (a) promotes stem elongation (review by Smith 1986), (b) enhances the allocation of dry matter to stem (Corré 1983a), (c) reduces total leaf area (McLaren and Smith 1978), (d) increases shoot to root ratio (McLaren and Smith 1978), and (e) decreases leaf thickness resulting from a reduction in cell density of mesophyll and a reduction in air-space volume of spongy mesophyll (Child *et al.* 1981). A low R:FR ratio also reduces chlorophyll contents per unit leaf area (McLaren and Smith 1978), and chlorophyll a:b ratio (Lee 1988; Chow *et al.* 1988). The decreases in chlorophyll a:b ratios reflect differences in chloroplast ultrastructure, and greater allocations to Photosystem II compared to Photosystem I reaction centres (Glick *et al.* 1985).

There have been very few studies on the effects of R:FR ratios for tree seedlings (Morgan *et al.* 1983; Kwesiga and Grace 1986; Warrington *et al.* 1989) and even fewer for seedlings which are more than a few months old. Warrington *et al.* (1989) have shown for relatively older materials of *Pinus radiata* that stem height and diameter, stem and needle dry weight, and apical dominance are markedly increased by a reduction in R:FR ratio. Growth responses of tree seedlings other than stem extension changes have been shown to be differentially affected between pioneer and climax species. Kwesiga and Grace (1986) have shown that relative growth rate resulting from an increased specific leaf area is enhanced with low R:FR ratios in pioneer species *Terminalia ivorensis*, whereas it is largely unaffected in climax species *Khaya senegalensis*. Photosynthesis is also known to be influenced by a reduction in R:FR ratio in some species. For example, Kwesiga *et al.* (1986) found that the rates of photosynthesis of shade leaves were higher when the R:FR ratio during growth was typical of forest shadelight. They proposed that there was an apparent direct influence of spectral quality on the functioning of a unit volume of leaf mesophyll. This result is consistent with that of Hoddinott and Hall (1982), who found 10% higher rates of photosynthesis under a R:FR ratio of 0.7 compared to one of 4.7 for *Phaseolus vulgaris*. Warrington *et al.* (1989) observed higher photosynthetic rates for *P. radiata* from the treatment with a low R:FR ratio. They have also found higher rates of dry matter accumulation under the low R:FR ratio and concluded that the increased dry matter accumulation was the result of reduced mutual shading of adjacent leaves as a consequence of photomorphogenetically-controlled internode lengths rather than of enhanced photosynthesis. In contrast to these results, Corré (1983a) found no influence of R:FR ratios on the rates of photosynthesis in a number of herbaceous plants.

In comparison to the studies on the effects of R:FR ratios, the effects of blue light on plant growth have been sporadically studied. There is a good evidence for responses to blue light (reviews by Voskresenskaya 1972, Thomas 1981, and Mohr 1986). A specific blue light response is generally ascribed to phytochrome and/or an unknown blue light photoreceptor. A small supplementary irradiation of blue light had a strong inhibitory effect on the growth in length of leaves of *Lolium multiflorum* growing in shadelight beneath a natural canopy (Casal and Alvarez 1988). Laskowski and Briggs (1989) and Warpeha and Kaufman (1989) found that blue light inhibits epicotyl elongation in *Pisum sativum* seedlings grown under continuous red light and concluded that the inhibition was not due to changes in Pfr concentration during treatment with blue light. A promotive effect of reducing blue light on stem extension growth has been observed in *Sinapis alba* and *Datura ferox*, when the first internode was simultaneously exposed to low R:FR ratios and low blue light levels (Ballaré *et al.* 1991). Mohr (1986) has shown that light absorbed by the blue light photoreceptor is necessary to maintain responsiveness to Pfr. With increasing age of the seedlings, the requirements for blue light increases strongly, and the fluence rate of blue light must exceed a certain threshold to become effective. Drumm-Herrel and Mohr (1991) have also shown that the action of blue light on stem elongation is related to the level of Pfr, and an expression of the effects of blue light is diminished if the level of Pfr is kept low.

Since the shadelight has a lower irradiance with a low R:FR ratio and a low proportion of blue light, the shade responses of seedlings may be the result of either decreased irradiance or changes in the light quality or both. In some cases, the responses to both quantity and quality are in the same direction but of different magnitude. For example, Chow *et al.* (1988) have shown that a growth light regime with a low R:FR ratio results in an additional drop in the chlorophyll a:b ratio below values obtained in low irradiance alone. In others, the responses are not necessarily in the same direction. For instance, a low R:FR ratio results in maximum stem extension rate at the expense of leaf development, whereas a low irradiance brings about maximum leaf development at the expense of stem development (Smith 1981).

### 1.2.2 Photosynthesis in tropical forest tree seedlings

In the forest understorey habitat, plants exhibit a variety of photosynthetic characteristics that enable them to maintain positive carbon balance under extremely low photon flux density (Boardman 1977; Björkman 1981). Among these, low rates of dark respiration (Table 1.1) and leaf life-span (review by Bazzaz 1991) are of ecological significance. Some authors have shown higher apparent quantum yields of photosynthesis in shade plants compared to sun plants, whereas reverse patterns of response have also been found in some studies (Table 1.1). However, several other studies have demonstrated that the apparent quantum efficiency is insensitive to growing conditions (Table 1.1; see also Sims and Pearcy 1989).

As regards to photosynthetic characteristics of species, the pioneer species have higher maximum photosynthetic rates than do non-pioneer species (Table 1.1). Furthermore, within a species, shade-grown seedlings generally have lower light saturated photosynthetic rates than do sun-grown seedlings. But there are exceptions such as *Hymenaea parvifolia* (see Table 1.1). In the forests, both sun and shade leaves may be found on the same individual. Their carbon gain capacities and contributions to the total carbon budget of that individual are probably different. Oberbauer and Strain (1986) compared seedling leaves to the leaves from three heights in the canopy of *Pentaclethra macroloba*. They have found that the changes in leaf characteristics along the canopy gradient paralleled the changes that could occur in seedlings grown on a light gradient. Oberbauer and Strain (1984) studied the photosynthetic capacity of 7 tree species in a moist tropical forest and related photosynthetic rates to successional status. They found that light saturated photosynthetic rates were related to the preferred light environments in the field. Plants preferring heavy shade had a mean photosynthetic rate of  $6.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ , those in canopy gaps  $11.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the species from large clearing  $27.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Further, the light saturation of plants from the clearing occurred at the photon flux greater than  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whilst for those from habitats receiving relatively lower daily photon flux, were reached at the much lower photon flux density. Recent field measurements show that pioneer tree species like *Ceiba pentandra* has photosynthetic attributes similar to weeds such as *Chromolaena odoratum* (Riddoch *et al.* 1991b). Riddoch *et al.* (1991b) have also shown that the apparent quantum yields of photosynthesis in the climax species do not differ from that in the pioneer species.

**Table 1.1:** Some photosynthetic characteristics of tropical forest tree seedlings grown under sun or high irradiance, and shade or low irradiance. NP = non-pioneer species, P = pioneer species;  $P_{\max}$  = maximum photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $R_d$  = dark respiration ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $\alpha$  = apparent quantum yield ( $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$ ).

Species	Ecological status	Growth irradiance	$P_{\max}$	$R_d$	$\alpha$
<b>Langenheim et al. (1984):</b>					
<i>Agathis robusta</i>	NP	sun	7.3	0.41	0.024
		shade	3.8	0.07	0.050
<i>A. microstachya</i>	NP	sun	4.5	0.26	0.018
		shade	3.2	0.05	0.038
<i>Hymenaea courbaril</i>	NP	sun	5.0	-	0.056
		shade	4.0	0.09	0.058
<i>H. parvifolia</i>	NP	sun	3.0	0.14	-
		shade	3.5	0.11	-
<i>Copaifera venezuelana</i>	NP	sun	7.6	-	0.065
		shade	7.0	0.11	0.100
<b>Oberbauer and Strain (1985):</b>					
<i>Pentaclethra macroloba</i>	NP	sun	6.5	0.48	0.027
		shade	5.3	0.15	0.047
<b>Kwesiga et al. (1986):</b>					
<i>Terminalia ivorensis</i>	P	high	8.0	0.95	0.038
		low	3.8	0.43	0.018
<i>T. superba</i>	P	high	8.2	0.32	0.034
		low	4.3	0.30	0.039
<i>Triplochiton scleroxylon</i>	P	high	9.3	1.28	0.096
		low	4.0	0.44	0.044
<i>Khaya senegalensis</i>	NP	high	4.0	1.53	0.100
		low	3.8	0.32	0.031
<b>Pearcy (1987):</b>					
<i>Argyrodendron peralatum</i>	NP	sun	8.1	0.37	0.043
		shade	5.7	0.22	0.051
<b>Thompson et al. (1988):</b>					
<i>Flindersia brayleyana</i>	NP	high	7.2	1.30	0.046
		low	6.5	0.70	0.043

Continuation of Table 1.1

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<b>Ramos and Grace (1990):</b>					
<i>Cordia alliodora</i>	P	sun	8.8	1.03	0.023
		shade	5.3	0.32	0.026
<i>Cedrella odorata</i>	P	sun	8.0	0.63	0.032
		shade	5.0	0.38	0.033
<i>Brosimum alicastium</i>	NP	sun	7.0	0.65	0.027
		shade	4.6	0.10	0.029
<i>Swietenia macrophylla</i>	NP	sun	6.2	0.88	0.026
		shade	4.6	0.46	0.029
<b>Riddoch et al.(1991a):</b>					
<i>Nauclea diderrichii</i>	P	high	6.9	1.36	0.035
		low	2.9	0.10	0.048
<i>Entandrophragma angolense</i>	NP	high	5.4	0.76	0.038
		low	2.8	0.40	0.047

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Gap formation by the falling of trees or branches in the canopy and the subsequent closure of those gaps create an extremely heterogeneous light environment in space as well as in time. Thus, a given seedling may be exposed to changes in light regime over the course of its lifetime, including increases as well as decreases. There is a large increase in light levels in the forest understorey habitats following a disturbance. Along with light levels, other physical variables like temperatures and vapour pressure deficits are also changed. The temperatures and the vapour pressure deficits are higher and more varied in the clearings, and are lower and less varied in the understorey habitats (review by Bazzaz 1991). Pearcy (1987) studied the possible effects of these physical variables on leaf photosynthesis, and showed that the photon flux density, rather than the temperatures or the leaf-air vapour deficits, is the most dominant factor controlling the leaf photosynthesis in the forests. Availability of soil nutrients may be as important as light. Thompson *et al.* (1988) and Riddoch *et al.* (1991a) have shown that leaf photosynthetic characteristics are significantly affected by nutrient supply. Even apparent quantum efficiency is highly reduced by the limited supply of nutrients (Thompson *et al.* 1988).

There have been very few studies on the acclimation potential of tropical forest tree seedlings to the changing light availability. However, Fetcher *et al.* (1983) examined the potential of pioneer species *Heliocarpus appendiculatus* and non-pioneer species *Dipteryx panamensis* for acclimation to changing light conditions. The seedlings, grown in full sun, partial shade (20% sun), and full shade (2% sun), were switched between environments. The growth of the pioneer species was more plastic than that of the non-pioneer species in response to changes in the light availability. In the high light, height growth was largely higher in *Heliocarpus* than in *Dipteryx*, but survival was higher in the latter than in the former. In a similar study, the seedlings of *Pentaclethra maculoba*, experimentally switched from 1% sun to full sun, showed severe photoinhibition and leaf abscission (Oberbauer and Strain 1985). Langenheim *et al.* (1984) had also observed photoinhibition and leaf damage when the shade seedlings (6% sun) of *Agathis robusta* and *A. microstachya* were experimentally transferred to the full sun. The leaves, which experienced photoinhibition, were retained by the plants, and had not recovered even after 12 months.

### **1.2.3 Ecological classification of tropical forest tree species**

Based on the differential responses to canopy shade, species are generally classified into two main ecological groups: shade intolerant or gap requiring species, and shade

tolerant or persistent species (Denslow 1980; Hartshorn 1980; Whitmore 1984). The first group is further sub-divided in species, which are typical shade intolerant and only regenerate in large gaps, where they complete their entire life cycle (pioneer species), and species, which may be shade tolerant in some stage(s) of their development, but require gaps to reach maturity (gap species)(Denslow 1980; Hartshorn 1980). Such a classification is usually used to make generalisations. In reality, the species almost certainly fall along a continuum in their light requirements.

The assignment of a species to one ecological group or another is usually based on the spatial and temporal distribution of species over microclimates (Denslow 1980; Hartshorn 1980; Brokaw 1985), on the growth rates and life span of adult trees (Lieberman *et al.* 1985; Primack *et al.* 1985), or on the survival rates of seedlings in the shade (Augspurger 1984). Recently, Swaine and Whitmore (1988) have proposed a simple division of tropical forest tree species into two groups, pioneer and non-pioneer (or climax), based on seed germination and seedling establishment. They suggest that seeds of pioneer species germinate only in canopy gaps open to the sky, whereas non-pioneer species have seeds that can germinate under forest shade or in gaps. More recently, Raich and Wooikhoon (1990) have studied germination behaviour of 43 tree species native to the lowland forests of Malaysia and showed that seeds of most gap-germinating species germinate to a degree in the forest understorey. They defined gap species as those that germinate best under a canopy opening, and non-pioneer species as those that germinate as well or better in the forest understorey. Their gap species includes both pioneer and non-pioneer species as suggested by Swaine and Whitmore (1988). Further, Popma and Bongers (1988) have used relative biomass growth rate in response to gap light conditions as an index for shade tolerance. They have shown that pioneers cannot persist under shade because of their negative net assimilation rates under light-limiting forest shade conditions. Taken together, it appears that gap species includes both pioneer species and those which germinate better in gap light conditions, but germinate in shade as well, and may be tolerant to shade in some stage(s) of their development, but mostly behave like pioneers.

### 1.3 Aims of the Project

Though a large fraction of the tropical forests lies in tropical Asia (Sedjo 1987), autecology of the species of these forests is poorly studied. The lack of knowledge on the species hinders domestication of indigenous species on one hand, and scientific management of regeneration on the other. The importance of this knowledge has been emphasised in recent reviews (Gómez-Pompa and Burley 1991; Nair 1991; Bazzaz 1991) for effective management of secondary forests, where light (and sometimes nutrient status of soil) plays a dominant role on the growth and competition of tree seedlings. The main aim of this project is, therefore, to characterise the responses of tree seedlings to light for a range of species from moist forests of tropical Asia.

Specifically, the work presented in this thesis has the following objectives:

- (a) to characterise the effects of light and nutrient supply on the growth of seedlings by growing them in simulated irradiance of understory habitats, disturbance gaps, and forest clearings;
- (b) to determine the effects of R:FR ratios on seedling development, simulating shadelight quality;
- (c) to assess the effects of a low proportion of blue light, as found in vegetational shadelight, on the development of seedlings;
- (d) to examine the potential of shade grown seedlings for acclimation to bright light, and to determine the physiological and anatomical changes occurred in the shade leaf during acclimation;
- (e) to determine the mechanism by which the fully expanded high-light leaf becomes photosynthetically acclimated to shade; and
- (f) to determine the effects of changing light availability on the growth of seedlings by exchanging them between simulated light conditions of understory habitat and disturbance gap.

In this thesis, the term 'low irradiance' is used for simulated shadelight with low photosynthetic photon flux density (PPFD) and low R:FR ratio, and the term 'high irradiance' for simulated daylight with high PPFD and high R:FR ratio.

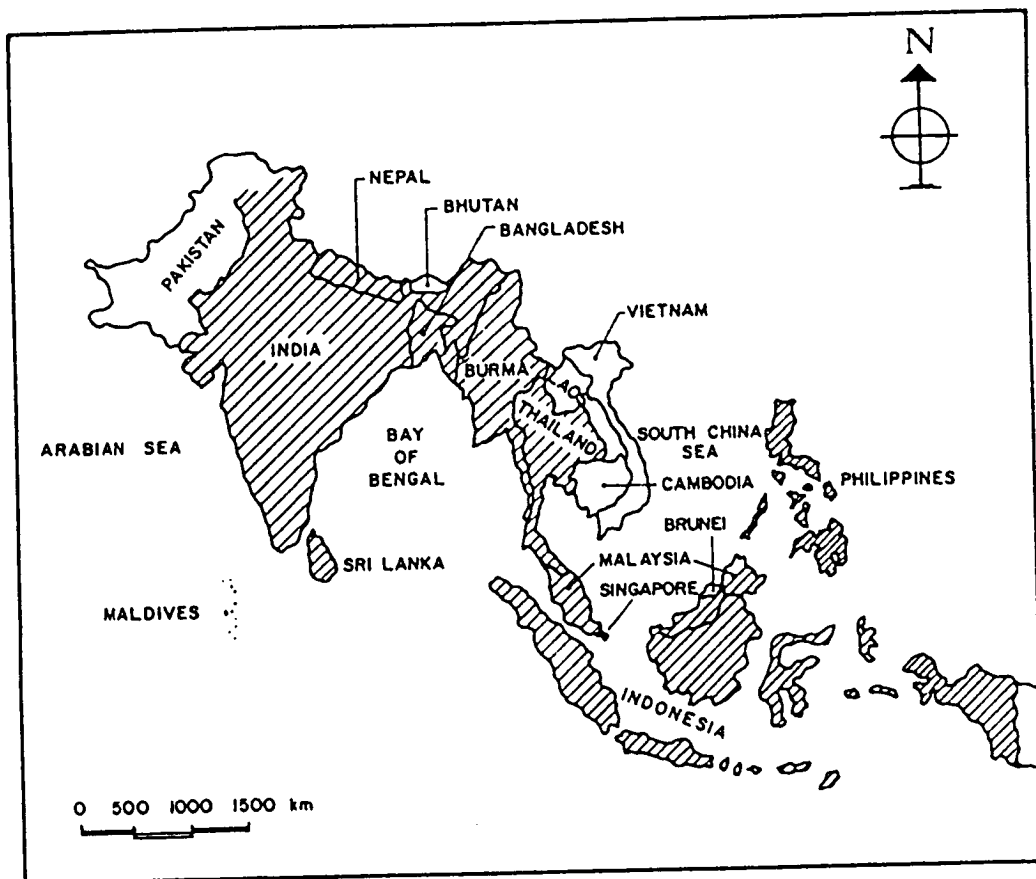
## **1.4 Choice of species: their distribution, biology and economic importance**

### **1.4.1 *Anthocephalus chinensis* (Lamk.) Rich. ex Walp., (Rubiaceae)**

This species has a wide distribution occurring from Nepal to New Guinea, and is found in India, Sri Lanka, Bangladesh, Burma, Brunei, Thailand, Malaysia, Indonesia and Philippines (Figure 1.1; Troup 1921; review by Fox 1971). It is a well-known plantation species in many parts of Asia. It is found to grow on alluvial ground along rivers and on swampy grounds. Extensive stands also occur on abandoned lands and logged areas, but it does not grow well on leached soil, even if soil physical conditions are good. It grows well, when interplanted with legumes like *Leucaena leucocephala* (Evans 1982). The tree can grow from sea-level to about 1000 m, in areas where the annual rainfall ranges from 150 mm to 5000 mm and the mean annual temperature is in between 3.3 °C and 37.7 °C (Whitmore 1984).

*A. chinensis* is botanically related to *Nauclea diderrichii*, an important West African timber tree of the Rubiaceae (see Fox 1971). It is a large deciduous (or semi-evergreen) tree with spreading branches and rather large shining leaves. It flowers and fruits at the age of 5 years. The fleshy fruit contains numerous seeds ( $20 \times 10^6$  per kg), which are distributed by bats and birds (Troup 1921), and wind (Richards 1952). Dispersal of seeds is also known to be affected by water (Fox 1971 and reference therein). This may be true for the origin of river-side stands. Rain water also plays an important role in the dispersal of seeds.

Seeds of this species require an after-ripening period of a few months. Old seeds germinate best in full sun, and fresh ones in shade (Fox 1971 and reference therein). The effects of light of different wavelengths on seed germination have been studied by Quintos *et al.* (1975). Seeds treated with white light gave the highest germination (78%) followed by red light (73%) and yellow light (59%). No seed germinated under the blue, and germination under continuous dark was 1% only. Seeds are known to persist in the soil under canopy for several months (Fox 1971). Natural reproduction



**Figure 1.1:** Countries of Asia showing distribution of *Anthocephalus chinensis*, *Bischofia javanica* and *Hopea odorata*. Shaded areas indicate the countries, where these species occur unless otherwise stated in the text.

is not found under established plantations or natural stands. Seedlings cannot persist in shade. They appear in open or logged areas in great abundance, approximately 45000 per ha (Troup 1921; Fox 1971).

Growth is very fast. Mean height of 3 m can be expected 1 year after planting and a mean increment of 2-3 m year<sup>-1</sup> can be expected for 6-8 years with a diameter increment of 13-76 mm year<sup>-1</sup> after that growth slows down, and becomes particularly slow after 20 years. Trees 38 m tall and 0.65 m in diameter can be expected on a 30 year rotation, yielding 350 m<sup>3</sup> timber per ha (Whitmore 1984).

*A. chinensis* produces industrial timber, which is called in the trade as 'Kadam'. Its wood is soft and mostly used in the match factories for making match boxes and splints, in the plywood industries for making tea-chests, packing boxes and flash doors. In many areas, it is grown in plantations to produce white, short-fibre pulp.

#### **1.4.2 *Bischofia javanica* Blume, (Euphorbiaceae)**

It is common in the tropical moist forests of India, Bangladesh, Burma, Malaysia and Philippines (Figure 1.1; Troup 1921; Richards 1952; Bor 1953; Burgess 1966). Usually, it is the characteristic species of fresh water swamp forests, where with other colonising tree species, it forms a seral community leading to the development of edaphic sub-climax (Troup 1921; Som Deva and Srivastava 1978). It also occurs in the evergreen forests and even in drier savannas. It is also found to grow on moist shady ravines, river banks and in the secondary forests of the region.

In moist localities, the tree is sometimes evergreen, whereas in dry situations, it becomes leafless or nearly so for a short time during the cold season. It usually fruits in great abundance. Seeds are small ( $92 \times 10^3$  per kg), and they germinate under favourable conditions irrespective of shade or sun. Seedlings develop satisfactorily in very moist ground, and they are able to struggle through grass and weeds, but their development is hindered thereby. They are capable of standing a fair amount of shade (Troup 1921).

Growth is moderately fast, sometimes as fast as 4 annual growth rings per 25.4 mm of radius, representing a mean annual girth increment of 4 cm (Troup, 1921). It attains a girth of 3 m and a height of 30 m or more (Burgess 1966).

Its timber is known in the trade as 'Bishop wood', sometimes called 'Red Cedar'. Its wood is red, moderately hard, durable particularly under water, and used for piles, bridge construction, buildings and railway sleepers. If used treated, it is an excellent sleeper and constructional wood (Trotter 1941).

#### **1.4.3 *Hopea odorata* Roxb., (Dipterocarpaceae)**

It occurs in the moist evergreen forests of India, Bangladesh, Thailand, Burma and Cochin China (Figure 1.1; Troup 1921). It typically grows on deep rich soil, most commonly along the banks of streams and in damp situations. The forests, in which it occurs, are not characterised by the presence of numerous forest grasses, but as a rule by a dense evergreen undergrowth. In its natural habitats, the absolute maximum shade temperatures vary from 36.7 °C to 40.6 °C and the absolute minimum from 7.2 °C to 15.7 °C, the annual rainfall ranges from 2286 mm to 5080 mm (Troup 1921).

It is a large evergreen tree reaching a height of 30-37 m with a girth of 3.7 m (Troup 1921). The species usually does not fruit regularly. Sometimes, there is heavy or gregarious flowering every few years, but in many cases, few flowers finally form fruits. Fruits are big ( $3 \times 10^3$  per kg), and mainly dispersed by winds. They germinate as soon as they fall. The seedlings are best established in shade, where soil is moist, and they stand a considerable amount of shade. Growth is relatively slow.

Its timber is called 'Hopea' in the trade. Its wood is yellow or yellowish brown, hard, and close- or even-grained, and typical strong durable constructional wood. The timber is much prized for furniture-making, boat-building and the construction of boats and large launches. It is also used for bridge work, piling, rafters, cart-building, railway sleepers, and many other purposes where their special qualities of great strength, hardness, and durability are required (Trotter 1941).

#### **1.4.4 Ecological status of the species and their seed source**

A review of the literature on growth rates, wood characteristics, germination behaviour, distribution in their natural habitats indicates that *Anthocephalus chinensis* is a typical pioneer species, whilst *Hopea odorata* is a non-pioneer or climax species (see section 1.2.3 and the preceding sections of 1.4). Existence in the secondary forests, colonising behaviour and growth rate suggest that *Bischofia javanica* is also a gap species. To distinguish *A. chinensis* from *B. javanica*, the former will be referred

to as a pioneer species and the latter as a gap species throughout the thesis although it should be realised that classification of the latter is provisional, pending further research, and that considerable ecotypic variation may exist.

Seeds for the experiments were obtained from moist semi-evergreen forests of Chittagong, Bangladesh. The seeds of each species were collected from 'mother trees' selected by Bangladesh Forest Research Institute, for normal seed collection. For each species, seeds from two individual trees were used in this study.

## CHAPTER 2

# Preliminary Investigations of the Growth of Seedlings of Two Contrasting Species in Relation to Light and Nutrient Supply

### 2.1 Introduction

Any understanding of regeneration and succession in tropical forests requires information on the responses of the constituent species to different levels of light, and particularly on their responses to gaps. Canopy gaps are areas of increased supply of resources, both light and nutrients. The tree species differ in their growth responses to the environmental conditions associated with canopy gaps of different sizes (Denslow 1980; Popma and Bongers 1988). This differentiation in growth responses to gaps may be most pronounced at the early stages of their growth.

With respect to this ecological differentiation, most tropical ecologists think of species in two groups: pioneers or gap species and non-pioneer or climax species based on seed germination and seedling establishment (Swaine and Whitmore 1988; Raich and Wooikhoon 1990). Popma and Bongers (1988) have used relative biomass growth rate in response to gap light conditions as an index for shade tolerance. However, the differential response of species to canopy gaps of different sizes and with different levels of photosynthetic photon flux is just beginning to be examined (review by Bazzaz 1991). Such studies should lead to a better understanding of the degree of differentiation, which apparently exists among species (Bazzaz and Pickett 1980).

Although light is the factor which has received most attention in seeking to evaluate seedling growth, nutrient status of soil may be as important as light. There have been very few studies on the effects of nutrient supply on growth of tropical forest tree seedlings. There is evidence that leaf morphology and physiology and hence the leaf photosynthetic capacity are changed with the supply of nutrient, particularly at higher light levels (Thompson *et al.* 1988; Riddoch *et al.* 1991a). A study on bioassay of nutrient limitation in a tropical rain forest soil shows that the relative growth rates of seedlings growing on fertilised soils collected from an undisturbed forest site are higher than those of seedlings growing on unfertilised ones (Denslow *et al.* 1987).

Many, but not all, tropical forest soils are nutrient-poor (review by Bruijnzeel 1991). The nutrient availability differs even in different areas within a single tree-fall gap (Orians 1982; Vitousek and Denslow 1986). Though supply of all nutrients is important (Grubb 1977), supply of N is particularly important as leaf photosynthesis is highly and positively correlated with the supply of this element (Thompson *et al.* 1988; Riddoch *et al.* 1991a). Thus, a plant may acclimate to a given irradiance and nutrient availability by physiological adjustments so as to increase carbon gain.

Previous studies suggest that gap species and climax species differ in their growth responses to light (Fetcher *et al.* 1983; Popma and Bongers 1988). The present study seeks to explore the possible interaction between light availability and nutrient supply. The hypothesis is that a fast growing gap species needs an enhanced supply of nutrients if it is to exploit full light. The differential responses of the species to irradiance and nutrient availability may influence the species colonisation patterns in the forests.

In this experiment, *Bischofia javanica* Blume and *Hopea odorata* Roxb. have been examined to characterise their growth responses to irradiance and nutrient supply. These species display contrasting distribution patterns in the succession of tropical forests. *Bischofia* is a characteristic species of fresh-water swamp forests, though its distribution ranges from drier savannas to moist evergreen forests (Troup 1921; Som Deva and Srivastava 1978). It produces small seeds, which germinate both in gaps and in forest shade, and has a fast growth rate. The big-seeded *Hopea*, on the other hand, is a climax species, and found typically on deep soil, most commonly along the banks of streams and in damp situations of tropical moist forests, where it grows slowly and is considered shade tolerant (Troup 1921).

In this study, an attempt was made to simulate forest light conditions, which as far as possible were typical of understory shadelight, small/medium, and wide disturbance gap light conditions. It is well known that forest shade is characterised by a reduced photosynthetic photon flux density (PPFD) and a low red to far-red ratio (R:FR ratio). Here, no attempt was made to separate these two factors, but care was taken to ensure that an appropriate reduction in R:FR ratio did accompany the reduction in the PPFD. In this way, the seedlings were grown under three irradiance levels providing them with high or low nutrient supply.

## 2.2 Materials and Methods

### 2.2.1 Plant materials and experimental design

Seed was germinated in seed-trays containing equal parts by volume of vermiculite and perlite. The seedlings were potted into 21 cm tall tubes (diameter 6.5 cm) filled with equal volumes of vermiculite and perlite, and randomly assigned to treatments on the bench in a glasshouse. At this point, the seedlings had attained a height of 2-3 cm (*Bischofia*) or 6-8 cm (*Hopea*) and their first true leaf had expanded fully. Starting date of experiment for the former was March 1989 and for the latter July 1990.

Six treatments, each with 8 seedlings, were set up in a randomised block design: three irradiance levels, low (dense shade), medium (partial shade), and high (full sun) (5, 50 and 100% daily PPFD), and two nutrient regimes, high and low. Here, no attempt was made to separate light quantity from light quality, but care was taken to ensure that an appropriate reduction in R:FR ratio did accompany the reduction in the PPFD.

### 2.2.2 Nutrition

A nutrient solution modified from Ingestad Solution for birch (Ingestad 1979) was used to feed the seedlings. This solution had already been used in this laboratory for the experiments with other tropical forest tree seedlings (Riddoch *et al.* 1991a). In this solution, the proportions of N, P and K were 100:16:55 by weight, and the ratio of nitrate-N to ammonium-N was 7:5. The composition of A and B stock solutions were as follows:

Solution A (g l<sup>-1</sup>): NH<sub>4</sub>NO<sub>3</sub> 140.2, KNO<sub>3</sub> 37.2, KH<sub>2</sub>PO<sub>4</sub> 41.3, K<sub>2</sub>SO<sub>4</sub> 14.0 .  
Solution B (g l<sup>-1</sup>): HNO<sub>3</sub> 1.6, H<sub>3</sub>BO<sub>3</sub> 0.57, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 2.5, Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O 24.3, Mg(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O 44.92, MnSO<sub>4</sub>.4H<sub>2</sub>O 0.81, CuCl<sub>2</sub>.2H<sub>2</sub>O 0.043, ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.064, Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O 0.008.

These stock solutions, when mixed in the proportion of 1.7A: 1B from 2-litre A solution and 1-litre B solution proportionately, provided 60 and 20 mg l<sup>-1</sup> of N for high and low nutrient regimes respectively. Nutrient solution was applied daily as irrigation to bring the medium to field capacity. The aim was to present all roots with this standard solution daily, and so solution was added until it began to flow from the drainage hole.

### 2.2.3 Design of 'shade-cover'

An individual 'shade-cover' was designed to shade seedlings under low and medium irradiance treatments. A 'shade-cover' was a plastic container (27.5 cm x 7.5 cm), cut at both the ends, with a celluloid filter, at the bottom end, fixed with a masking tape (Figure 2.1). The filter was fixed to the tube in such a way (forming a curvature) that some space was left on the two sides of the filtered end for ventilation. Moreover, as the tube of the 'shade-cover' was larger in diameter than the tube with the seedling, enough space was left, when the former was fitted to the latter.

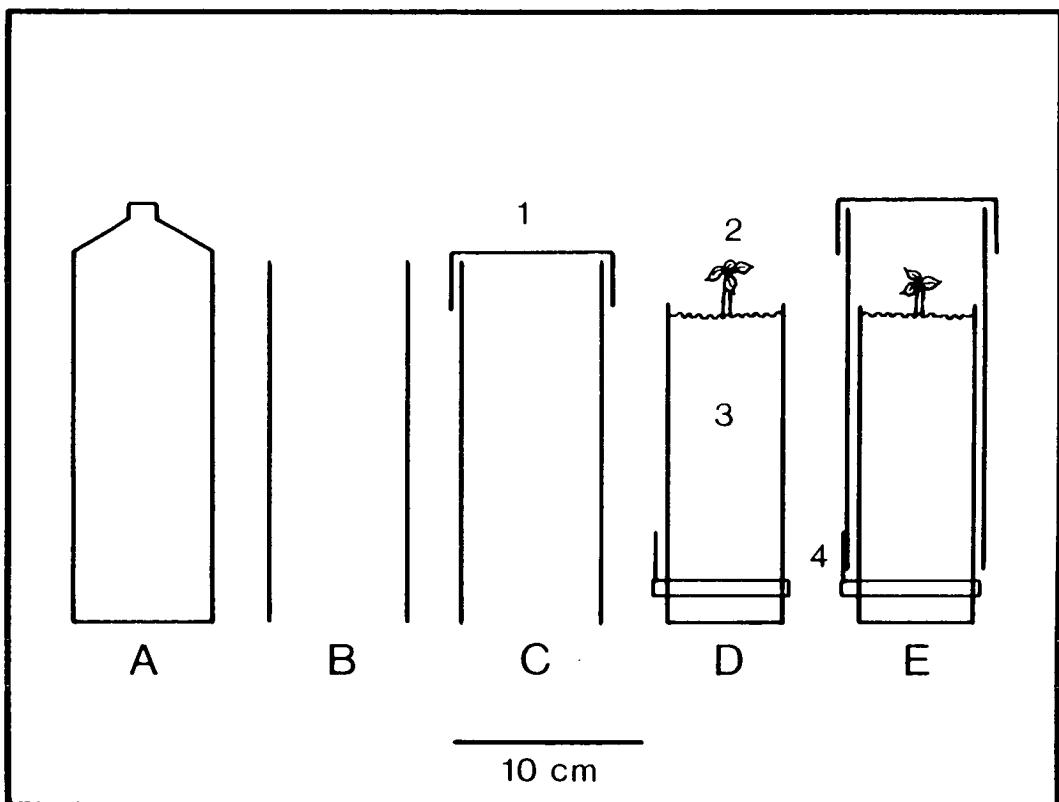
Blue green filter (chromoid 116, strand Lighting, Middlesex, UK) and clear filter (Overhead projector transparency 2500.5.999, Folex, Solihull, West Midlands, UK) were used in conjunction with the shading effect of the tube itself for low and medium irradiance treatments respectively.

The 'shade-cover' was attached to the seedling tube with a plastic clip, thus enabling it to be lifted upwards progressively as the seedling grew taller to minimise the effects of size advantage, where appropriate.

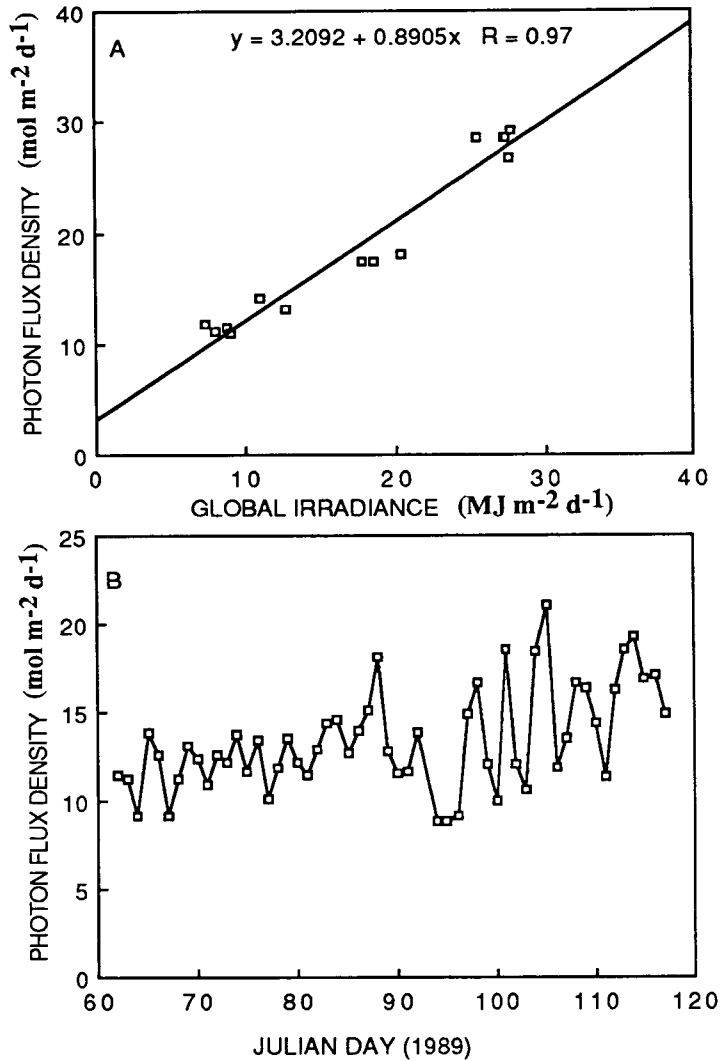
### 2.2.4 Light, temperature and humidity

A quantum sensor (Li-190 SB Li-Cor., Inc., Lincoln, USA) was used to measure photosynthetic photon flux density. The total daily photosynthetic photon flux density (DPF) on the experimental bench and inside the 'shade-covers' was recorded by using calibrated quantum sensors and a datalogger (Deltalogger, Delta-T devices, Delta-T Co. Ltd., Cambridge, UK). Based on 2 weeks' data, the percentage daylight inside 'shade-cover' was calculated.

During the experiment with *Bischofia*, the DPF on the experimental bench was recorded for 2 weeks. Total daily global irradiance data were obtained from East Craigs Meteorological Station, 9 km (NW) away from the experimental site. Using a regression equation, derived from the two sets of corresponding values, DPF on the experimental bench for the whole experimental period was calculated (Figure 2.2). During the experiment for *Bischofia*, overhead lamps (MBFRU mercury fluorescent lamp, 400 W, General Electric Co. Ltd., England, UK) were used at 12 h cycles. The irradiance and the photoperiod on the experimental bench varied according to natural rhythm.



**Figure 2.1:** Diagrammatic representation of the construction of 'Shade-cover' used for shading seedlings. A: a plastic container (27.5 cm × 7.5 cm); B: the container cut on the top and at the bottom into a tube; C: the tube with a celluloid filter (1) on the top; D: a growth tube with a seedling (2) growing in a medium (3) of equal volumes of vermiculite and perlite; E: the seedling under 'shade-cover' fixed to the growth tube by a plastic clip (4).



**Figure 2.2:** Light climate on the experimental bench during growth of *Bischofia javanica* seedlings. A: a regression equation between total daily global irradiance at nearby meteorological station and total daily photosynthetic photon flux density inside glasshouse; B: total daily photosynthetic photon flux density on the experimental bench estimated from a knowledge of global irradiation.

A red/far-red ratio sensor (SKR 110, Skye Instruments Ltd., Powys, Wales, UK) was used to measure R: FR ratio, defined as photon fluence rate centred at 660 and 730 nm respectively (the wavebands were not symmetrical around the wavelengths of maximum transmittance, being -13 nm and +8 nm with respect to 660 nm for red, and -18 nm and +6 nm with respect to 730 nm for far-red). The R:FR ratios were measured throughout a day, and the mean ratio for each treatment was calculated from 10 readings.

Temperature and relative humidity were partially controlled in the glasshouse. The day temperatures were usually about 25 °C, whilst the night temperatures were about 20 °C. The relative humidity was usually over 50%.

During the experiment for *Hopea*, light, temperature, and vapour pressure deficit on the experimental bench (Figure 2.3) were recorded by a datalogger. For DPF measurements, calibrated quantum sensor was used. By using thermocouples (Copper-Constantan thermocouple wire, British standard BIO BS 1843 Type T, T. C. Ltd., Uxbridge, UK), 'dry-bulb' temperatures ( $T_a$ ) and 'wet-bulb' temperatures ( $T_w$ ) were recorded. The vapour pressure deficit ( $\delta e$ ) was calculated as:

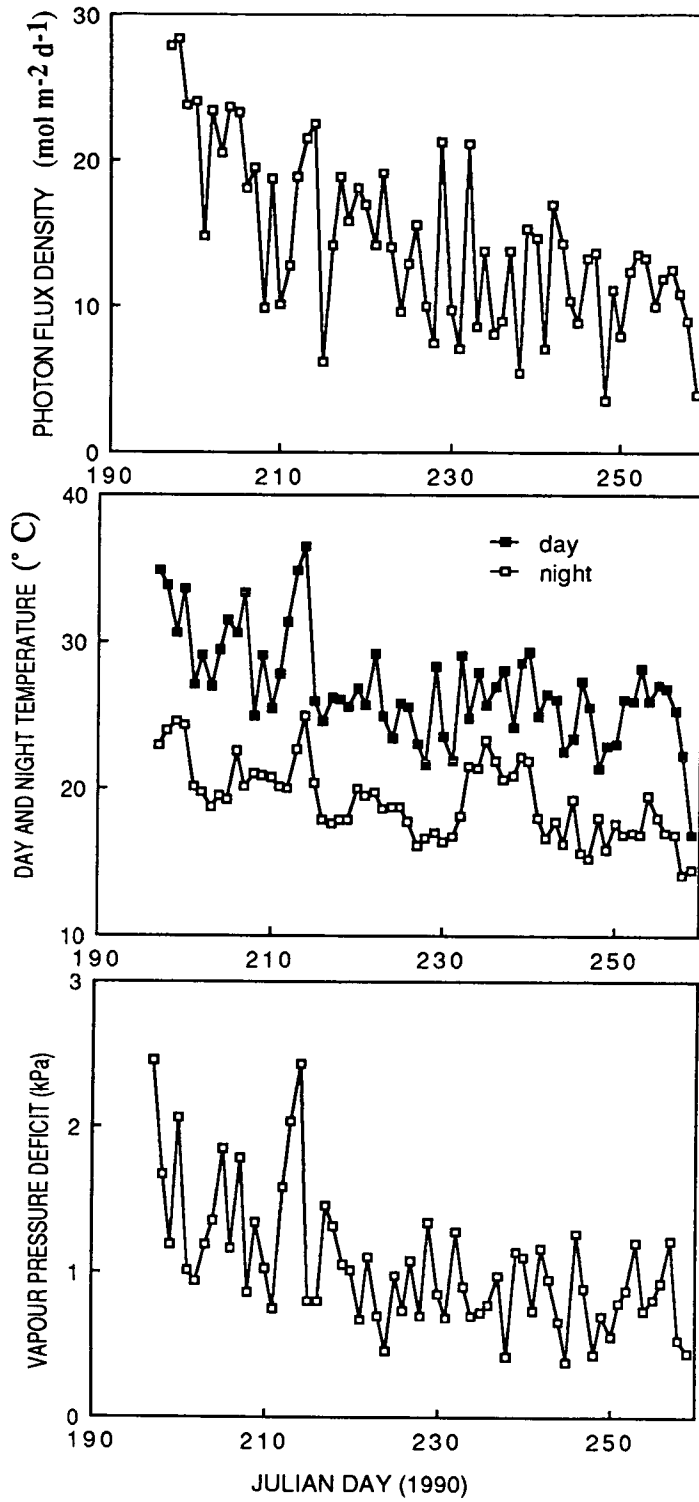
$$\delta e = e_s(T_a) - e$$

where  $e_s(T_a)$  = saturation vapour pressure (kPa) at  $T_a$ , and  $e$  = actual vapour pressure (kPa).

$$e = e_s(T_w) - \gamma(T_a - T_w)$$

where  $e_s(T_w)$  = saturation vapour pressure (kPa) at  $T_w$ , and  $\gamma$  = psychrometric 'constant', taken as 0.08 kPa °C<sup>-1</sup> for the unspirated 'wet-bulb'.

Air temperatures on the bench and inside the 'shade-covers' were also recorded (Table 2.1) by using thermocouples and a datalogger for 8 days during the experimental period. The vapour pressure deficits under 'shade-covers' and on the experimental bench were also determined for 24 h by using thermocouples and a datalogger (Figure 2.4). The aim was to explore the differences in air temperatures and vapour pressure deficits between treatments.



**Figure 2.3:** Total daily photosynthetic photon flux density, mean day and night temperatures, and vapour pressure deficit on the experimental bench during growth of *Hopea odorata* seedlings.

**Table 2.1:** Air temperatures (° C) under different irradiance treatments. Mean of 8 days  $\pm$ SD.

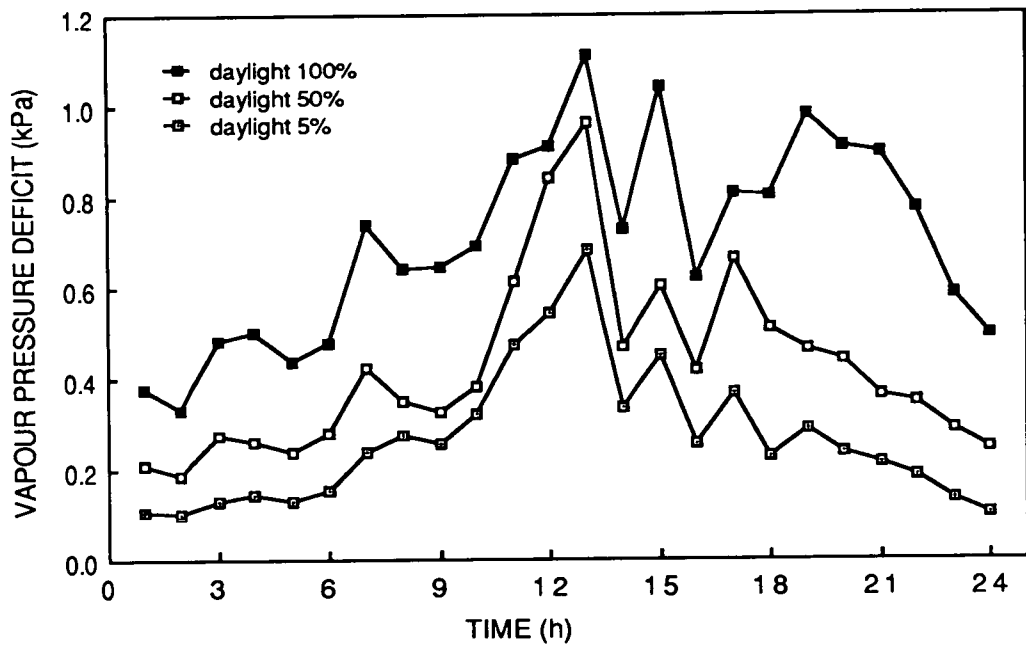
percentage daylight	100		50		5	
	day	night	day	night	day	night
	29.3	18.7	31.6	19.0	31.1	19.1
	(1.6)	(0.9)	(2.0)	(1.0)	(1.8)	(1.0)

### 2.2.5 Measurements of spectral transmittance of the celluloid filter

Filters may fade over weeks and months in bright light. Spectral transmittance changes in the blue green filter after 9 weeks of exposure to light in the glasshouse were measured by using a spectroradiometer (model 6000, Monolight Instruments Ltd., Surrey, UK) comprising of system controller (6810 OSA module, 6811 ADC module, 6830 HeNe module, 6841 Interface Card-IBM At., 6850 Std. software 200-1500 nm), scanning monochromator 6162, tungsten halogen light source with IR filter 6130, integrating sphere 6118, and PMT unit 6171+6173A. The integrating sphere and the stabilised light source (tungsten halogen light source) were used during measurements. The same reference buffer was used for the measurements. The transmittance spectra are presented in Figure 2.5

### 2.2.6 Data collection and analyses

At the start of the experiment ( $t_1$ ), 8 seedlings were harvested. Finally ( $t_2$ ), the seedlings under treatments were harvested after 8 weeks (*Bischofia*) and 9 weeks (*Hopea*) of growth. The seedlings were harvested before their leaves became over-crowded inside the 'shade-covers'. At the time of final harvest, the height of the tallest seedling was 13.3 cm in *Bischofia* and 18.7 cm in *Hopea*. The leaf area of each seedling was determined, and the samples were dried in the oven at 70 °C for dry weight assessments.



**Figure 2.4:** Vapour pressure deficits under the high (100% daylight), medium (50% daylight) and low (5% daylight) irradiance treatments. The vapour pressure deficit was calculated from the hourly 'dry-bulb' and 'wet-bulb' temperatures- the averages of sample readings recorded at 5 min intervals.

From the raw data, relative growth rate (*RGR*) and net assimilation rate (*NAR*) were calculated following pairing and ranking methods described by Hunt (1978). The following equations (Hunt 1978) were used:

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{\ln A_2 - \ln A_1}{A_2 - A_1}$$

( $W_2$  = plant dry weight at  $t_2$ ;  $W_1$  = plant dry weight at  $t_1$ ;  $A_2$  = leaf area at  $t_2$ ;  $A_1$  = leaf area at  $t_1$ ;  $t$  = time).

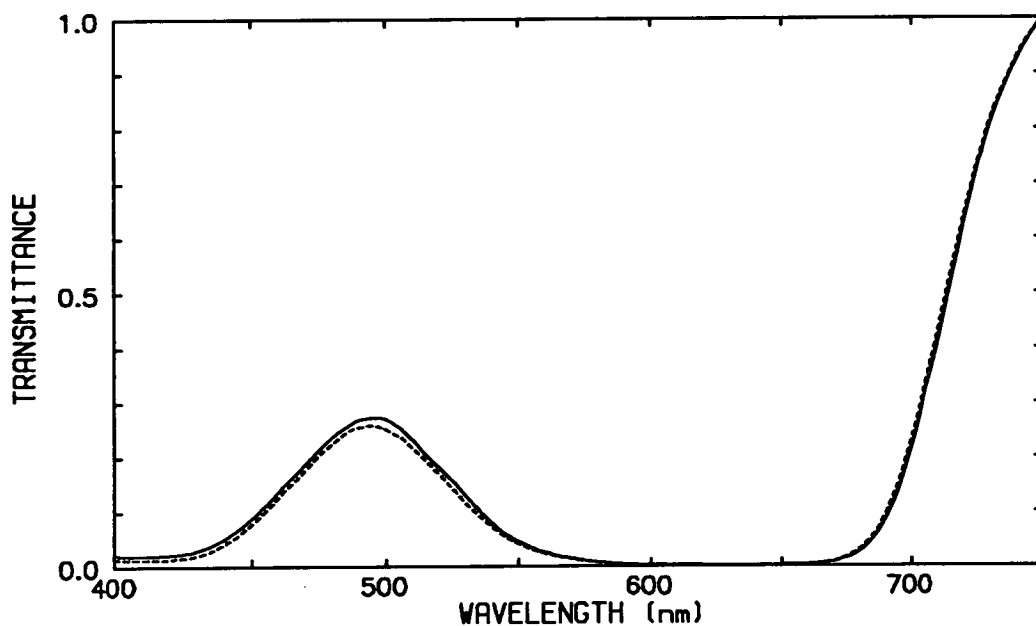
The equation used for *NAR* is only valid when plant dry weight and leaf area are linearly related (Hunt 1978). Linear regression between plant dry weight and leaf area shows that they were highly and positively correlated ( $r = 0.91$ ,  $n = 48$ ,  $P < 0.001$ ).

From the data at the final harvest, the following variables were derived: leaf area ratio (*LAR*, leaf area/total plant dry weight), specific leaf area (*SLA*, leaf area/ leaf dry weight), leaf weight ratio (*LWR*, leaf dry weight/total plant dry weight), stem weight ratio (*SWR*, stem dry weight/ total plant dry weight), root weight ratio (*RWR*, root dry weight/total plant dry weight), and specific stem length (*SSL*, stem length/stem dry weight). The variation in each parameter was explored by analysis of variance.

## 2.3 Results

### 2.3.1 Microclimate

Daily integrated photosynthetic photon flux density (DPF) on the experimental bench ranged mostly between 8 to 21 mol m<sup>-2</sup> d<sup>-1</sup> (Figure 2.2 and 2.3) with a R:FR ratio of 1.08 ±SD 0.04, and this range of irradiance was for the high irradiance treatment. The DPF in the clearing of one tropical rain forest ranged from 13.7 to 33.9 mol m<sup>-2</sup> d<sup>-1</sup> with R:FR ratio of 1.17-1.28 (Chazdon and Fetcher 1984). The DPF range on the experimental bench was lower than that found in Chazdon and Fetcher's clearing. In a large gap (approximately 500 m<sup>2</sup>) with nearly 7 h insolation daily, the relative DPF ranged between 38.6 and 53.4% of the full sun (Popma and Bongers 1991).



**Figure 2.5:** Spectral transmission of the chromoid blue green filter after exposure to light for 9 weeks in the glasshouse (*broken line*) and the unused one (*solid line*). The same reference buffer was used for the measurements. Total transmittance values (400-700 nm), R:FR ratios (660:730  $\pm$  10 nm) and B:R ratios (400-500 nm : 600-700 nm) are: 22.6, 0.004 and 5.23 for unused filter, and 21.2, 0.004 and 4.33 for used one respectively.

Compared to this, the relative DPF on the experimental bench ranged between 23.6 and 61.9% of the tropical DPF (33.9 mol m<sup>-2</sup> d<sup>-1</sup> on the upper range, Chazdon and Fetcher 1984). So, the high irradiance treatment, used in this experiment, was close to the light condition of a large gap in a tropical forest.

Using 'shade-cover' with clear neutral and blue green filters, it was possible to reduce irradiance to 50 and 5% for the medium and the low irradiance levels respectively. As such, the DPF ranged mostly between 4.0 and 10.5 mol m<sup>-2</sup> d<sup>-1</sup>, and 0.40 and 1.05 mol m<sup>-2</sup> d<sup>-1</sup> in the medium and the low irradiance treatments respectively. The transmitted light through the wall of the 'shade-cover' tube also influenced the light quality inside, and hence in the medium irradiance treatment, a R:FR ratio of 0.86 ±0.06 was found. The spectral distribution of light under the blue green filter was significantly deficient in red and blue, just as is vegetational shadelight. Thus, the R:FR ratio under the low irradiance treatment was 0.16 ±0.01. Chazdon and Fetcher (1984) have recorded the DPF range from 3.86 to 13.07 mol m<sup>-2</sup> d<sup>-1</sup> (R:FR ratio, 0.59-1.14) in gaps (400 m<sup>2</sup>), and 0.15 to 1.05 mol m<sup>-2</sup> d<sup>-1</sup> (R:FR ratio, 0.17-0.69) in understorey habitats of a tropical forest. In this way, the high, medium, and low irradiance treatments used in this experiment roughly matched the light conditions in large gaps, small gaps and understorey habitats respectively.

Typically, air temperatures are highest in the large gaps and lowest in the forest understorey habitats. The mean maximum temperatures range from 27 to 34.7 °C in the large gaps, and 24.8 to 28.5 °C in the forest understorey habitats; the mean minimum temperatures range from 16.0 to 24.0 °C in the large gaps, and 17.0 to 21.9 °C in the forest understorey habitats (Bongers *et al.* 1988). The exact simulation in respect of temperatures in the high, medium, and low irradiance treatments was not obtained. There was no significant difference in temperatures between the treatments (Table 2.1). However, the mean day and night temperatures during the experimental period were similar to those recorded in the tropical forests (see Bongers *et al.* 1988).

The saturation vapour pressure deficit ( $\delta e$ ) was highest in the high irradiance treatment and lowest in the low irradiance treatment over the period of 24 h, and in this respect, the medium irradiance treatment was in between the two other irradiance treatments (Figure 2.4). Typically, the  $\delta e$  is highest in the large gaps and lowest in the forest understorey habitats (Bongers *et al.* 1988). So, in this respect, the high, medium and low irradiance treatments were almost similar to the large gaps, the small gaps and the understorey habitats of the tropical forests.

Spectral transmittance of the chromoid blue green filter used in the low light treatment was slightly changed over a period of 9 weeks (Figure 2.5). This change occurred only in the blue part of the spectrum without almost no change in the red resulting in a slight decrease in the B:R ratio. This spectral transmission change of the filter was not significant and hence might be considered negligible.

Because of the differences in seed availability, the species were not studied at the same time. However, the difference in microclimate between the two environments was not appreciable.

### 2.3.2 Growth analysis

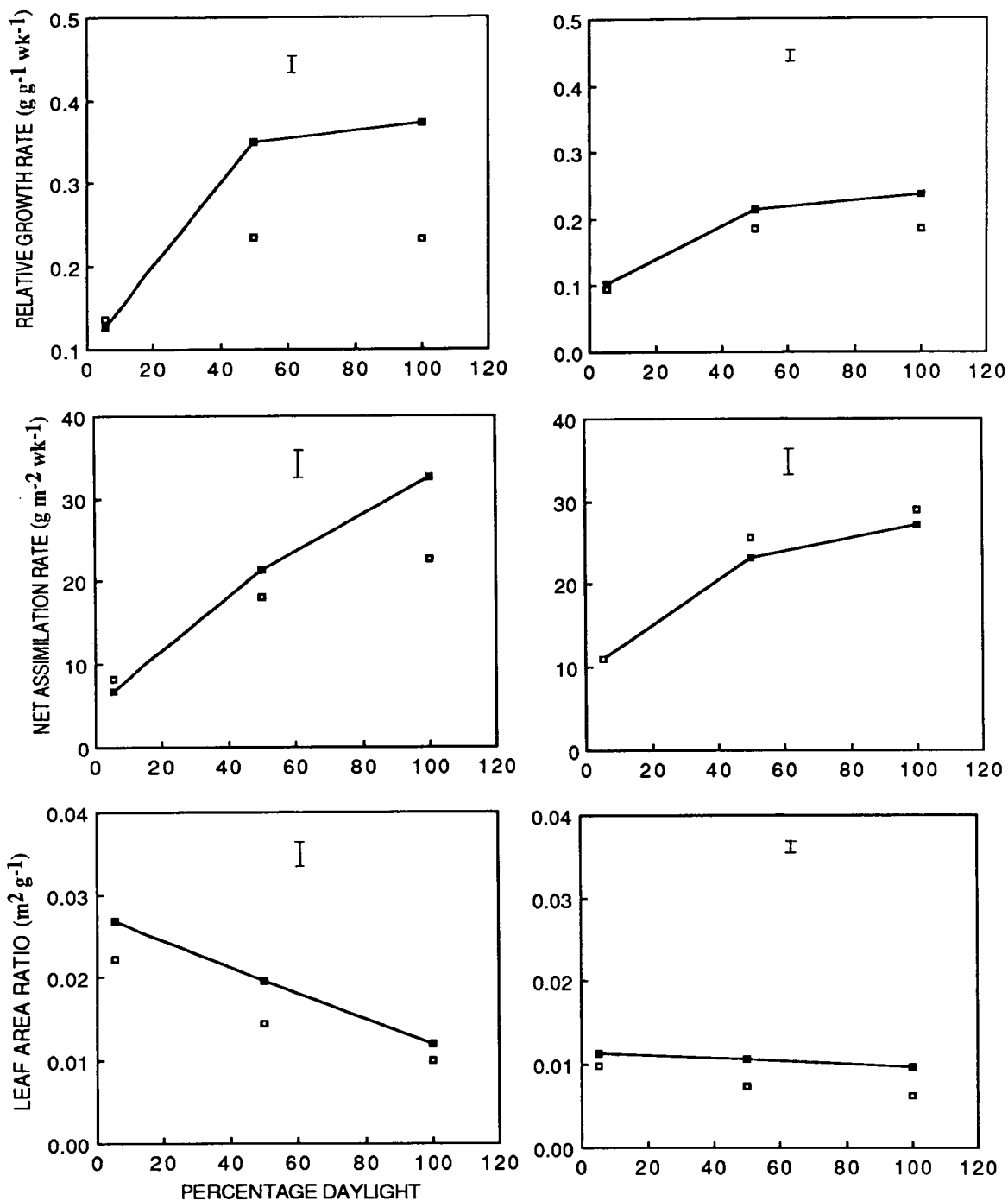
The irradiance treatments used in this study were different in their spectral quality, particularly in R:FR ratios. In general, the *RGR* increased with increasing irradiance levels (Figure 2.6). It was highest in the seedlings of the high irradiance and lowest in the seedlings of the low irradiance treatment. The seedlings of the medium irradiance treatment took the intermediate position. The *RGR* values were higher in *Bischofia* than in *Hopea*. The *RGR* was also affected by nutrient supply. In the high and the medium irradiance levels, seedlings receiving high nutrient supply attained higher *RGR*, the peak being at the high irradiance. At the low irradiance, the *RGR* was not affected by nutrient supply. The differences in *RGR* between high and low nutrient regimes were larger in *Bischofia* than in *Hopea*. Significant interactions of light and nutrient in both the species indicate that the effects on *RGR* were shared by both the factors at the higher irradiances (Table 2.2).

The differences in *RGR* were due to the differences in both *NAR* and *LAR* (Figure 2.6). Light availability had opposing effects on *NAR* and *LAR*. An increase in the photon flux resulted in an increase in *NAR*, but a decrease in *LAR*. Although the seedlings in the high irradiance had a lower *LAR*, the *RGR* of these seedlings was relatively high, because of a relatively high *NAR*. At the low irradiance, the lower *NAR* was partially offset by an increase in *LAR*, particularly in *Bischofia*. In this species, the *NAR* was also significantly affected by nutrient supply at higher irradiance levels. In *Hopea*, this response was not appreciable as is evident from the non-significant *F* for nutrient supply (Table 2.2).

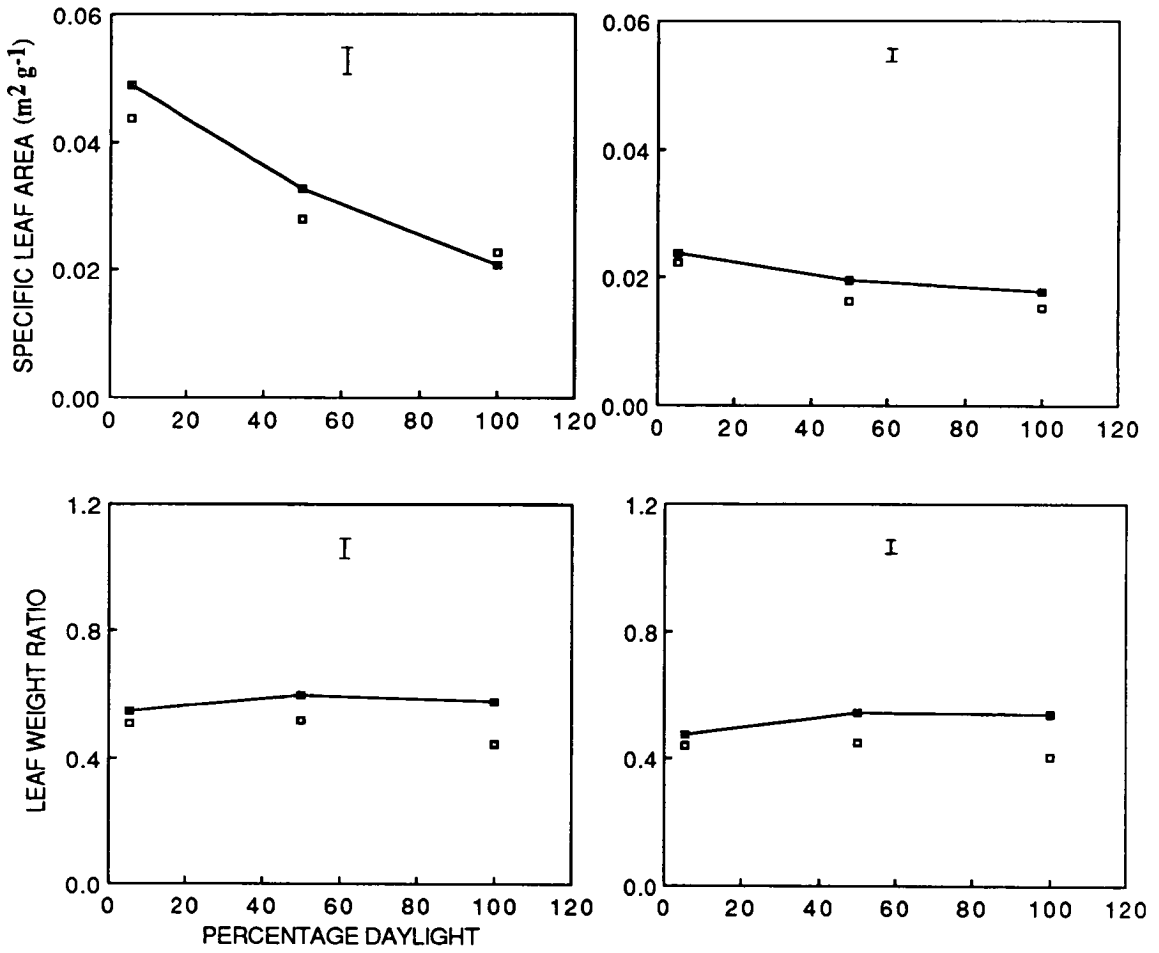
**Table 2.2:** The effect of light and nutrient on growth parameters of *B. javanica* and *H. odorata*. Two-way Block ANOVA (for details see Appendix A). (\*\*\*)  $P < 0.0001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; ns, not significant at  $P < 0.05$ )

Probability : Factors:	P			
	Position	Nutrient	Light	Nutrient*Light
<b><i>B. javanica</i></b>				
relative growth rate	ns	***	***	***
net assimilation rate	ns	***	***	***
leaf area ratio	ns	***	***	ns
specific leaf area	ns	*	***	*
leaf weight ratio	ns	***	ns	ns
specific stem length	ns	**	***	***
stem weight ratio	ns	**	***	*
root weight ratio	ns	***	***	ns
<b><i>H. odorata</i></b>				
relative growth rate	ns	***	***	***
net assimilation rate	ns	ns	***	ns
leaf area ratio	ns	***	***	*
specific leaf area	ns	***	***	ns
leaf weight ratio	ns	***	**	**
specific stem length	ns	*	***	ns
stem weight ratio	ns	*	***	ns
root weight ratio	ns	***	**	***

The differences in *LAR* were the results of the differences of both *SLA* and *LWR* (Figure 2.7). The species showed similar pattern of response in respect of *SLA*. The *SLA* was highest in the low irradiance and lowest in the high irradiance. The seedlings at the medium irradiance took the intermediate position. The interaction of light and nutrient on *SLA* was significant in *Bischofia*, and not in *Hopea* (Table 2.2). The *LWR* was the parameter, which was relatively less affected by both irradiance levels and nutrient supply (Figure 2.7). However, in *Bischofia*, the *LWR* was not significantly affected by irradiance, but influenced by nutrient supply, particularly at higher irradiances. Hence, the interaction of light and nutrient on this parameter in



**Figure 2.6:** Relative biomass growth rate, net assimilation rate, and leaf area ratio of the gap species *B. javanica* (left) and the climax species *H. odorata* (right) under high and  $\square$  low nutrient regimes. Mean of 8 seedlings; the seedlings of *Bischofia* and *Hopea* were grown under treatments for 8 and 9 weeks respectively. Vertical bar in each graph indicates LSD<sub>0.05</sub>.



**Figure 2.7:** Specific leaf area and leaf weight ratio of *B. javanica* (left) and *H. odorata* (right) under ■ high and □ low nutrient regimes. Other particulars are the same as Figure 2.6.

*Bischofia* was not significant. In *Hopea*, on the other hand, the *LWR* was affected by both irradiance levels and nutrient supply, higher being at the high nutrient regime. The interaction of light and nutrient on the *LWR* in *Hopea* was significant.

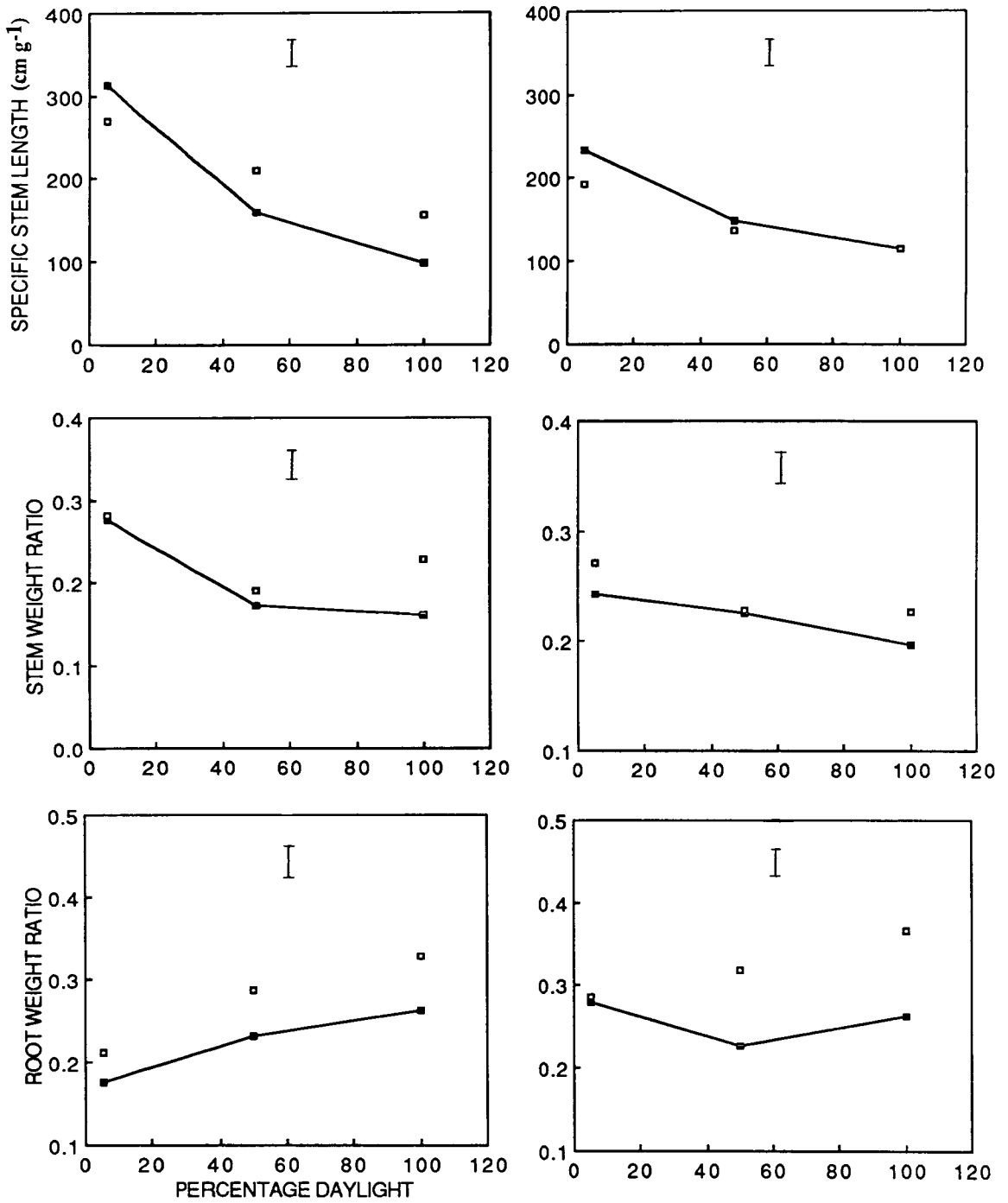
### 2.3.3 Supporting data on growth

Allocation patterns of dry matter were affected by both irradiance levels and nutrient supply (Figure 2.8). The *SSL* was decreased with the increase in irradiance. In the higher irradiance levels, the *SSL* values of the seedlings receiving low nutrient supply were higher than (*Bischofia*), or equal to (*Hopea*), those of the seedlings growing under high nutrient supply, whereas at the low irradiance, the seedlings growing at the low nutrient regime showed lower *SSL* values than their high-nutrient counterparts. This means that the seedlings receiving low nutrient supply produced the same length of stem with more material at the low irradiance.

The irradiance levels had opposing effects on *SWR* and *RWR* (Figure 2.8). An increase in irradiance brought about a decrease in *SWR* and an increase in *RWR*. The *SWR* was increased in seedlings receiving low nutrient supply. In *Bischofia*, this was only significant in the seedlings growing at the high irradiance. The low nutrient supply enhanced root growth as indicated by the increased *RWR*. This enhancement was intensified with the increase in irradiance. In *Bischofia*, the seedlings receiving high nutrient supply also showed an increased root growth with the increase in irradiance, whilst in *Hopea*, this trend disappeared in seedlings receiving high nutrient supply. The leaf expansion was also affected by nutrient supply. The ratio between leaf areas of low to high nutrient treatments were 0.87, 0.28 and 0.27 in *Bischofia*, and 0.83, 0.53 and 0.42 in *Hopea* for low, medium and high irradiance regimes respectively. This means that leaf expansion was restricted by low nutrient supply, a constraint, which intensified as the irradiance increased. This effect of low nutrient supply was more pronounced in *Bischofia* than in *Hopea*.

## 2.4 Discussion

Differences in seedling growth in response to irradiance and nutrient supply are reflected in the differences in *RGR*. At the low irradiance, the species maintained a positive *RGR*, but they displayed contrasting modes of adaptation to the low irradiance. In *Bischofia*, there was a large increase in *LAR* resulting from a significant



**Figure 2.8:** Specific stem length, Stem weight ratio, and root shoot ratio of *B. javanica* (left) and *H. odorata* (right) under ■ high and □ low nutrient regimes. Other particulars are the same as Figure 2.6.

increase in *SLA* (Figure 2.7). It appears that the formation of thinner leaves resulted in a higher *SLA*, and at the same time, there was a decrease in *RWR* (Figure 2.8). The dry matter not used in root growth benefited the stems and petioles, and not the foliage (see also Corré 1983b), and hence the *RWR* decreased in favour *SWR* (Figure 2.8). The seedlings of *Bischofia* showed almost no change in *LWR* over the range of irradiance levels used (Figure 2.7). An increase in *SLA* combined with an almost equal *LWR* led to an increasing *LAR*, and this relative increase in leaf area compensated, at least partially, for a lower photosynthesis per unit leaf area under this irradiance level. In this way, *LAR* made a significant contribution in maintaining a positive *RGR*. The seedlings of both pioneer and non-pioneer species show such an adaptation to low irradiance (Corré 1983b; Popma and Bongers 1988). The value of *LAR*, however, has no effect on *RGR*, when *NAR* is very low, and this generally happens with pioneer seedlings growing in deep forest shade (see Popma and Bongers 1988).

In *Hopea*, *LAR* in the low irradiance followed the slight increase in *SLA*, because *LWR* did not increase, rather it was slightly decreased (Figure 2.7). A small change in *SLA* indicates that the leaf thickness was not largely changed in the low irradiance. As shown in Figure 2.6, a positive *RGR* in the low irradiance was highly influenced by *NAR*, and weakly by *LAR*. Moreover, *NAR* was significantly higher in the seedlings of *Hopea* than those of *Bischofia* at the low irradiance. This indicates that net photosynthetic rate per unit leaf area might be higher in the seedlings of *Hopea* than those of *Bischofia*. The increase in net photosynthetic rate per unit area results from higher amounts of rate-limiting constituents at the chloroplast level, such as RuBP carboxylase (Björkman 1981). This can be achieved by a higher number of chloroplasts per unit leaf area distributed among the relatively denser mesophyll volume. The number of chloroplasts per unit area have been found to increase by an increase in tissue density, and hence the number of chloroplasts per unit area in a leaf with denser mesophyll tissue are relatively high (Syvertsen and Smith 1984) resulting in higher amounts of carboxylation enzymes per unit leaf area (Sinclair *et al.* 1977). Thus, the seedlings of *Hopea* had adapted to a low irradiance by increasing physiological component, *NAR* without bringing about a large change in the morphological component, *LAR*.

These growth responses are in accordance with previous studies on growth of forest tree seedlings (Oberbauer and Strain 1985; Popma and Bongers 1988). Their non-pioneer tree seedlings also displayed a positive *NAR* in the forest understorey habitats.

In examining the relevance of this comparison, it appears that the forest understorey light conditions in those studies were 1% (Oberbauer and Strain 1985) and 1.5% (Popma and Bongers 1988) of DPF under full sun. Compared to their forest understorey light conditions, the low irradiance treatment used in this experiment was roughly 2.1% of the tropical DPF, which is comparable to those encountered in the forest understorey habitats in the tropical forests (see Chazdon and Fetcher 1984). Kwesiga (1984) conducted a similar experiment with the seedlings of pioneer species *Terminalia ivorensis* and climax species *Khaya senegalensis* by growing them under a range of irradiance levels, which were almost the same as the present experiment. The *NARs* displayed by the seedlings of *Bischofia* and *Hopea* at the higher irradiance levels are comparable to those recorded for *Terminalia* and *Khaya* seedlings growing at the high irradiance, but his *Terminalia* seedlings displayed a negative *NAR* under the low light level. Such a negative *NAR* has also been found for the seedlings of pioneer species *Cecropia obtusifolia*, growing under a low irradiance (Popma and Bongers 1988).

At the higher irradiance levels, the low nutrient supply caused a large decrease in *RGR*. A lower *LAR* appeared to be largely responsible for this decrease in the seedlings of *Hopea*, because *NAR* remained almost unaffected with the nutrient supply. But in the seedlings of *Bischofia*, the decreased *RGR* was the consequence of a lower *LAR* and a lower *NAR*. The differences in *NAR* between the seedlings receiving high and low nutrient supply might be attributed, at least partly, to the differences in the photosynthetic capacity of their leaves. The photosynthetic apparatus of the plants receiving a low nutrient supply is markedly less efficient than those receiving a high nutrient supply under the high light conditions (Robson and Parsons 1978). The high nutrient supply, on the other hand, lifts N status of leaves regardless of irradiance during growth, and photosynthetic processes respond accordingly (Thompson *et al.* 1988). The leaves acclimated to the low irradiance require less RuBP carboxylase, hence less N, to sustain light saturated photosynthesis in that light condition compared with the leaves of those seedlings growing in the higher irradiance levels (Björkman 1981). With this advantage, the seedlings receiving low nutrient supply displayed almost the same *NAR* as their high-nutrient counterparts under the low irradiance.

At the high irradiance, the seedlings receiving low nutrient supply produced light green foliage. Thompson *et al.* (1988) found significantly lower amounts of chlorophylls per unit leaf area and hence lower rates of photosynthesis in the seedlings of their high irradiance plus low nutrient regime for *Flindersia brayleyana*. Limitation of

photosynthates may bring about changes in the allocation patterns of dry matter. The substantial increase in *SSL* in the seedlings growing under the higher irradiance levels plus low nutrient supply might be attributed to the lower supply of photosynthates. This means that the seedlings receiving low nutrient supply produced the same length of stem with less material at the higher irradiance levels compared to their high-nutrient counterparts. Conversely, under the low irradiance, the seedlings growing at the high nutrient regime displayed a higher *SSL* than those under the low nutrient treatment. Speculation is that the stem cell enlargement was possibly enhanced with the supply of high nutrient resulting in a relatively low density stem tissue, and hence a higher *SSL* value. As a consequence of low nutrient supply, the *RWR* increased greatly at the expanse of leaf and/or stem (see also Corré 1983c; Thompson *et al.* 1988).

The results reported in this chapter suggest the differential growth responses of the species in response to irradiance levels and nutrient supply. These differential growth responses support the original hypothesis: the growth of gap species is more plastic than that of climax species. At the high irradiance, gap species *Bischofia* will be able to increase growth rate, by a large physiological adjustments rather than by morphological adjustments, and the expression of full potential of the species will be limited in the nutrient-poor sites. The growth of the climax species *Hopea*, on the other hand, will be relatively less plastic in response to nutrient status of soils even at the high irradiance.

## CHAPTER 3

# Stem Extension Growth of Tree Seedlings in Response to Red to Far-red Ratio

### 3.1 Introduction

Vegetational shadelight is different from unfiltered daylight in its spectral quality. Due to preferential absorption of red (R) light by chlorophylls, the photon fluence rate centring on the wavelength 660 nm is reduced in relation to photon fluence rate at 730 nm (Smith 1982). This red to far-red photon (R:FR) ratio is approximately 1.17-1.28 under full sun light and as small as 0.10 under forest canopies (Chazdon and Fetcher 1984; Lee 1987). Under vegetation, a drop in R:FR ratio never occurs without simultaneous reduction in photon fluence rate (Smith 1981). However, in seedling stands formed by plants of similar stature, a reduction in R:FR ratio within the stand may occur without an appreciable change in photon fluence rate. This reduction in R:FR ratio is accounted for by the increased far-red radiation (FR) reflected by leaves of neighbouring plants (Ballaré *et al.* 1989).

There is overwhelming evidence that the photoreceptor implicated in the R and FR morphogenetic responses is phytochrome (review by Smith 1986). Although phytochrome responds to all radiation between approximately 350 and 750 nm, it is in the R and FR wavebands that the quantum effectiveness is greatest (Holmes 1981). As a result, phytochrome acts to detect the changes in the R:FR ratios and modulates plant development accordingly. Species differences have been found for the responses shown to R:FR ratios, and in many cases these are systematically related to their distribution in the natural habitats. Species from open habitats are more responsive to R:FR ratios than the species which typically grow in vegetational shadelight (Morgan and Smith 1979; Smith 1981; Corré 1983a; review by Smith 1986).

Herbaceous species show an increase in stem extension rate (Morgan and Smith 1978; Casal *et al.* 1987; Ballaré *et al.* 1990) with concomitant increase in stem weight ratio (Corré 1983a) and decrease in leaf area ratio (Smith 1981) in response to low R:FR ratio. This capacity for morphogenetic adjustments appears to have a adaptative value. In a competitive situation for light, such as fast-growing seedling stands, an increased

stem extension in response to low R:FR ratio may result in young leaves reaching a better lit stratum within the seedling canopy.

There have been very few studies on the morphogenetic responses to low R:FR ratio for tree seedlings (Morgan *et al.* 1983; Kwesiga and Grace 1986; Warrington *et al.* 1989) and even fewer for seedlings, which are more than a few months old (Warrington *et al.* 1989). However, in studies of the growth responses of *Pinus radiata* to artificial light spectra, a large effect on stem extension growth was observed that has been directly related to the R:FR ratio and thus to the phytochrome photoequilibrium of the light environment (Morgan *et al.* 1983; Warrington *et al.* 1989). Plant growth responses other than stem extension changes have been shown to be differentially affected by R:FR ratios between seedlings of pioneer and climax tree species from tropical forests (Kwesiga and Grace 1986).

In this study, the growth responses of *Anthocephalus chinensis* Rich. ex Walp. and *Hopea odorata* Roxb. to R:FR ratios are reported. The species are contrasting in their natural distribution. *Anthocephalus* is a fast-growing, small-seeded pioneer species (Fox 1971), whilst *Hopea* is a relatively slow-growing, big-seeded climax species (Troup 1921).

The whole study comprised of a series of experiments: (a) experiment 1, a long-term experiment, where the seedlings were grown under high, medium, and low R:FR ratio regimes, separating the R:FR ratios from the photosynthetic photon flux density (PPFD); (b) experiment 2, stem extension rates were measured keeping the stem or leaf under low and high R:FR ratios; (c) experiment 3, stem extension rates were measured by irradiating the internodes of young seedlings with supplementary FR.

## **3.2 Materials and Methods**

### **3.2.1 Experiment 1: Seedling growth under contrasting R:FR ratio regimes**

The methods of raising seedlings in this experiment were similar to those described in Section 2.2.1, except that this experiment was carried out in a growth cabinet. The potted seedlings were placed on the experimental bench and randomly assigned to treatments. There were 3 treatments, each with 6 seedlings: high, medium, and low

R:FR ratio regimes. At this point, the *Anthocephalus* seedlings were 6 months old, but very small; the height ranged between 0.8 and 1.1 cm, and most of the seedlings had 3 pairs of leaves. *Hopea* seedlings were of about 1 month old; their height ranged from 6 to 9 cm, and their first true leaf was not fully expanded.

### **3.2.1.1 Nutrition**

The materials and methods of nutrition were similar to those described in Section 2.2.2, except that nutrient solution used here to feed the seedlings provided 60 mg l<sup>-1</sup> of N. Application of nutrient solution was regular- once a day.

### **3.2.1.2 'Shade-covers' and the light climate they produced**

An individual 'shade-cover' was used to shade each seedling. The 'shade-cover' was made following method illustrated in Figure 2.1. Three types of filters (Strand Lighting, Middlesex, UK) namely, silver black scrim (cinelux 270), moss green celluloid filter (chromoid 122) and blue green celluloid filter (chromoid 116) were used to obtain high, medium and low R:FR ratio regimes respectively. Neutral muslin and nylon fabrics were used, where appropriate, to adjust the PPFD under 'shade-covers' so that the treatments differed in R:FR ratios and not PPFD (see Table 3.1).

A quantum sensor (Li-190SB Li-cor., Inc. Lincoln, USA) was used to measure the PPFD. Light spectra were measured by using a spectroradiometer (model 6000, Monolight Instruments Ltd., Surrey, UK) comprising of system controller (6810 OSA module, 6811 ADC module, 6830 HeNe module, 6841 I/Face Card-IBM At., 6850 Std. software 200-1500 nm), scanning monochrometer 6162, tungsten halogen light source with IR filter 6130, integrating sphere 6118, and PMT unit 6171+6173A. The integrating sphere was used during measurements. Glass fibre optic light guide, instead of integrating sphere, was used when light spectra at the internode level within 'shade cover' were measured. R:FR ratios, defined as the ratio of photon fluence rate in 10 nm wide wavebands centred at 660 nm to 730 nm, were calculated from the light spectra. The PPFDs and R:FR ratios under 'shade-covers' are shown in Table 3.1 and the light spectra of R:FR ratio regimes are presented in Figure 3.1.

**Table 3.1:** The materials used for the 'shade-covers' and light climate they produced in the growth cabinet. R:FR photon ratio = 660:730  $\pm$ 10 nm calculated from light spectra. PPFDs under all the 'shade-covers' were measured (6 per treatment).

Filters	PPFD $\pm$ SD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	R:FR ratio
1 layer silver black scrim, 1 layer plastic netting, 2 layers muslin, 4 layers nylon fabric	39.8 $\pm$ 1.6	1.01
1 layer moss green filter, 3 layers nylon fabric	39.8 $\pm$ 1.6	0.32
1 layer blue green filter	39.3 $\pm$ 0.8	0.07

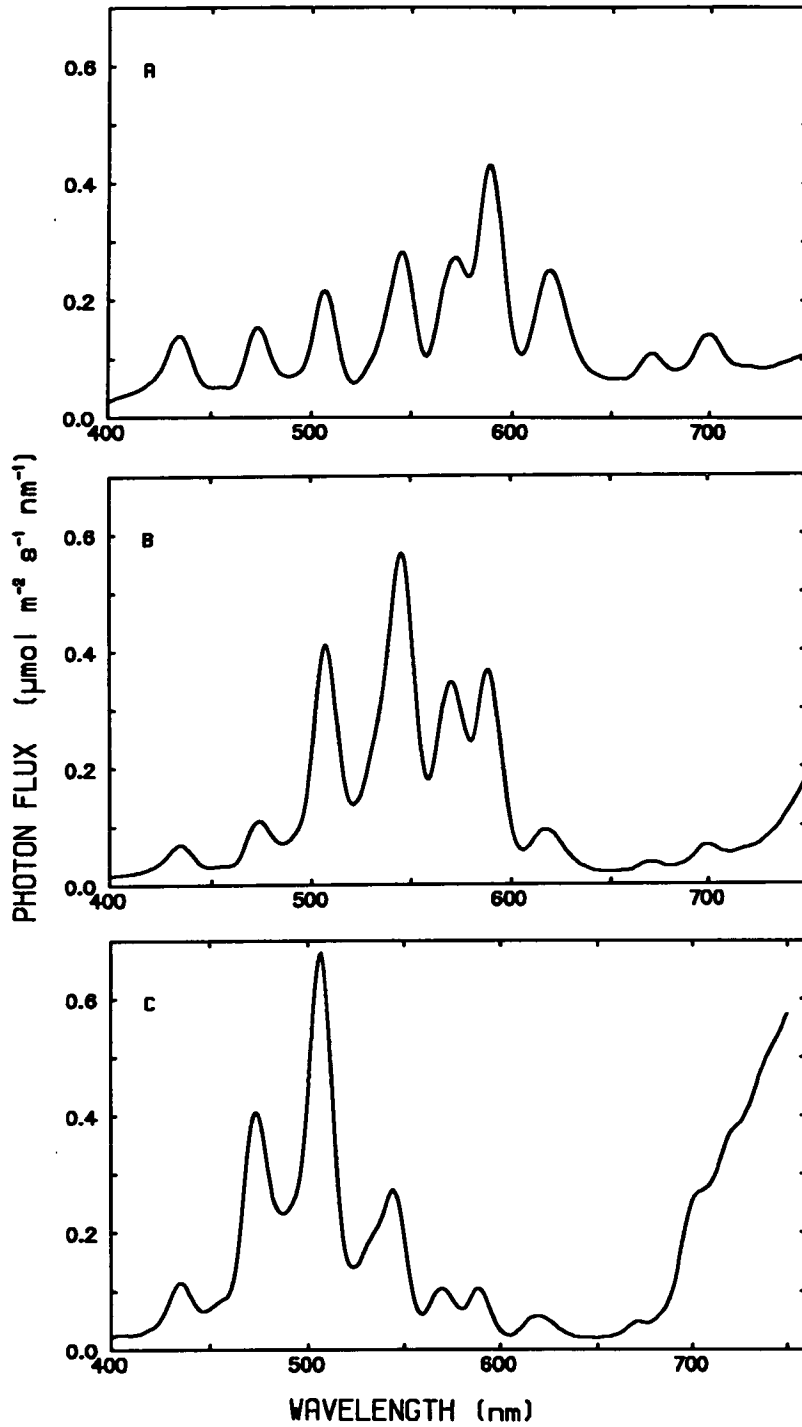
### 3.2.1.3 Growth cabinet and growth conditions

The lamps used in the growth cabinet were metal halides fluorescent kolorarc 400 W MBIF/H with 60 W incandescent bulbs (Thorn Lighting, London, UK). On the bench, the PPFD was 650  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Other growth conditions were: 12 h photoperiod, day and night temperatures 26 and 20  $\pm$ 0.5  $^{\circ}\text{C}$  respectively, relative humidity 85 $\pm$ 5%; the air flow of about 1  $\text{m s}^{-1}$  was horizontal and turbulent in the cabinet.

### 3.2.1.4 Data collection and analyses

Seedling height was recorded weekly throughout the experiment. At the end of 4 weeks of growth, the seedlings were harvested. Leaf area and the dry weight of root, stem and leaf of each seedling were recorded following methods described in Section 2.2.6.

Using the data, the following variables were derived: stem weight ratio (*SWR*, stem dry weight/total plant dry weight), specific stem length (*SSL*, stem length/stem dry weight), leaf area ratio (*LAR*, leaf area/total plant dry weight), specific leaf area (*SLA*, leaf area/leaf dry weight), and leaf weight ratio (*LWR*, leaf dry weight/total plant dry weight). The variation in each parameter was explored by analysis of variance.



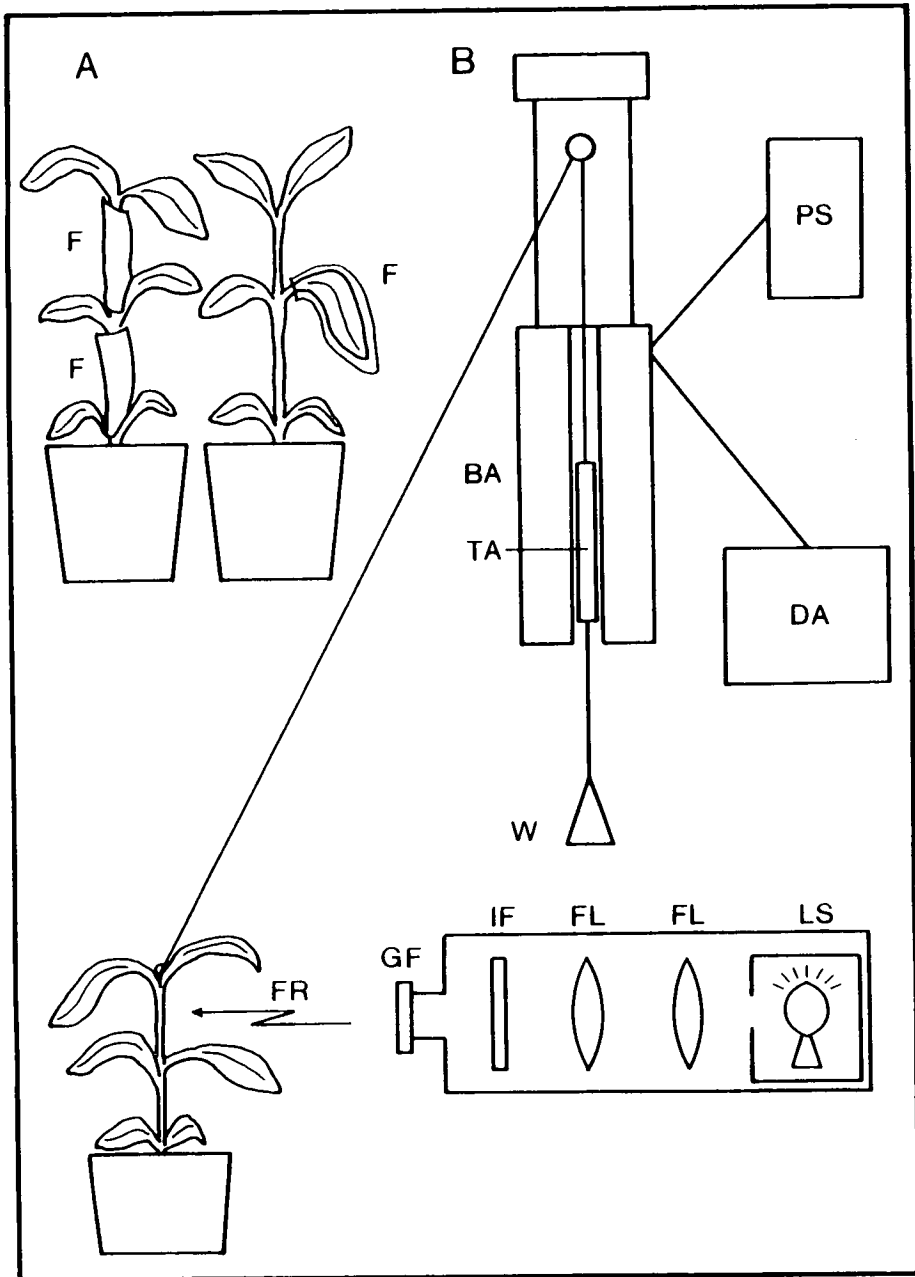
**Figure 3.1:** Spectral photon flux density within 'shade-covers' inside growth cabinet. A: high R:FR ratio, 1.01; B: medium R:FR ratio, 0.32; and C: low R:FR ratio, 0.07

### 3.2.2 Experiment 2: Measurements of stem extension rate by LVDT

The seedlings, after transfer from seed-trays to the growth tube as described in Section 2.2.1, were grown under white light (WL) for 2 weeks before measurements. PPF on the bench was reduced to about  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  putting muslin fabric just below the ceiling. Other growth conditions were the same as described in Section 3.2.1.3. At the time of the measurements, *Anthocephalus* seedlings were 3 to 4 cm tall with 3 or 4 pairs of leaves; *Hopea* seedlings were 7 to 9 cm tall with 2 or 3 leaves. By this time, the hypocotyl extension growth ceased and the extension growth was mostly confined to the internodes.

Stem extension rate was measured by a linear voltage displacement transducer (LVDT, Sangamo DG/5.0/S; Schlumberger, West Sussex, UK) as illustrated in Figure 3.2. Two LVDTs were used at the same time. Each LVDT was mounted in an adjustable supporting stand, with the armature running freely through the barrel. The armature was attached to a cotton thread stretched at both the ends. The seedling was fastened to the one end (tied around just below the stem tip) and a counterbalance weight was attached to the other end of the armature. The thread was passed through a small pulley wheel for the free movement of the armature. The counterbalance weight eliminated all the slack in the system. Anti-vibration pads/mattings were used, wherever necessary, to minimise the vibration noise. During the measurements, the growth cabinet was not opened, and the data were recorded by digital multimeter (model 1905 a, Thurlby Electronics Ltd., England, UK) placing it in an adjacent room. Although the LVDT allowed very fine resolution of displacement, the absolute extension rates of the seedlings were so low that fine resolution of the timebase was not possible. The extension rates were calculated over 10-minute time periods in order to avoid vibration-caused signal noise.

Neutral clear celluloid filter (cinelux 430) and blue green celluloid filter (chromoid 116) were used to simulate high and low R:FR ratio regimes respectively (Table 3.2). They were employed in such a way as to target individual parts of the plant. Each internode was covered separately, and care was taken to keep the entire internode under the filter (Figure 3.2). Similarly, each leaf was put into a pocket of filters leaving the edges open for ventilation (Figure 3.2). To eliminate the blue part of the spectrum, an orange celluloid filter (cinelux 458) was used with the blue green filter (Table 3.2). Muslin and nylon fabrics were used to adjust the PPF (Table 3.2). The light spectra under



**Figure 3.2:** Diagrammatic representation of a linear voltage displacement transducer (LVDT). **A:** internode or leaf covered with celluloid filter for the measurement of stem extension rates of seedlings; **B:** LVDT and the source of far-red radiation. BA = transducer barrel; DA = data acquisition; F = celluloid filter; FL = focussing lens; FR = far-red radiation; GF = glass filter; IF = interference filter; LS = light sources; PS = power supply; TA = transducer armature; W = weight.

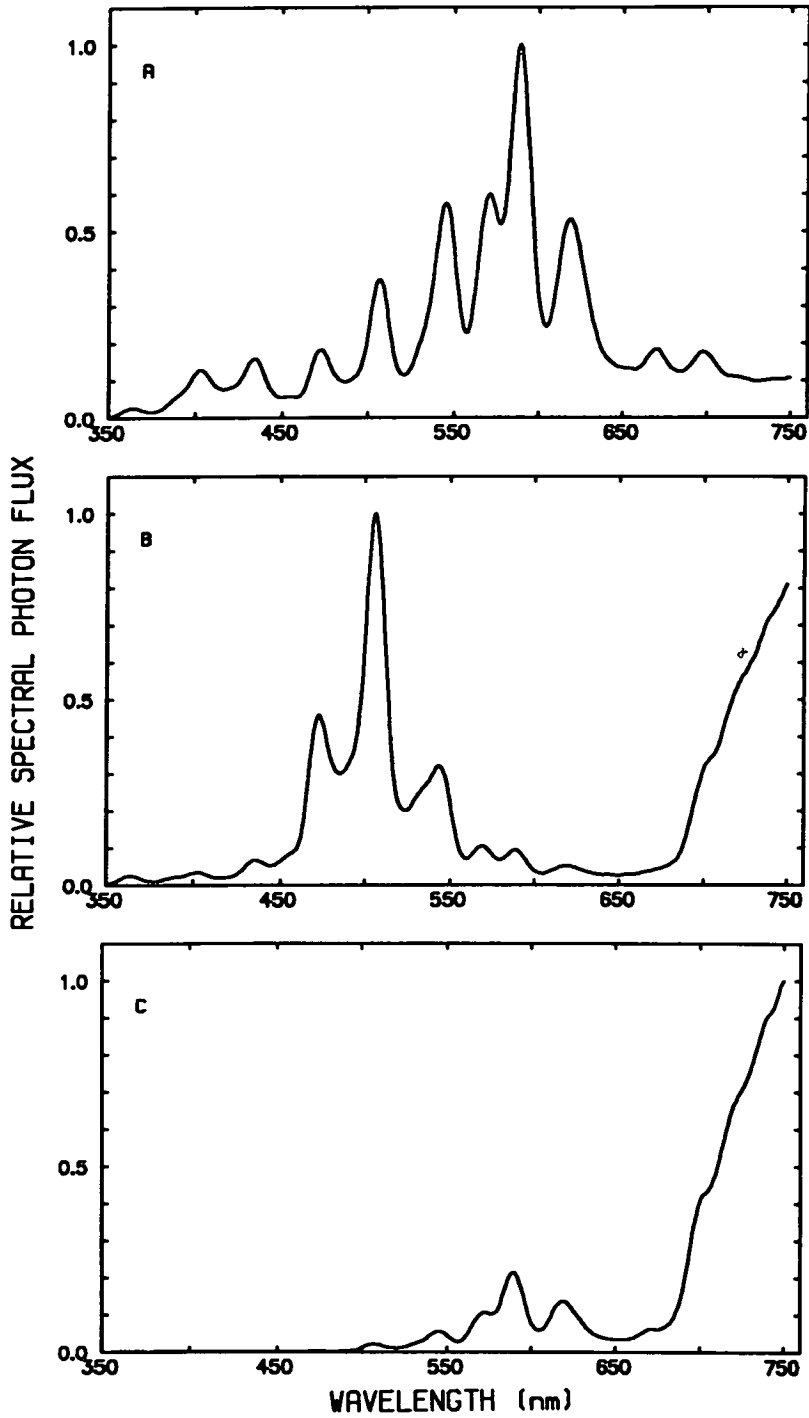
**Table 3.2:** The materials used for shading the internode or the leaf and the light climate they produced in the growth cabinet. Particulars on R:FR ratios are the same as Table 3.1.

Organ exposed	Filters	PPFD $\mu\text{mol m}^{-2} \text{s}^{-1}$	R:FR ratio
internode	1 layer clear neutral filter, 1 layer muslin fabric	36.4	1.45
"	1 layer blue green filter	35.9	0.05
"	1 layer blue green filter, 1 layer deep orange filter	11.2	0.05-blue
"	1 layer blue green, 1 layer clear filter, 1 layer muslin fabric	10.6	0.05+blue
leaf	1 layer clear filter, 2 layers muslin	45.0	1.45
"	1 layer blue green filter	44.8	0.07

the treatments (Figure 3.3) were measured as described in Section 3.2.1.2. The light spectra for leaf treatments were similar to those shown for high and low R:FR ratio regimes (Figure 3.1).

### 3.2.3 Experiment 3: Extension rates by irradiating stem with supplementary FR

The measurement procedure and the growth conditions here were the same as described in the preceding section. The terminal internode of the seedling was irradiated by FR from a slide projector (Pradovit, Leitz, West Germany) mounted with an interference filter (FRmax = 730 nm, 25 mm diameter; Glen Spectra, Middlesex, UK). The light source within the projector was from a 250 W tungsten-halogen bulb; the beam was then focused by a lens system, via an aluminium iris, onto the interference filter (see Figure 3.2). The whole assembly was cooled by an electric fan within the system. The incoming FR beam was further passed through a neutral glass filter (Frew-Smith Ltd., Irvine, UK) to reduce the FR fluence rate to desired level. Light spectra of the background white light (WL) and FR are shown in Figure 3.4.



**Figure 3.3:** Relative spectral photon flux of light transmitted through celluloid filters (light that laterally impinged the internode). A: clear neutral filter, R:FR ratio 1.45; B: blue green filter, R:FR ratio 0.05; C: blue green filter plus deep orange filter, R:FR ratio 0.05

The terminal internode was irradiated with FR from one side only, but the opposite side of the internode was also illuminated by reflecting FR from an aluminium foil placed behind the stem. The seedling was completely exposed to WL. At the time of irradiation with FR, it was not possible to exclude, at least, a part of terminal pair of leaves from irradiation of added FR. The period of measurement before FR treatment was 1 h and the period of FR treatment was also 1 h. The measurement was continued for another 2 h after FR switch-off. During the measurement, the growth cabinet was not opened, and the projector switch turned 'on' or 'off' from the adjacent room (see also Section 3.2.2).

### 3.3 Results

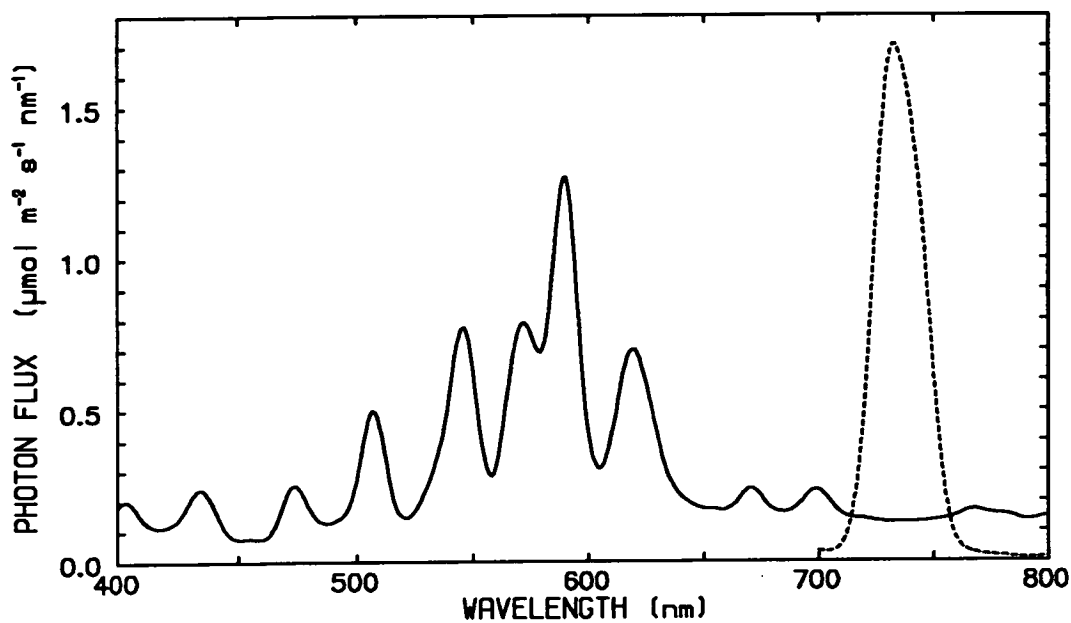
#### 3.3.1 Light climate

By using celluloid filters, it was possible to simulate a range of light spectra within the growth cabinet (Figure 3.1), resulting in transmitted light with different R:FR ratios under 'shade-covers' (Table 3.1). The silver black scrim is porous, and hence does not change the spectral quality of transmitted light under it, whereas the moss green and blue green celluloid filters markedly affect the R:FR ratios. Muslin and nylon fabrics have almost no effect on R:FR ratio. Thus, by using the neutral fabrics, it was possible to obtain almost same PPFD under different R:FR ratio regimes (Table 3.1).

Within the 'shade-cover', the transmitted light through the filters used was influenced by that through the tube wall as well. However, the R:FR ratio values under treatments used here were clearly different from each other, and almost similar to those found in natural conditions (see Chazdon and Fetcher 1984; Lee 1987).

Similarly in the experiment 2, by using neutral clear filter and blue green filter, it was possible to simulate high and low R:FR ratios respectively (Table 3.2). The blue part of the spectrum of the transmitted light through the blue green filter was eliminated by using an orange filter with it (Figure 3.3). Thus, it was possible to eliminate the possible effect of the blue part of the spectrum from that of a low R:FR ratio.

In experiment 3, the FR irradiation at a photon fluence rate of about  $45 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $730 \pm 25 \text{ nm}$ ) in a background WL of about  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  resulted in a drop in R:FR ratio from 1.45 (WL R:FR ratio) to 0.13 (see Figure 3.4). In this way, it was



**Figure 3.4:** Spectral photon flux density of white light (WL) and supplementary FR. Fluence rate of supplementary FR at about  $45 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $730 \pm 25 \text{ nm}$ ) to a background WL of about  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  reduced the R:FR ratio ( $660:730 \pm 10 \text{ nm}$ ) from 1.45 (WL R:FR ratio) to 0.13 at internode level.

possible to change the R:FR ratio at the internode level in a background WL in the growth cabinet.

### **3.3.2 Stem extension growth on long-term whole plant exposure to R:FR ratios**

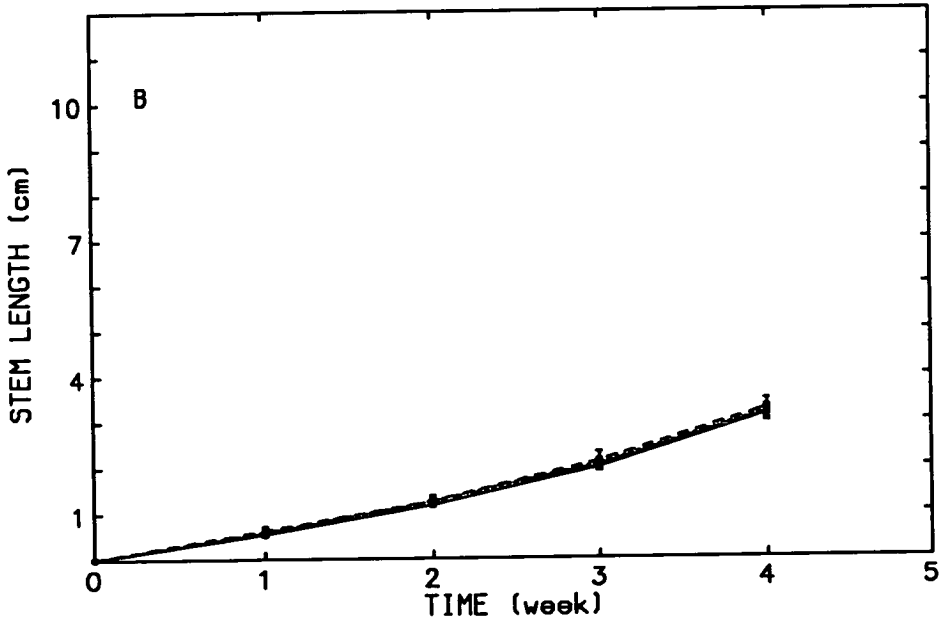
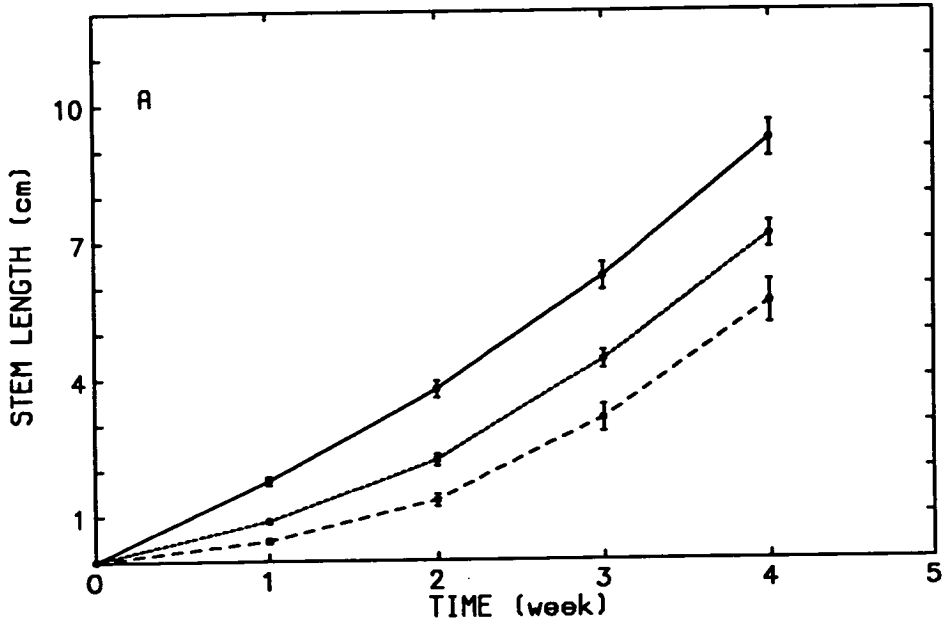
The seedlings of pioneer species *Anthocephalus* and climax species *Hopea* displayed differential stem extension growth in response to R:FR ratios (Figure 3.5). For *Anthocephalus*, stem extension growth increased with a decrease in R:FR ratio. The seedlings, growing under high R:FR ratio, showed lowest extension growth, while the extension growth was highest in seedlings growing at a low R:FR ratio. The medium R:FR ratio seedlings occupied intermediate position.

This scenario of extension growth in *Anthocephalus* became slightly different when the weekly increases in stem length were examined (Figure 3.6). At low R:FR ratio regime, the increases in stem length were largely highest at the beginning of the experiment. The seedlings under this treatment also maintained a higher rate of extension growth throughout the experiment. The extension rate at high and medium R:FR ratio regimes was enhanced dramatically after 2 weeks of growth, and towards the end of the experiment, the extension rates between these treatments were not significantly different.

For *Hopea*, the seedlings growing under different R:FR ratio regimes did not show significantly different stem extension growth (Figure 3.5), and even the weekly increases in stem length did not significantly differ from one treatment to another (Figure 3.6).

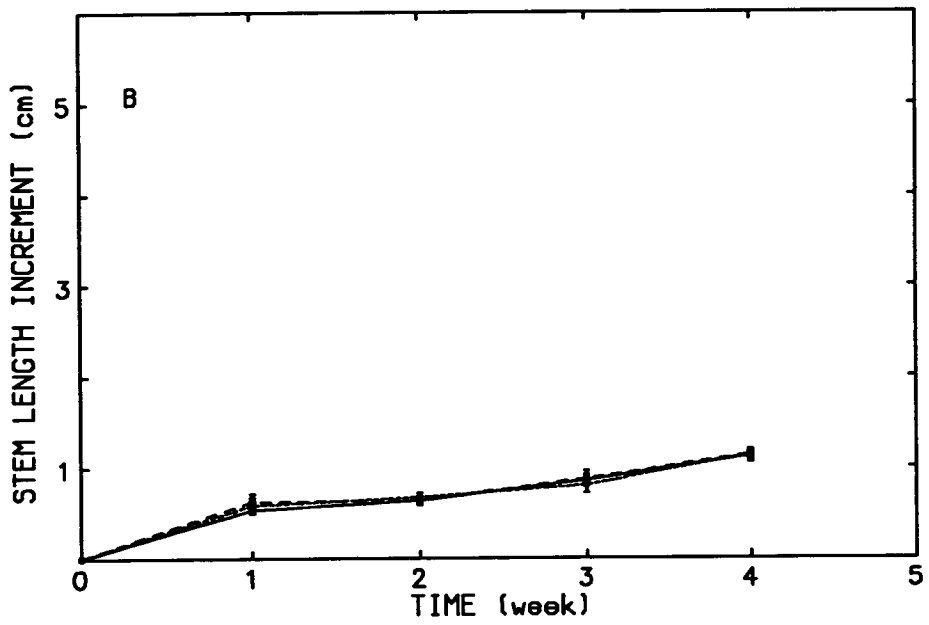
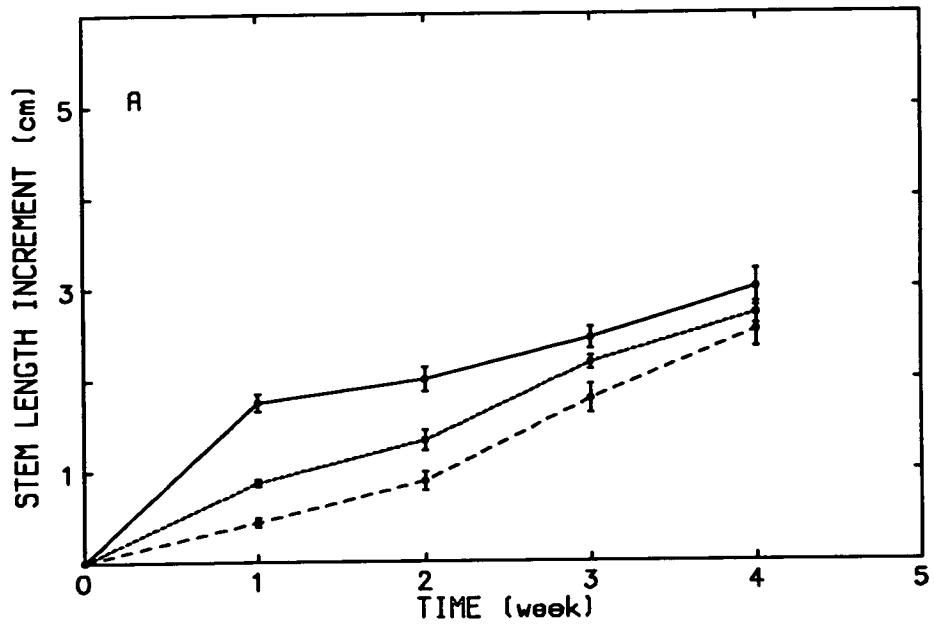
### **3.3.3 Stem extension rates on short-term exposure of stem or leaf to contrasting R:FR ratios**

Short-term exposure of the stem to high or low R:FR ratios again showed differential extension rates for *Anthocephalus*, and not for *Hopea* (Table 3.3). *Anthocephalus* seedlings exhibited substantially increased rates of stem extension, when the internodes were subjected to a low R:FR ratio in comparison to the rates displayed when the internodes were kept at a high R:FR ratio. Further, when the leaf instead of internode was irradiated by a beam of high or low R:FR ratio, the seedlings showed



**Figure 3.5:** Stem extension growth of *Anthocephalus chinensis* (A) and *Hopea odorata* (B) seedlings under high (long broken line), medium (short broken line) and low (solid line) R:FR ratio regimes. Means of 6 seedlings; vertical bar indicates standard error of mean.





**Figure 3.6:** Weekly increment in stem length of *Anthocephalus chinensis* (A) and *Hopea odorata* (B) seedlings under R:FR ratio regimes. Other particulars are as in Figure 3.5.

no significant difference in stem extension rates. This indicates that the internodes might act as the effective site of perception and response to a low R:FR ratio.

The response of an increased stem extension to a low R:FR ratio for *Anthocephalus* became more clear when the blue light was eliminated from the low R:FR ratio beam by using a deep orange filter with the blue green filter. A beam with a low R:FR ratio without blue, directed at the internode did not elicit significantly different extension rates from those displayed when the internodes were exposed to a beam with a low R:FR ratio plus blue light (Table 3.3).

**Table 3.3:** Stem extension rates ( $\mu\text{m min}^{-1}$ ) of *Anthocephalus chinensis* and *Hopea odorata* seedlings. Stem extension rate = slope of regression lines (regression of stem length upon time); mean of 4 seedlings  $\pm$  SE; Paired *t*-test was used to assess differences between treatment means.

organ exposed	R:FR ratio high	R:FR ratio low	Low R:FR ratio +blue	Low R:FR ratio -blue	<i>t</i> value	<i>P</i> (2-tail)
<i>H. odorata</i>						
internode	1.12 $\pm$ 0.17	1.16 $\pm$ 0.13			0.42	0.702
<i>A. chinensis</i>						
internode	1.24 $\pm$ 0.13	2.37 $\pm$ 0.13			4.85	0.017
"			2.64 $\pm$ 0.33	2.66 $\pm$ 0.32	0.65	0.564
leaf	1.34 $\pm$ 0.25	1.33 $\pm$ 0.25			1.49	0.233

### 3.3.4 Response of extension rate to supplementary FR

The onset of FR was followed by a marked increase in extension rate, reaching a sharp peak within 30 min, after which a sharp decline was observed (Figure 3.7). A second peak was seen, reaching a level intermediate between the WL rate and that of the first peak, by about 50 min after the onset of FR. There was a decrease in extension rate following FR switch-off, but this decrease was more gradual than the increase following the onset of FR. The mean time for extension rate to return to the value

observed before the FR switch-on was approximately 30 min. During the FR treatment, the extension rates were invariably higher than the WL rates.

From Figure 3.7, it appears that there was a carry-over effect after the FR switch-off. But all the six seedlings measured did not show such response. Moreover, analysis of variance of the slopes of the regression lines shows that there was no significant difference between the WL rates before switch-on and after FR switch-off, whilst the rates during FR treatment were substantially higher (Table 3.4).

**Table 3.4:** Stem extension rates ( $\mu\text{m min}^{-1}$ ) of *Anthocephalus chinensis* seedlings (terminal internode was irradiated with supplementary FR in background white light, WL). Stem extension rate = slope of regression lines (regression of stem length upon time); Mean of 6 seedlings  $\pm$ SE. Means preceded by the same letter are not significantly different from each other at  $P < 0.05$  (ANOVA and Duncan's multiple range test).

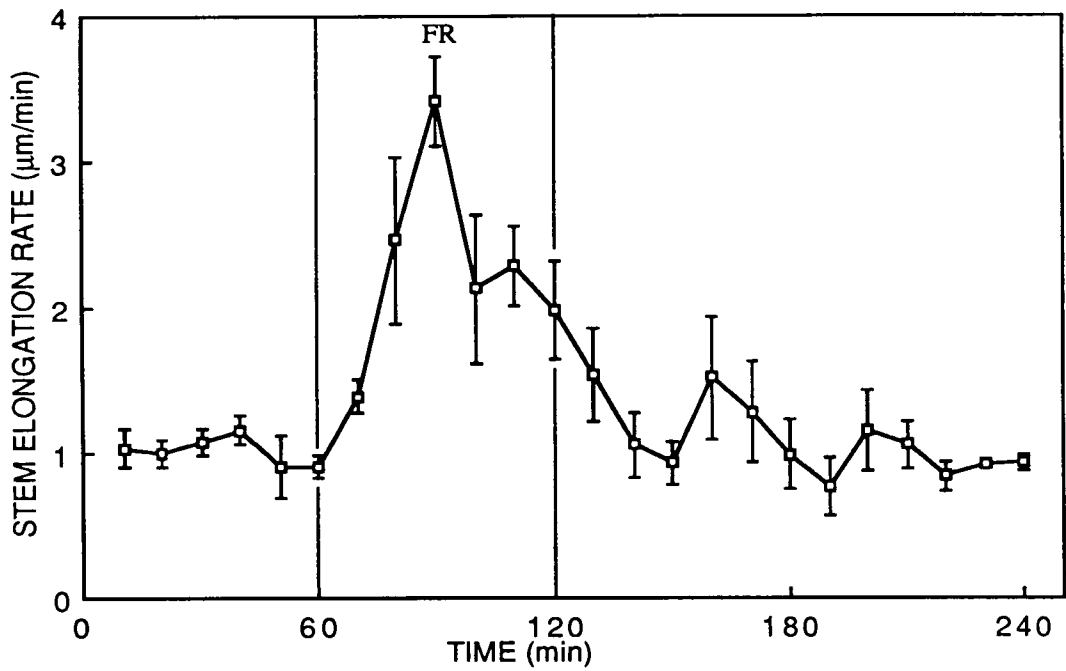
WL rate before treatment	Rate during FR treatment	WL rate after treatment	<i>F</i>	<i>P</i>
b 1.06 $\pm$ 0.10	a 2.48 $\pm$ 0.33	b 1.04 $\pm$ 0.09	16.0	0.0001

### 3.3.5 Other morphological responses on long-term exposure to R:FR ratios

*Anthocephalus* seedlings displayed large morphogenetic adjustments in response to low R:FR ratio, whilst *Hopea* seedlings did not show any such marked adjustment to R:FR ratios (Table 3.5). In *Anthocephalus*, *SWR* increased greatly under low R:FR ratio, and it was lowest in the high R:FR ratio. Conversely, *LAR* was highest at the high R:FR ratio and lowest at the low R:FR ratio. Further, *SLA* was not significantly affected by R:FR ratio, but *LWR* was largely influenced in accordance with *LAR*. *SSL*, which is usually sensitive to the levels of photon irradiance, was not significantly affected by R:FR ratios. Plants subjected to the medium R:FR ratio treatment did not significantly differ from those in the low R:FR ratio regime in these morphological responses.

**Table 3.5:** Characteristics of *Anthocephalus chinensis* and *Hopea odorata* seedlings grown under different R:FR ratios. Mean  $\pm$ SE of 6 seedlings. Means preceded by the same letter are not significantly different from each other at  $P < 0.05$  (ANOVA and Duncan's multiple range test).

R:FR ratios	high	medium	low	F	P
<b><i>A. chinensis</i></b>					
stem weight ratio, SWR (mg mg <sup>-1</sup> )	b 0.180 (0.014)	a 0.220 (0.008)	a 0.224 (0.008)	5.6	0.015
leaf area ratio, LAR (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.366 (0.013)	b 0.275 (0.010)	b 0.302 (0.013)	15.5	0.0001
specific leaf area, SLA (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.643 (0.012)	a 0.642 (0.023)	a 0.594 (0.021)	2.1	0.153
leaf weight ratio, LWR	a 0.655 (0.019)	b 0.612 (0.005)	b 0.613 (0.007)	4.1	0.038
specific stem length, SSL (cm mg <sup>-1</sup> )	a 0.298 (0.013)	a 0.313 (0.008)	a 0.279 (0.008)	2.9	0.086
<b><i>H. odorata</i></b>					
stem weight ratio, SWR (mg mg <sup>-1</sup> )	a 0.237 (0.011)	a 0.247 (0.007)	a 0.240 (0.008)	0.3	0.691
leaf area ratio, LAR (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.161 (0.010)	a 0.164 (0.005)	a 0.163 (0.008)	0.03	0.968
specific leaf area, SLA (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.310 (0.012)	a 0.314 (0.008)	a 0.312 (0.008)	0.05	0.953
leaf weight ratio, LWR	a 0.519 (0.020)	a 0.520 (0.005)	a 0.523 (0.012)	0.03	0.970
specific stem length, SSL (cm mg <sup>-1</sup> )	a 0.357 (0.013)	a 0.343 (0.017)	a 0.332 (0.017)	0.6	0.549



**Figure 3.7:** Effect of supplementary FR on stem elongation rates of *Anthocephalus chinensis* seedlings. Mean of 6 seedlings  $\pm$ SE. Area between two vertical lines indicates the period of FR treatment.

### 3.4 Discussion

The main effect of a reduction in R:FR ratio was a large increase in stem extension growth in pioneer species *Anthocephalus*, not in climax species *Hopea* (Figure 3.5). The significantly higher stem extension rates, when the internodes were subjected to a low R:FR ratio (Table 3.3) as well as the increased extension rates, when the terminal internode was irradiated with supplementary FR (Figure 3.7 and Table 3.4) also support this long-term observation. R:FR ratio is directly related to the phytochrome photoequilibrium, Pfr/P (review by Smith 1986). Low R:FR ratios strongly reduce the Pfr/P (Morgan and Smith 1976; Morgan *et al.* 1980; Child and Smith 1987; Ballaré *et al.* 1989), and the long-term stem extension rate is an inverse linear function of Pfr/P (Morgan and Smith 1976). This linearity has been found for a wide range of herbaceous species (review by Smith 1986), and also for tree seedlings of pioneer species *Pinus radiata* (Morgan *et al.* 1983; Warrington *et al.* 1989).

The accelerated stem extension rates in medium and high R:FR ratio regimes, particularly after 2 weeks of growth in *Anthocephalus* (Figure 3.6) could be interpreted as the result of a reduction in R:FR ratio at internode level. In fact, the first internode was shaded by the expanding laminae of the terminal pair of leaves. Spot readings on light spectra at stem level just below the laminae showed a drop in R:FR ratios. These reduced R:FR ratios were 0.23, 0.13 and 0.04 in high, medium and low R:FR ratio regimes respectively, measured towards the end of the experiment. The shading of the first internode by the leaves belonging to the same seedlings has also been reported for *Datura ferox* growing under sunlight (Ballaré *et al.* 1988).

An increased extension rate, when the internodes were exposed to low R:FR ratio (Table 3.3) indicates that the internodes might be the site of perception and response. Such a response has also been reported for light-grown seedlings of *Sinapis alba* and *D. ferox* (Ballaré *et al.* 1991). The extension rates, when internodes were subjected to low R:FR ratio, were not significantly different from those when the internodes were exposed simultaneously to low R:FR ratio and low blue light (Table 3.3). Thus the effect of low R:FR ratio appeared to be independent of a low proportion of blue light received by the internodes. Similar response has also been found in *S. alba* and *D. ferox* growing under sunlight (Ballaré *et al.* 1991). The induction of low R:FR ratio on the young leaves (when the leaves instead of stem were subjected to low R:FR ratio) failed to bring about any detectable change in stem extension rate (Table 3.3). Morgan *et al.* (1980) had found an increased stem extension rates in light-grown *S.*

*alba*, when the leaf or stem was irradiated with supplementary FR; the response was very rapid, when the stem alone was irradiated. In their experiment, a lag period of 3-4 h followed FR irradiation of the leaf, before an increase in stem extension rate was detectable. There have been no other comparable data for tree seedlings. Moreover, the experimental treatments of Morgan *et al.* (1980) were quite different from that used here. Hence, it is difficult to conclude whether the perception of low R:FR ratio by younger leaves and its effect on stem extension rate is species-specific; it is known that the longer term responses to added FR vary markedly between species (Morgan and Smith 1978).

The increased stem extension rate in young *Anthocephalus* seedlings following irradiation with supplementary FR (Table 3.4) might be the result of an abrupt drop in R:FR ratio at the internode level (Figure 3.4). Such a drop in R:FR ratio locally generates low Pfr/P in the internodes, which, in its turn, brings about an increased stem extension rate (Morgan *et al.* 1980; Child and Smith 1987; Casal and Smith 1989). Such abrupt increases in stem extension rates following irradiation of stem with FR have also been reported for *Chenopodium album* (Morgan and Smith 1978) and *S. alba* (Morgan *et al.* 1980; Child and Smith 1987). The adding FR to constant background WL increases the phytochrome cycling rate concomitant with decreasing Pfr/P, and in this way, phytochrome cycling is involved in the increased stem extension rate in response to supplementary FR in WL, but these responses are directly depend on the concentration of Pfr (Child and Smith 1987).

The increase in extension rate following irradiation of stem with FR occurs after a short lag period. The accurate determination of the lag periods for *Anthocephalus* seedlings was difficult because of normal biological variability within the experimental individuals and time-base resolution (see also Child and Smith 1987). Within the limits of resolution available, the latent period after switch-on was seen to be less than 10 min, similar to the value already reported for *C. album* (Morgan and Smith 1978) and *S. alba* (Child and Smith 1987).

Previous studies on *Phaseolus vulgaris* (Nakata and Lockhart 1966) and *Helianthus annuus* (Garrison and Briggs 1975) suggest that far-red light enhances the internode extension growth by stimulating the rate of both cell division and cell enlargement. The increased stem extension growth of *Anthocephalus* seedlings growing under low R:FR ratio might be the result of the increased rates of cell division and cell enlargement. However, the rapid response of stem extension rate following FR

irradiation might be almost entirely due to the increased rates of cell elongation (see review by Smith 1986).

The concomitant increase in *SWR* with an increased stem extension growth in seedlings growing under low R:FR ratio (Table 3.5) might be the result of the increases in dry matter allocation to stem (see also Corré 1983a). In *Anthocephalus*, the difference in *SWR* between high and low R:FR ratio regimes was substantial. It seems that higher *SWR* was the result of the extra demand for energy made by the rapidly elongating stem (see also Corré 1983a). The lower *LAR* under low R:FR ratio indicates that the increased stem extension was at the expense of the development of leaf area (see also Smith 1981; review by Smith 1986). This allocation pattern became more clear when the components of *LAR* were examined. The *LWR*, not the *SLA*, responded in accordance with *LAR* (Table 3.5). Further, from the non-significant difference in *SSL*, it appears that the increased stem extension growth under low R:FR ratio was more the result of a change in dry matter allocation between plant organs than that of a reduction in stem thickness. It may be that *SSL* primarily depends on the amount of energy fixed by the plants, and therefore, it depends more on the quantity of light than on its quality. This is an important difference between the effects of light quantity and light quality (see also Smith 1981).

In this experiment, *SLA* was not significantly affected by R:FR ratio even in pioneer species *Anthocephalus*. This result is consistent with that reported for *C. album* (Morgan and Smith 1981). In contrast, a reduction in R:FR ratio brought about an increase in *SLA* in pioneer tree seedlings of *Terminalia ivorensis* and almost no effect on this response in climax tree seedlings of *Khaya senegalensis* (Kwesiga and Grace 1986). But neither the *Terminalia* nor the *Khaya* displayed an increased or a decreased stem extension growth in response to low R:FR ratio. It is, however, probable that leaf developmental response and possibly the developmental response as a whole is species-specific.

## CHAPTER 4

# Responses of Tree Seedlings to the Blue light of Simulated Shadelight

### 4.1 Introduction

Vegetation selectively absorbs the blue and red wavelengths of light, but transmits some of the green and most of the far-red (Holmes 1981). This attenuating effect of vegetation brings about a marked reduction in the quantity of radiation in the blue waveband (B) with simultaneous reduction in photosynthetic photon flux density (PPFD) and red to far-red photon (R:FR) ratio. Only 1% of the radiation in the 400-500 nm waveband incident at the top of the canopy may reach the ground (Holmes 1981).

It is well-known that the photoreceptor implicated in the R and FR morphogenetic responses is phytochrome (see review by Smith 1986), whilst a specific B light response is ascribed to phytochrome and/or B photoreceptor (Meijer 1968; Gaba and Black 1979; Thomas and Dickinson 1979; Ritter *et al.* 1981; review by Mohr 1986). Currently, it is hypothesised that in species from open habitats an unknown B photoreceptor measures light quantity, whilst phytochrome measures light quality (review by Smith 1986).

Previous studies on herbaceous plants suggest that blue light strongly affects stem elongation, leaf expansion, leaf/shoot and root/shoot ratios and shoot relative growth rate of seedlings (Warrington and Mitchell 1976; review by Gaba and Black 1983, and Mohr 1986; Drumm-Herrel and Mohr 1991) even under low irradiance (Warrington and Mitchell 1976). Recently, Casal and Alvarez (1988) have recorded a significant effect of supplementary B light on the leaf growth of *Lolium multiflorum* in shadelight beneath a natural canopy. These responses indicate that the natural B waveband of shadelight may affect the growth and development of seedlings. There have been few studies on long-term growth responses of seedlings to the blue part of the shadelight and no studies in the particular case of tropical forest tree seedlings.

In the present experiment, an attempt was made to simulate forest shadelight: low PPFD, low R:FR ratio and low B light. The latter attribute was varied over a range:

high, medium and low. Keeping the low PPFDs and low R:FR ratios almost the same, it was possible to characterise the responses of tree seedlings of pioneer species *Anthocephalus chinensis* Rich. ex Walp., gap species *Bischofia javanica* Blume, and climax species *Hopea odorata* Roxb. to different proportions of B light of simulated shadelight.

## **4.2 Materials and Methods**

### **4.2.1 Plant materials and experimental design**

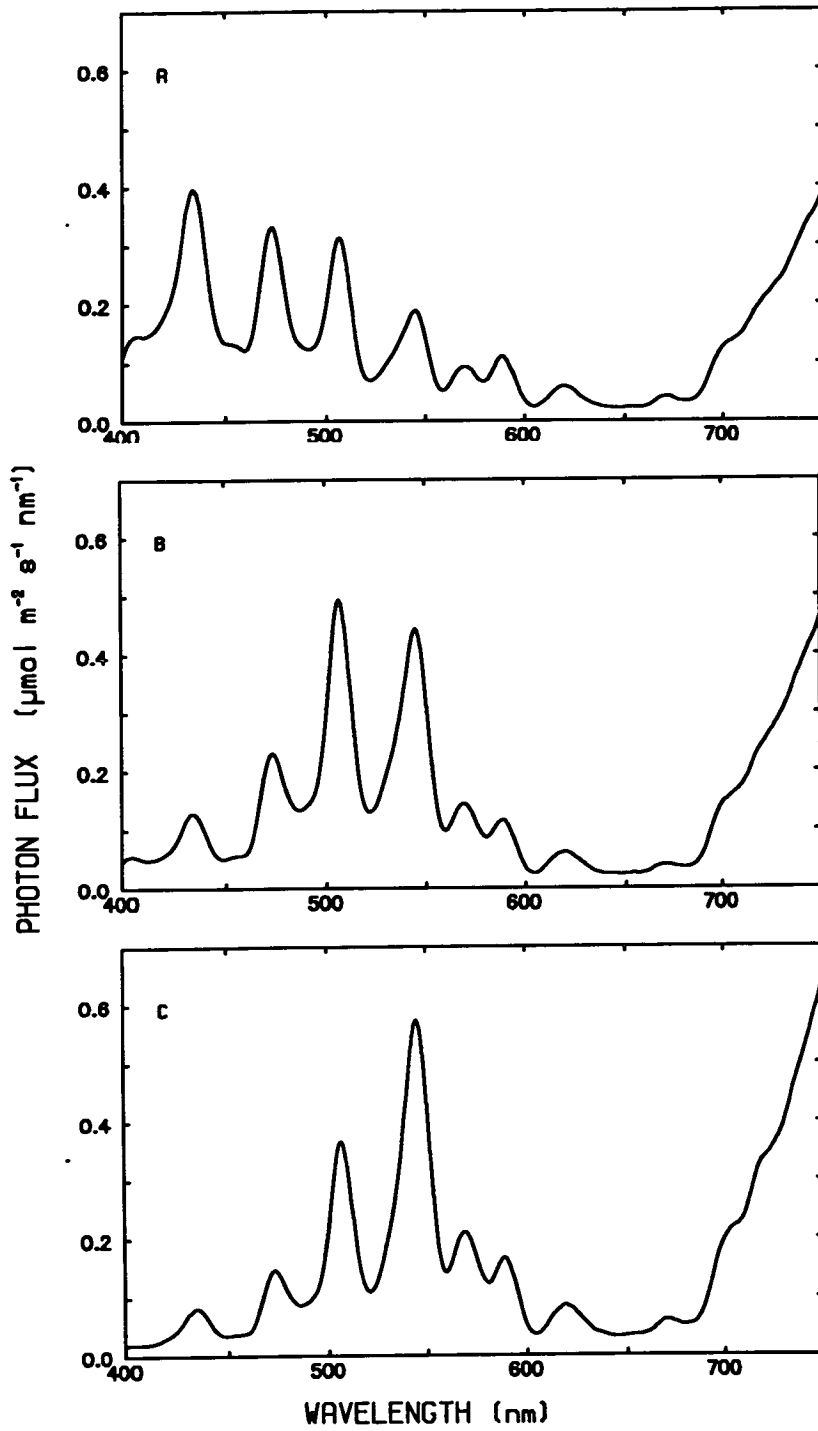
The methods of raising seedlings in this experiment were similar to those described in Section 2.2.1 except that the experiment was conducted in a growth cabinet. Seedlings were transferred to the growth tubes from seed-trays and randomly assigned to treatments on the experimental bench. There were three treatments, each with 6 seedlings: high, medium and low B light regimes. At this point, the height of *Anthocephalus* seedlings ranged in between 1 and 2 cm and they had 2 or 3 pairs of leaves; the height of *Bischofia* seedlings ranged from 1.8 to 2.6 cm and their first true leaf had not fully expanded; the *Hopea* seedlings were in between 7.8 and 9.7 cm and most of them produced 3 leaves.

### **4.2.2 Nutrition**

The materials and methods of nutrition were similar to those described in Section 2.2.2. The nutrient solution applied to the seedlings contained 60 mg l<sup>-1</sup> of N. The application of nutrients was daily- once a day.

### **4.2.3 'Shade-covers' and the light conditions they produced**

An individual 'shade-cover' was used to shade each seedling. The 'shade-cover' was made following the method illustrated in Figure 2.1. Three combinations of celluloid filters (Strand Lighting, Middlesex, UK) were used, keeping the bright blue filter (cinelux 441) common. Other filters were: light rose filter (cinelux 407), light amber filter (cinelux 402) and medium amber (cinelux 404). Neutral muslin and nylon fabrics were used, where appropriate, to adjust the PPFD under 'shade-covers' (Table 4.1). The light spectra (Figure 4.1), the PPFDs and R:FR ratios (Table 4.1) were measured following the methods described in Section 3.2.1.2.



**Figure 4.1:** Spectral photon flux density of high (A), medium (B) and low (C) blue light irradiance regimes inside growth cabinet.

**Table 4.1:** The materials used in 'shade-covers' and the light climate they produced in the growth cabinet. PPFDs under all 'shade-covers' were measured keeping the sensor at the same distance from the filter-top; mean of 6 'shade-covers' per treatment  $\pm$ SD. Blue light (400-500 nm), ratio of blue (400-500 nm) to red (600-700 nm) and ratio of red (660  $\pm$ 10 nm) to far-red (730 nm  $\pm$  10 nm) were calculated from light spectra.

Filters	Blue light ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	B:R ratio	R:FR ratio	PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
1 layer bright blue filter, 1 layer light rose filter, 1 layer muslin, 3 layers of nylon fabric	20.1	4.97	0.10	33.3 $\pm$ 0.6
1 layer bright blue filter, 1 layer light amber filter, 1 layer muslin, 2 layers of nylon fabric	10.8	2.52	0.10	33.1 $\pm$ 0.6
1 layer bright blue filter, 1 layer medium amber filter	5.8	1.11	0.10	33.7 $\pm$ 0.2

#### 4.2.4 Growth cabinet and growth conditions

This experiment was conducted in the same growth cabinet as described in Section 3.2.1.3. The PPFD and other growth conditions were the same as described in Chapter 3.

#### 4.2.5 Data collection and analyses

The height of all the seedlings were recorded at the start of the experiment. The seedlings were harvested at the end of 5 (*Bischofia* and *Hopea*) or 8 (*Anthocephalus*) weeks of growth under the various treatments. Leaf area, stem length and the dry weight of stem and leaves of each seedling were recorded following methods described in Section 2.2.6.

From the raw data the following variables were derived: leaf area stem length ratio (leaf area/stem length), specific stem length (*SSL*, stem length/stem dry weight) and specific leaf area (*SLA*, leaf area/leaf dry weight). The relative height growth rate ( $RGR_h$ ) was calculated according to the equation (Hunt 1978):

$$RGR_h = \frac{\ln H_2 - \ln H_1}{t_2 - t_1}$$

where  $H$  = seedling height, and  $t$  = time.

### 4.3 Results

#### 4.3.1 Light climate

By using three combinations of celluloid filters, it was possible to simulate a range of light spectra with different proportions of B light with almost the same R:FR ratios (Table 4.1). Muslin and nylon fabrics have almost no effect on light spectra. Thus by using the neutral fabrics, it was also possible to obtain almost the same PPFD under different B light irradiance regimes.

The B light irradiances (400-500 nm) with 20, 11 and 6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  as high, medium and low B light regimes respectively (Table 4.1) roughly match the natural B

light in vegetational shadelight (see Holmes 1981). For example, under a stand of *Solanum bonariensis*, natural B light (400-500 nm) has been found to be  $12 \mu\text{mol m}^{-2} \text{s}^{-1}$  at midday (Casal and Alvarez 1988). Further, the R:FR ratio under the B light regimes was 0.10, which was similar to that found in deep forest shade in the tropical forests (see Lee 1987). The integrated daily PPFD under the shadelight was  $1.4 \text{ mol m}^{-2} \text{ d}^{-1}$ , and almost the same for all the treatments. This value of daily PPFD was also in accordance with that found in tropical forest understory habitats (see Chazdon and Fetcher 1984).

#### 4.3.2 Responses to blue light

Data on developmental responses of *Anthocephalus*, *Bischofia* and *Hopea* seedlings are shown in Table 4.2. The species did not show significant difference in  $RGR_h$  in response to differences in the amount of B light. A reduction in the amount of B light influenced the leaf area per unit stem length in pioneer species *Anthocephalus*, gap species *Bischofia*, and not in climax species *Hopea*. This response was more pronounced in *Anthocephalus* than in *Bischofia*.

A reduction in B light had also affected *SSL* in *Anthocephalus* seedlings. *SSL* in this species significantly increased indicating that the seedlings under low B light produced the same stem length with less material. *SSLs* in *Bischofia* and *Hopea* seedlings were not affected by the differences in the amount of B light. The seedlings at the high B light regime did not significantly differ from those under the medium B light regime in respect of variables examined.

The seedlings of *Anthocephalus* and *Bischofia* showed substantially higher *SLA* values than those of *Hopea*, but this parameter was not significantly affected by the differences in the amount of B light.

#### 4.4 Discussion

The stem extension growth was not significantly affected by different proportions of B light in the seedlings of climax species *Hopea*, and same was true for the seedlings of

**Table 4.2:** Characteristics of *Anthocephalus chinensis*, *Bischofia javanica* and *Hopea odorata* seedlings grown under different proportions of blue light. Mean of 6 seedlings  $\pm$ SE; means preceded by same letters are not significant at  $P < 0.05$  (ANOVA and Duncan's multiple range test).

Blue light:	high	medium	low	F	P
<b>A. chinensis</b>					
relative height growth rate, $RGR_h$ (cm cm <sup>-1</sup> wk <sup>-1</sup> )	a 0.205 (0.016)	a 0.227 (0.015)	a 0.206 (0.019)	0.5	0.597
leaf area per unit stem length (cm <sup>2</sup> cm <sup>-1</sup> )	a 6.51 (0.78)	a 6.36 (0.43)	b 4.32 (0.40)	4.7	0.026
specific stem length, $SSL$ (cm mg <sup>-1</sup> )	a 0.269 (0.013)	a 0.265 (0.009)	b 0.350 (0.020)	10.8	0.001
specific leaf area, $SLA$ (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.553 (0.015)	a 0.540 (0.016)	a 0.542 (0.017)	0.2	0.838
<b>B. javanica</b>					
relative height growth rate, $RGR_h$ (cm cm <sup>-1</sup> wk <sup>-1</sup> )	a 0.217 (0.016)	a 0.246 (0.012)	a 0.223 (0.011)	1.4	0.278
leaf area per unit stem length (cm <sup>2</sup> cm <sup>-1</sup> )	a 3.47 (0.27)	a 2.66 (0.33)	a 2.53 (0.29)	2.9	0.084
specific stem length, $SSL$ (cm mg <sup>-1</sup> )	a 0.522 (0.018)	a 0.546 (0.031)	a 0.573 (0.027)	0.9	0.415
specific leaf area, $SLA$ (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.483 (0.016)	a 0.464 (0.013)	a 0.467 (0.026)	0.3	0.773
<b>H. odorata</b>					
relative height growth rate, $RGR_h$ (cm cm <sup>-1</sup> wk <sup>-1</sup> )	a 0.108 (0.007)	a 0.100 (0.007)	a 0.102 (0.011)	0.3	0.779
leaf area per unit stem length (cm <sup>2</sup> cm <sup>-1</sup> )	a 3.12 (0.29)	a 3.28 (0.16)	a 3.01 (0.12)	0.4	0.649
specific stem length, $SSL$ (cm mg <sup>-1</sup> )	a 0.264 (0.024)	a 0.297 (0.028)	a 0.316 (0.026)	1.0	0.397
specific leaf area, $SLA$ (cm <sup>2</sup> mg <sup>-1</sup> )	a 0.238 (0.013)	a 0.256 (0.006)	a 0.242 (0.010)	0.8	0.456

pioneer species *Anthocephalus* and gap species *Bischofia* (Table 4.2). Their ecological status was reflected in their  $RGR_h$ : the rates in *Anthocephalus* and *Bischofia* seedlings were higher than those of *Hopea*, and both the pioneer and the gap species showed similar rates of extension growth. The extension rates of *Anthocephalus* seedlings can be comparable to those when the seedlings of this species were grown under low R:FR ratio (see Chapter 3).

It is unlikely that at this low R:FR ratio (0.10) phytochrome did not play a role in the extension growth of the pioneer tree seedlings. This is because the different levels of B light were obtained without affecting the R and FR components. Moreover, there is evidence that a reduction in R:FR ratio substantially promotes the stem extension growth of *Anthocephalus* seedlings (see Chapter 3).

Laskowski and Briggs (1989) and Warpeha and Kaufman (1989) found that B light inhibits epicotyl elongation in *Pisum sativum* seedlings grown under continuous R light and concluded that inhibition was not due to changes in Pfr concentration during the treatment with B light. Mohr (1986) argues that light absorbed by B photoreceptor is necessary to maintain responsiveness to Pfr. With increasing age of the seedlings the requirement for B light increases strongly, and the fluence rate of B light must exceed a certain threshold to become effective. Hence, under low irradiance the B light effect on stem extension growth is unlikely (review by Mohr 1986). Drumm-Herrel and Mohr (1991) have also shown that the action of B light on stem elongation is related to the level of Pfr, and that an expression of the effect of B light is diminished if the level of Pfr is kept low. Supplementing the B-deficient light ( $94 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) to *Sinapis alba* seedlings, Casal and Smith (1988) have concluded that B light does not inhibit internode elongation, rather restores the promotive effect of low R:FR ratio. Further, Ballaré *et al.* (1991) have observed a promotive effect of reducing B light on stem extension growth in *S. alba* and *Datura ferox*, when the first internode was exposed simultaneously to low R:FR ratios and low levels of B light, without a significant interaction of B light and FR, and concluded that the effect of R:FR ratio on stem elongation is independent of the fluence rate of B light received by the internodes.

The restriction of the leaf expansion in the seedlings of pioneer species under low B light condition, as is evident from the values for leaf area per unit stem length (Table 4.2), might be, at least partially, attributed to the limitation of photosynthates under this condition. This effect has become more clear when the SSLs are compared: the

SSLs were substantially higher in *Anthocephalus* seedlings growing at the low B light regime. This means that the seedlings of *Anthocephalus* under low B light regime produced the same length of stem with less materials. It is known that chlorophyll has a peak of absorption in the B region of the spectrum (see Whatley and Whatley 1980) and it is not unlikely that under the low irradiance supplied suboptimal levels of photosynthates.

A certain amount of B light is necessary to maintain high quantum yield of photosynthesis (see review by Voskresenskaya 1972), and possibly this requirement was intensified under vegetational shadelight, where R light was also significantly deficient. The B light is known to activate the biosynthesis of protein, ribonucleic acids, and chlorophylls, principally chlorophyll b, and also bring about photoactivation of nitrate reductase (review by Voskresenskaya 1972 and Thomas 1981). It is not unlikely that such regulatory roles of B light might also have contributed to the photosynthetic carbon metabolism in the seedlings under high and medium B light regimes.

# CHAPTER 5

## Photoinhibition and Light Acclimation in Seedlings of *Bischofia javanica*

### 5.1 Introduction

Seedlings of tropical trees are exposed to very large increases in light when a gap in the canopy forms. Typically, photon flux density is increased by a factor of  $\times 25$  (Chazdon and Fetcher 1984). It is well known that exposure of shade-adapted leaves to high light results in photoinhibition of photosynthesis (Kok 1956; Critchley and Smillie 1981; Langenheim *et al.* 1984; Oberbauer and Strain 1985). This photoinhibition is manifested by a reduced quantum yield and light saturated capacity of photosynthesis, a reduced electron transport activity and altered chlorophyll fluorescence characteristics. These effects are generally considered to result from the inactivation of reactions associated with photosystem II (PSII) (see review by Powles 1984 and Bolhar-Nordenkamp *et al.* 1989). At room temperature, virtually all the fluorescence emission arises from chlorophylls associated with PSII and hence the fluorescence induction curve is clearly dominated by changes in PSII fluorescence (Baker and Horton 1987). The chlorophyll fluorescence characteristics thus provide valuable information on stress effects on leaves, and in recent years, fluorescence techniques have been widely applied in studies on photoinhibition (e.g. Critchley and Smillie 1981; Greer *et al.* 1986; Demmig and Björkman 1987; Ögren 1988).

Although photoinhibition has been recognised for a long time, very little is known about the mechanisms of adaptation of shade leaves to strong light and the time courses over which plants repair photosynthetic capacity after periods of photoinhibition (see review by Powles 1984). Such adaptations of shade grown seedlings of tree species may affect survival and growth, and could be critical in determining the successional status of a species. In this way, the potential for photosynthetic acclimation of the constituent species may influence the species composition of a newly-formed gap.

In this study, the capacity of fully developed shade leaves of *Bischofia javanica* Blume for acclimating to strong light was examined. There have been very few studies of this acclimation process (Bunce *et al.* 1977; Syvertsen and Smith 1984; Sebaa *et al.* 1987; Bauer and Thöni 1988), and no studies for the particular case of tropical trees.

The distribution of *B. javanica* ranges from drier savannas and evergreen climax forests to freshwater swamp forests, where with other colonising species it forms a seral community leading to the development of edaphic sub-climax (Troup 1921; Som Deva and Srivastava 1978). It germinates and establishes under forest shade, but becomes canopy-dominant or emergent as an adult tree in the open (Troup 1921; Dakshini 1965).

For the present study, *Bischofia* seedlings were grown under simulated shade light in a controlled environment. After transfer of these 'shade' grown seedlings to a higher light regime, chlorophyll fluorescence induction kinetics, net photosynthesis and changes in leaf chlorophylls and leaf anatomy were examined in fully developed shade leaves. The results are discussed in the context of its seedling establishment in the tropical forest ecosystem.

## **5.2 Materials and Methods**

### **5.2.1 Plant materials**

The seedlings were raised in seed-trays as described in Section 2.2.1 except that the trays were kept in a growth cabinet. After transfer to the tubes, the seedlings were placed in a random design on the bench of the growth cabinet. At this point, the seedlings had attained a height of 3 to 4 cm and their first leaf was fully expanded.

### **5.2.2 Nutrition**

The materials and methods of nutrition were similar to those described in Section 2.2.2, except that all the seedlings were provided with same level of nutrients: 60 mg l<sup>-1</sup> of N. This concentration of nutrient solution was attained by gradually increasing the concentration over a period of 7 days starting from a very low concentration. This was done to prevent injury to the small seedlings. Application of nutrient solution was regular - once a day in the low-light cabinet and twice a day in the high-light cabinet.

### **5.2.3 Growth cabinets and growth conditions**

The materials used in the growth cabinets and the growth conditions were the same as described in Section 3.2.1.3. A wooden frame with blue green filter (chromoid 116, Strand Lighting, Middlesex) was placed just below the ceiling of one cabinet to

simulate forest shadelight. The photosynthetic photon flux density (PPFD) and the light spectra were measured following methods described in Section 3.2.1.2.

Celluloid filter may fade over weeks and months in bright light. Spectral transmittance changes in the blue green filter, after 6 months of exposure to light in the growth cabinet were measured according to the methods described in Section 2.2.5. The transmittance spectra are presented in Figure 5.1.

#### **5.2.4 Growth period**

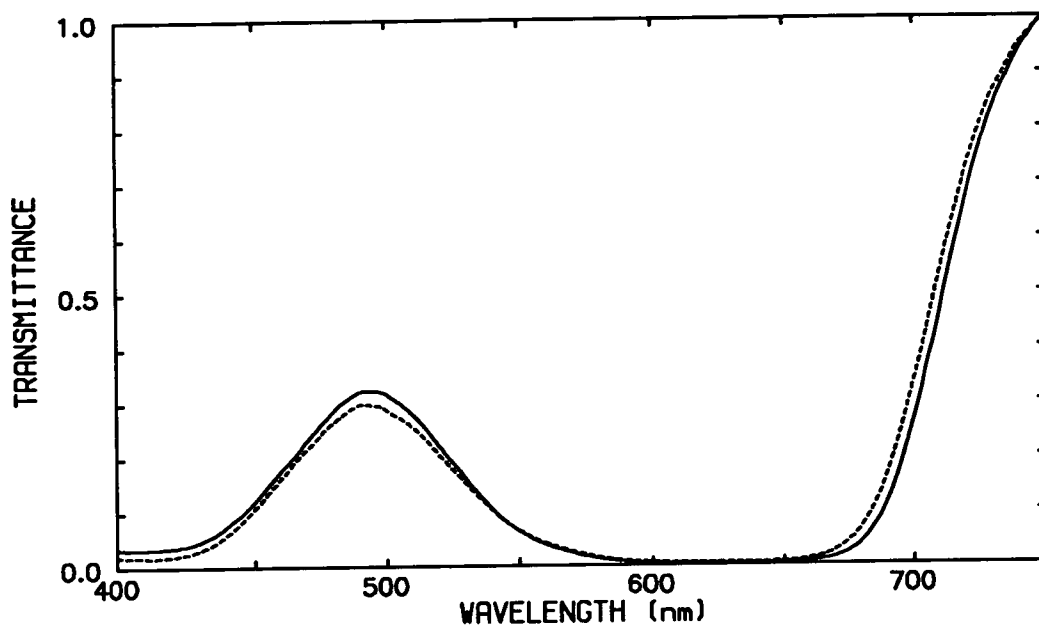
The seedlings were grown in the low-light cabinet for 8 weeks. Then they were transferred to the high-light cabinet. At the time of transfer, most of the seedlings had produced at least 8 fully expanded leaves. The seedlings, on the bench, were relocated regularly to minimise the possible effects of location. The treatments were: leaf growing in low light continuously (L), leaf developed at low light before transfer to high light (LH), and leaf developed under high light on the same plant as LH treatment (H).

#### **5.2.5 Leaf chlorophyll fluorescence and net photosynthetic rate**

Chlorophyll fluorescence induction kinetics were measured in intact attached leaves by a Plant Stress Meter (PSM 102 89, Biomonitor S.C.I. AB, Sweden). A fully developed leaf (8th from the first true leaf) was labelled on 5 seedlings and measured over time. The pre-set light level and run-time for all the measurements were  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  and 10 s respectively, and the dark adaption period was 15 min.

The same leaf in each seedling was used for the measurement of net photosynthetic rate ( $P_n$ ). The gas analyser used was the LCA2 with the Broadleaf PLC assimilation chamber and the ADC data logger (Analytical Development Co. Ltd., Hoddesdon, England).  $P_n$  was measured at  $600 \pm 8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  in the growth cabinet. The leaf chamber was held in a fixed horizontal position by using a stand inside the growth cabinet. The seedlings were adjusted so that the leaves fitted into the chamber, and received the photon irradiance at the time of measurement.  $P_n$  was calculated by using formulae described by Long and Hallgren (1985).

Calibration of the gas analyser was done by using a set of precision mixing pumps



**Figure 5.1:** Spectral transmission of the chromoid blue green filter after exposure to light for about 6 months in the growth cabinet (*broken line*) and the unused one (*solid line*). The same reference buffer was used for the measurements. Total transmittance values (400-700 nm), R:FR ratios (660:730  $\pm$  10 nm) and B:R ratios (400-500 nm : 600-700 nm) are: 28.8, 0.007 and 4.79 for unused filter, and 28.1, 0.014 and 2.91 for used one respectively.

(Wosthoff, Bochum, FRG). The humidity sensor was checked regularly with a water vapour generator (WG 600, Analytical Development Co. Ltd., Hoddesdon, UK). The quantum sensor was checked against a standard quantum sensor (190SB Li-Cor. Inc., Lincoln, Nebraska, USA).

### **5.2.6 Leaf chlorophyll contents and leaf weight per unit area**

Leaf discs (0.5 cm<sup>2</sup>), taken by a cork borer, were macerated with a small amount of acetone and fine sand in a pestle and mortar. The extract for chlorophyll determination was spun using a bench centrifuge for 3 min at an acceleration of 1600 g. Avoiding direct light, the extract was diluted with further 80% acetone to a known volume, and then a reading of absorbance was taken by using a spectrophotometer (SP800, Unicam, Sweden). The chlorophyll a and b concentrations were calculated from the absorbance values using the equations of Ziegler and Egle (1965) quoted by Šesták (1971).

Leaves of the same age-group were used for determination of leaf chlorophyll contents over time. Sampling was done from 5 seedlings at a time, selecting a single leaf from which 20 leaf discs were collected. Half was used for chlorophyll determination and the other half was put in the oven for 3 days at 70 °C for dry weight and leaf weight per unit leaf area ( $L_w$ ) determination.

### **5.2.7 Leaf anatomy**

For determination of stomatal density, a surface replica from the lower surface was made with transparent varnish. Counts of stomatal densities on the replica were made with a calibrated grid in the eyepiece of the microscope. Sampling was done on 5 seedlings and counts of stomatal impressions were made covering a microscopic field of 0.145 mm<sup>2</sup> on each leaf. Thickness of leaf and of palisade and spongy layers were derived from hand-cut transverse sections of leaf viewed under a light microscope.

## **5.3 Results**

### **5.3.1 Light climate in the growth cabinets**

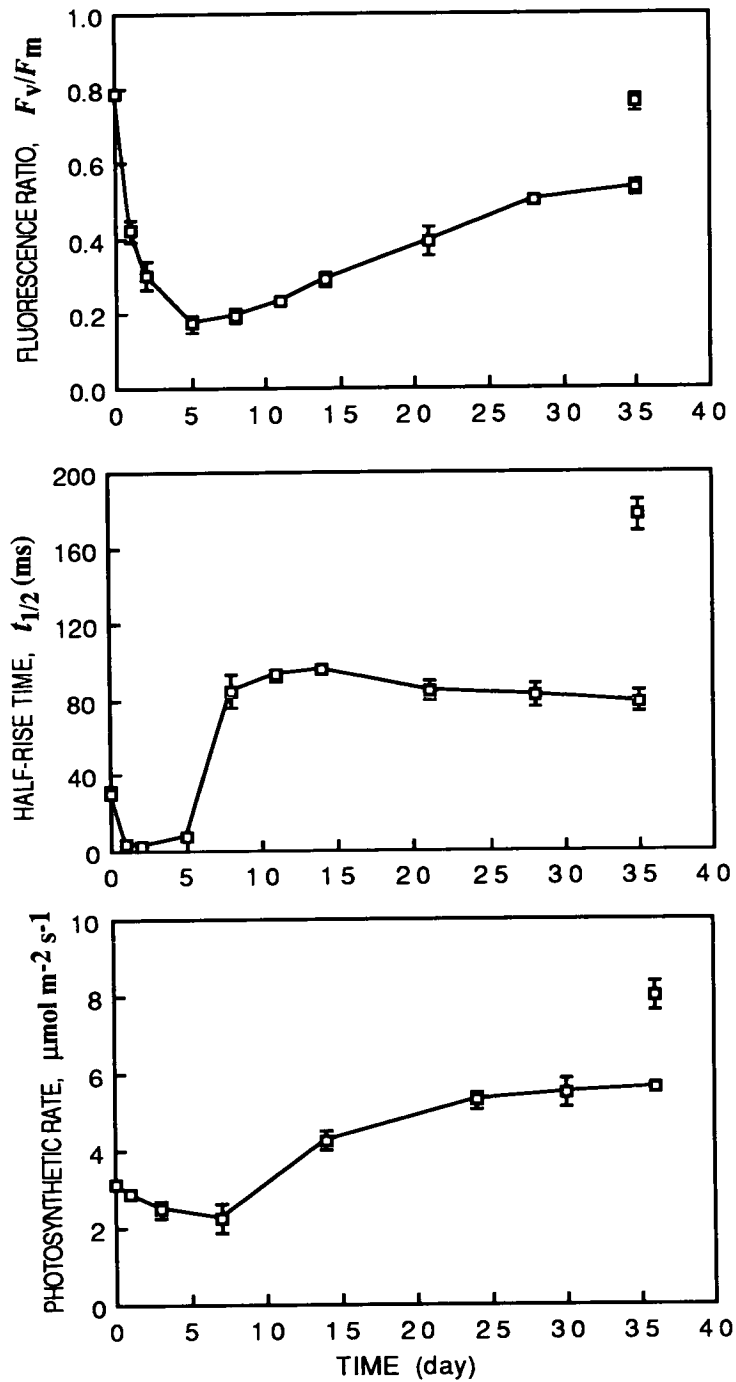
The light spectra in the low-light and the high-light growth cabinets were similar to those shown in Figures 3.1C and 3.4 (for white light) respectively. The PPFDs of 40

and  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  with R:FR ratios of 0.10 and 1.45 (660:730  $\pm 10$  nm) in the low-light and the high-light cabinets simulated the natural daylight and vegetational shadelight respectively (see Chazdon and Fetcher 1984; Lee 1987). Blue light (400-500 nm) in the low-light and the high-light cabinets was about 186 and  $14 \mu\text{mol m}^{-2} \text{s}^{-1}$  respectively. The amount of blue light in the low-light cabinet was fairly typical to that of the natural shadelight, but in the high-light cabinet it was less than that found in the open sun light (see Holmes 1981). However, the difference between the two cabinets in respect of the quantity of blue light was large.

Spectral transmittance of the chromoid blue green filter used in the low-light cabinet was changed over a period of about 6 months (Figure 5.1). Mostly, the changes occurred in the red part of the spectrum with almost no change in the far-red region resulting in an increase in the R:FR ratio. The ratio of blue to red (B:R ratio) was decreased. This change in B:R ratio was due to a decreased transmission in the blue and an increased transmission in the red part of the spectrum. Interestingly, the spectral transmission changes did not appreciably change the total transmittance. Thus it might be expected that the PPFD in the low-light cabinet was not significantly changed by the spectral transmission changes of the filter. However, the spectral transmittance changes were not large enough to alter substantially the quality of shadelight. Moreover, these changes occurred over a long time of exposure (about 6 months), and in the present experiment, the seedlings were grown in this cabinet for 8 weeks only. Hence, the possible effects of these spectral transmission changes of the filter might be considered negligible.

### 5.3.2 Chlorophyll fluorescence characteristics

The chlorophyll fluorescence ratio ( $F_v/F_m$ ), which reflects the photochemical efficiency of photosystem II (PSII), was high in the low (L) light leaf prior to exposure to the high (H) light. When the L light seedlings were exposed to the H light, the  $F_v/F_m$  of the fully developed leaves decreased immediately after transfer and continued to decrease further over several days (Figure 5.2). This decline in  $F_v/F_m$  indicates a loss of photochemical efficiency (Björkman and Demmig 1987). After 4 days,  $F_v/F_m$  began increasing gradually and there was no further increase after 28 days. The final value of  $F_v/F_m$  in the light-acclimating leaf (LH) did not attain the value of the L light leaf before exposure or that for the newly-formed leaf under the H light (H light leaf).



**Figure 5.2:** The ratio of the variable fluorescence to maximum fluorescence ( $F_v/F_m$ ), the half-rise time of variable fluorescence ( $t_{1/2}$ ) and net photosynthetic rate ( $P_n$ ) at  $600 \pm 8 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the low-light leaf transferred to the high light (LH leaf) of *B. javanica* during light acclimation to the high light. Mean  $\pm$ SE of 5 leaves; the same leaves of 5 seedlings were measured over the period. The first measurement after exposure (the second data point) was taken after 1 h of exposure. The scattered values are for the newly-formed leaf under the high light (H light leaf).

The decrease in  $F_v/F_m$  was paralleled by a corresponding decrease in  $t_{1/2}$  (Figure 5.2). This parameter is a function of the rate of the photochemical reaction and the pool size of electron acceptors on the reducing side of PSII, including the plastoquinone pool (Öquist and Wass 1988). The  $t_{1/2}$  started increasing almost at the same time the  $F_v/F_m$  had started, but reached its maximum long before the  $F_v/F_m$ . The  $t_{1/2}$  exceeded the value for the L light leaf before exposure by a factor of more than double but was far less than the value for the H light leaf.

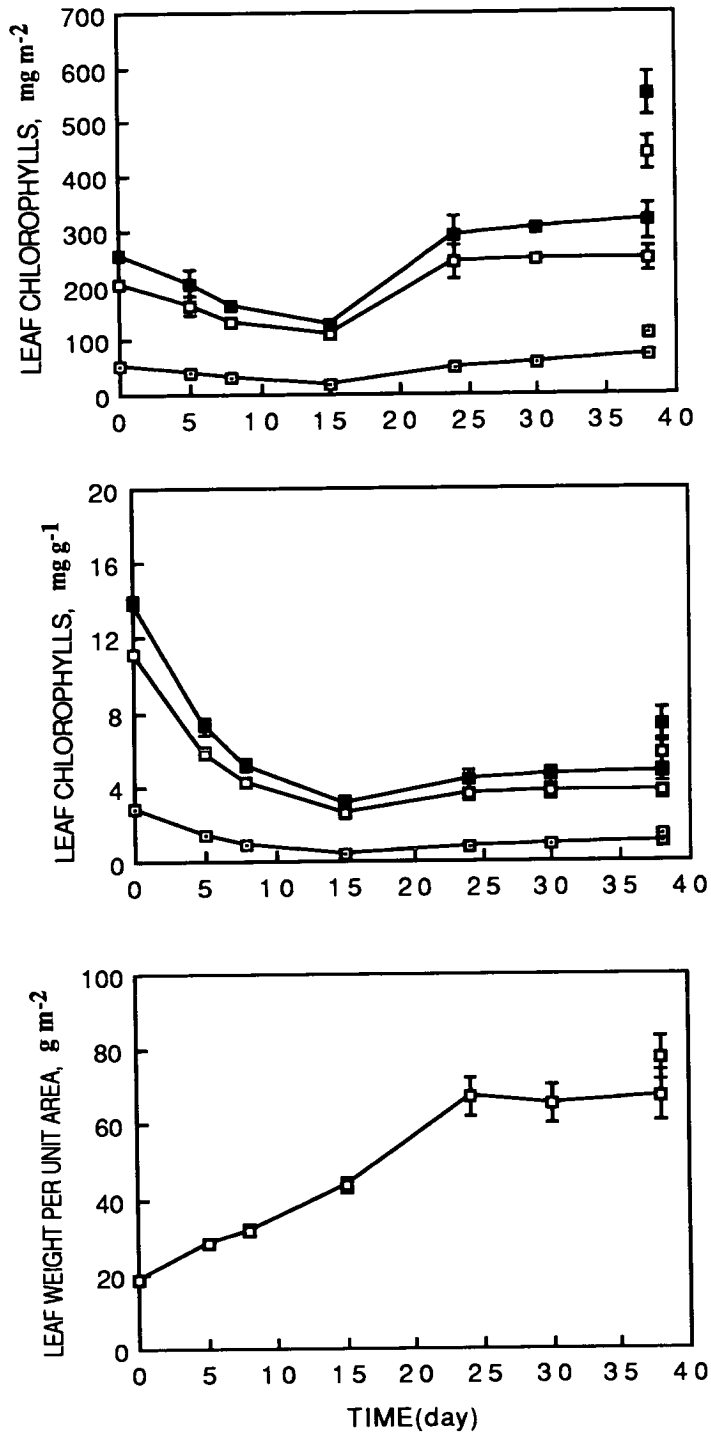
Both the changes in  $F_v/F_m$  and those in  $t_{1/2}$  indicate a partial recovery of photoinhibitory damage over time. Parallel measurements on the L light leaves, serving as controls, measured over the same period showed no appreciable change in  $F_v/F_m$  or  $t_{1/2}$ .

### 5.3.3 Changes in net photosynthesis

Like  $F_v/F_m$ , the net photosynthetic rate ( $P_n$ ) decreased immediately after exposure to the H light and continued further to decline over several days (Figure 5.2). An increase in  $P_n$  commenced towards the end of 5 days, and within 14 days it exceeded the  $P_n$  before exposure by more than 37% and reached its maximum within 28 days, when it was higher than that before exposure by 75%. But the final  $P_n$  was less than that of the H light leaf by more than 30%. The trend in the  $P_n$  during light acclimation was similar to that displayed in the  $F_v/F_m$ .

### 5.3.4 Changes in leaf chlorophylls and leaf weight per unit leaf area

Exposure to the H light brought about changes in the leaf chlorophylls and the leaf weight per unit leaf area (Figure 5.3). Leaf chlorophylls (a and b) per unit leaf area decreased on exposure, and unlike the  $F_v/F_m$ , the  $t_{1/2}$  and the  $P_n$ , it reached its minimum towards the end of 14 days, when it was 50% of that before exposure. An increase in chlorophylls was immediate after severe bleaching and after 28 days, there was no further increase. Chlorophyll contents per unit leaf weight also decreased gradually until day 14, and the final level did not reflect the substantial recovery because of compensating variations in the leaf weight per unit leaf area (Figure 5.3).



**Figure 5.3:** Leaf chlorophylls and leaf weight per unit leaf area ( $L_w$ ) of the low-light leaf transferred to the high light (LH leaf) of *B. javanica* during light acclimation to the high light. Mean  $\pm$ SE of 5 leaves; leaves of the same age-group from different seedlings were harvested over the period; the last values represent the leaves measured for chlorophyll fluorescence and net photosynthetic rates. The scattered values represent newly-formed leaf under the high light (H light leaf). Symbols are:  $\blacksquare$ , chlorophylls a and b;  $\square$  chlorophyll a;  $\square$ , chlorophyll b.

The leaf weight per unit leaf area ( $L_w$ ) increased gradually on exposure to the H light. This increase in the  $L_w$  became steady within 24 days of exposure, and the final  $L_w$  of the light acclimated (LH) leaf approached that of the H light leaf (Figure 5.3).

### 5.3.5 Morphological changes

When the seedlings were growing under the L light, the leaf surface was almost at right angles to the incident light maximising light interception by the leaf surface. On transfer to the H light, there was a distinct change in the position of lamina. The lamina adopted a downward position from less than its midpoint forming a curvature along the midrib thus minimising the interception of incident light. This did not appear to be the result of water stress, but an active movement. The developing leaves stopped growing and became compactly crowded at the apex within the first few days. This induced the sprouting of lateral buds, and within a short time, several short epicormic shoots with a cluster of smaller leaves appeared on the lower part of the stem. Towards the end of the 2nd week, the younger leaves on the tip started opening and ultimately resumed growth. When several new leaves were formed, and the epicormic shoots were shaded by the upper new leaves, the formation and growth of the epicormic shoots became restricted.

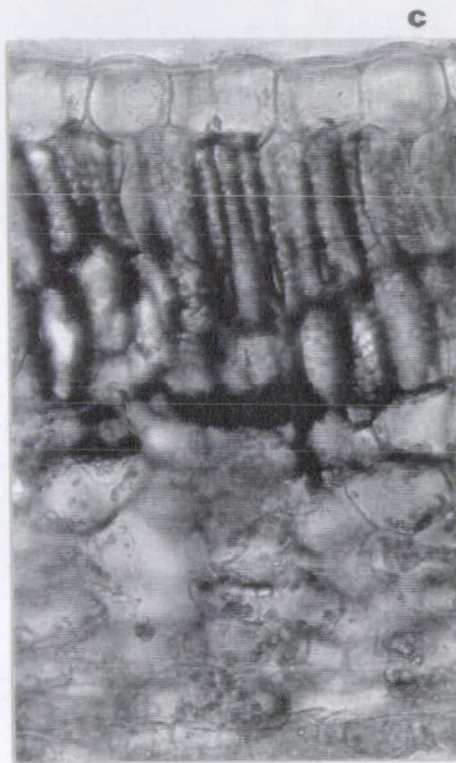
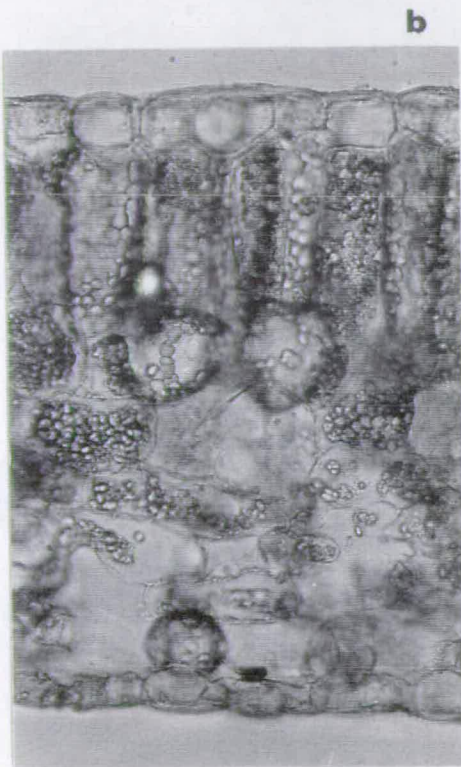
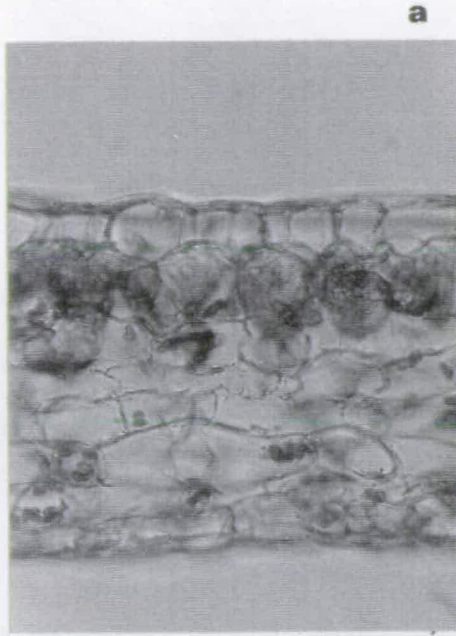
No visible symptom of leaf damage was observed during the first few days. At the end of the first week, the LH leaves turned pale green and within a week showed a network of chlorotic areas on the surface. During the 4th and 5th weeks, re-greening developed over the surface particularly along the veins, but greened areas did not eliminate the chlorosis completely. These leaves became distinctly different from their appearance before exposure to the H light and remained so till the end of the experiment.

### 5.3.6 Changes in leaf anatomy

Although the leaves were fully expanded before the exposure to the H light, the leaf thickness, during light acclimation, was increased by about 45%, and the leaf tissues became denser without changing the leaf area. The LH leaf had one layer of palisade cells, and the changes in leaf thickness were mainly due to the substantial expansion of palisade layer (Table 5.1 and Plate 5.1). The palisade/spongy ratio was increased by more than 80% over the ratio before the exposure. Stomatal density was not affected during light acclimation.

**Table 5.1:** Leaf anatomy of the low-light control leaf (L), the light-acclimated leaf (LH), and the newly-formed leaf under the high light (H) of *Bischofia javanica*. Mean ( $\pm$ SE) of 5 leaves; variables representing the light-acclimated leaf were from the same leaves measured for chlorophyll fluorescence and net photosynthetic rate. Means preceded by the same letter are not significantly different from each other at  $P < 0.05$  (ANOVA and Duncan's multiple range test).

Variables	L	LH	H	F	P
stomatal density (mm <sup>-2</sup> )	b 158 (6)	b 159 (3)	a 215 (7)	37.7	0.0001
leaf thickness ( $\mu$ m)	c 180.8 (4.5)	b 261.4 (6.1)	a 312.8 (15.6)	44.3	0.0001
palisade thickness ( $\mu$ m)	c 44.9 (3.9)	b 104.3 (6.7)	a 154.4 (7.7)	75.6	0.0001
spongy thickness ( $\mu$ m)	a 97.7 (7.4)	a 121.4 (3.4)	a 113.5 (8.3)	3.3	0.0710
palisade/spongy ratio	c 0.47 (0.05)	b 0.86 (0.07)	a 1.37 (0.05)	57.1	0.0001



**Plate 5.1:** Transverse sections of leaves from *Bischofia javanica* seedlings grown in the growth cabinets. Magnification, x 312. **a:** low-light control leaf, L; **b:** fully expanded low-light leaf after 6 weeks of exposure to the high light, LH; **c:** newly-formed leaf in the high light, H.

Under the H light, the L light seedlings produced a substantially thicker leaf (H light leaf) with higher stomatal density and palisade/spongy ratio than the LH leaf, but the thickness in spongy tissue did not differ significantly. The palisade cells formed 2 distinct layers in the H light leaf (Plate 5.1).

#### 5.4 Discussion

Shade leaves, only one hour after the transfer to the H light, exhibited a depression in the chlorophyll fluorescence ratio ( $F_v/F_m$ ) with concomitant decline in pool size of electron acceptors on the reducing side of PSII ( $t_{1/2}$ ) and in net photosynthesis ( $P_n$ ) (Figure 5.2). The H light, therefore, resulted in photoinhibitory damage to the primary photochemical reactions of PSII. Any reduction in the  $F_v/F_m$  ratio is a quantitative indicator of the reduction in the efficiency of the primary photochemistry of PSII, irrespective of whether the reduction in the  $F_v/F_m$  is caused by an increase in initial chlorophyll fluorescence ( $F_o$ ) or by a decrease in variable fluorescence ( $F_v$ ). An increase in  $F_o$  is characteristic of destruction of PSII reaction centres, whereas a decline in  $F_v$  may indicate the increase in non-photochemical quenching (Baker and Horton 1987). The decline in the  $F_v/F_m$ , in the present experiment, was caused mostly by an increase in  $F_o$  indicating the inactivation of PSII reaction centres. These trends in fluorescence are in agreement with previous studies in which leaves had been exposed to a step-wise increase in light (e.g. Demmig and Björkman 1987).

Transfer to the H light brought about chlorophyll bleaching resulting in a substantial decline in leaf chlorophyll content (Figure 5.3). This decline is more rapid when the chlorophyll content is expressed as per unit leaf weight because of correspondingly rapid increase in the leaf weight per unit leaf area. For the same reason, the increase in chlorophyll content (area basis) as a result of synthesis after severe bleaching disappeared when expressed per unit leaf weight. Chlorophyll bleaching reached its maximum towards the end of the second week (Figure 5.3) by which time the pool size of electron acceptors ( $t_{1/2}$ ) had increased to its maximum (Figure 5.2) and the recovery from photoinhibition had proceeded substantially as indicated by an increase in the  $F_v/F_m$  and the  $P_n$  (Figure 5.2). These results agree with the fact that photoinhibition is not the consequence of chlorophyll bleaching but rather that the bleaching occurs only after a certain degree of photoinhibition has occurred (Satoh 1970; Björkman 1981; a review by Powles 1984). The decrease and increase in chlorophyll content over time also support the visual observations of bleaching and

recovery. Such response of bleaching and recovery has also been reported in *Citrus* (Syvertsen and Smith 1984). In *Bischofia*, a steady state in chlorophyll content, during the recovery phase, was attained earlier than in *Citrus*, and the leaf chlorosis did not disappear completely.

The increase in leaf weight per unit leaf area ( $L_w$ ) in the LH leaf was immediate and then it gradually approached that of the H light leaf (Figure 5.3). Changes in the  $L_w$  are partially attributable to the differences in mesophyll thickness but are also caused by the differences in the contents of starch, sugars and inorganic solutes (see Björkman 1981). Increase in the  $L_w$  immediately after transfer, when leaf thickness did not change, might be a result of the accumulation of starch and other cell components under the H light conditions. The final  $L_w$  of the LH leaf did not significantly differ from that of the H light leaf, in spite of significantly thicker mesophyll tissue in the latter. This indicates that an increase in the  $L_w$  might be caused by an increase in the leaf density (weight per unit volume) rather than the leaf thickness (see also Syvertsen and Smith 1984).

The fully developed leaves, that had formed under the L light, were able to increase the  $P_n$  immediately after photoinhibition (Figure 5.2). This recovery had also been reflected by an increase in the  $F_v/F_m$  and the  $t_{1/2}$  at the same time. The increase in the  $P_n$  in the LH leaf results from the higher amounts of rate-limiting constituents at the chloroplast level, such as RuBP carboxylase (Björkman 1981). This can be achieved by a higher number of chloroplasts per unit leaf area distributed among an increased mesophyll volume. The number of chloroplasts per unit surface has been found to increase almost proportionately with leaf thickness (Louwerse and Zweerde 1977) and the amount of carboxylation enzyme per unit surface is increased accordingly (Sinclair *et al.* 1977). An increase in leaf thickness has frequently been observed in leaves developed under the H light (Table 5.1, see also Björkman 1981; Nobel and Walker 1985). Here significant increases in leaf thickness were observed in leaves fully developed under the L light and then transferred to the H light, resulting mostly from thicker palisade layer (Table 5.1 and Plate 5.1). Anatomical rearrangements in fully expanded leaves during acclimation to the H light have also been observed in several herbaceous species (Bunce *et al.* 1977; Sebaa *et al.* 1987) and only a few woody species (Syvertsen and Smith 1984; Bauer and Thöni 1988).

It seems that the orientation of leaf on exposure to the H light was an adaptation to minimise the interception of incident H light by the leaf surface. Such leaf movements

on exposure to the H light to minimise photoinhibition have also been reported by Powles and Björkman (1982). The crowding of tender leaves on the stem tip and the formation of epicormic shoots on the lower part of the stem possibly reflect the disruption of apical dominance. The visual observations on resumption of apical dominance and the faster growth at the end of crucial phase of photoinhibition are consistent with the acclimation of physiological characteristics in the light-acclimated LH leaves. The formation of epicormic shoots, possibly to compensate for the photoinhibition of photosynthesis along with the light acclimation of the LH leaves and the formation of new 'sun type' leaves, represent the whole plant adaptation to the H light.

In examining the relevance of light conditions used in this experiment with those encountered in natural forests, it may be mentioned here that unlike natural condition, the photon irradiance was almost constant over the photoperiod. Some studies, however, have investigated acclimation of morphological and photosynthetic characteristics under different levels of integrated as well as instantaneous PPFD (Chabot *et al.* 1979; Nobel and Hartsock 1981). These studies demonstrate that photosynthesis and leaf structure are determined by the integrated PPFD rather than by the peak PPFD.

The anatomical, physical, and physiological characteristics of *B. javanica* leaves in response to the L light and the H light during growth indicate how they can acclimate to different light conditions. The changes noted in leaf characteristics during light acclimation indicate that the leaves can acclimate even after the leaves are fully developed. These responses are analogous to changes that might occur on the forest floor when a gap in the canopy is formed. Moreover, the potential for photosynthetic acclimation to the changed light conditions may enable the seedlings, growing under forest understorey habitats, to utilise sunflecks more efficiently.

The changes in species composition in a disturbance gap depends on survival and competitive ability of the successional species. The survival of tree seedlings following gap formation depends mostly on competition from successional species with high photosynthetic rates (Bazzaz and Pickett 1980). The photosynthetic acclimation in *B. javanica* may make its seedlings more competitive following disturbance.

## CHAPTER 6

# Photosynthetic Shade Adaptation in the Seedlings of *Bischofia javanica*

### 6.1 Introduction

Light conditions in the forest understorey are strongly affected by seasonal changes in the canopy cover. With the expansion of foliage in the canopy, the photosynthetic photon flux density (PPFD) typically drops to as little as 1 to 2% of those before canopy closure or of those in the open (Chazdon and Fetcher 1984; Lee 1989). These changes in light conditions are particularly pronounced in the deciduous and semi-evergreen forests (review by Chazdon 1988). Moreover, the gap formation by the falling of a tree or branch and the subsequent closure of those gaps creates an extremely heterogeneous light environment in space as well as in time. Thus, a given seedling may be exposed to changes in light regime over the course of its lifetime, including sudden increases as well as gradual decreases.

It is well known that under contrasting light regimes, leaves have different anatomical, morphological and biochemical properties leading to differences in apparent rates of photosynthesis (e.g. Bunce *et al.* 1977; Patterson *et al.* 1978; Hoflacher and Bauer 1982; Langenheim *et al.* 1984; Eliáš and Čiamporová 1986). Very little is known about whether the seedlings growing in the higher light conditions (e.g. before canopy closure) respond to a change towards lower light conditions (e.g. after canopy closure) by acclimation of current leaves to the new light environment, or whether they discard their leaves and replace them with shade-adapted ones (review by Bazzaz 1984, Chazdon 1988 and Bazzaz 1991). The replacement of leaves developed before canopy closure is slow and difficult in a light-limited understorey habitat (Clark and Clark 1985). In tropical forest tree seedlings, leaf-level and whole-plant carbon gain are influenced by leaf life span. The leaf life span is about 6 to 9 months in deciduous species and more than 1 year in evergreen species (Clark and Clark 1985; Chazdon 1988). Thus, the capacity of the high-light leaves of a given species for adaptation to shadelight may positively affect seedling survival and growth in the light-limited forest understorey habitats.

Studies on herbaceous species show that fully expanded leaves of certain species retain some capacity to adapt to altered light environments (e.g. Bunce *et al.* 1977; Sebaa *et al.* 1987). There have been very few studies on woody species (Syvertsen and Smith 1984; Oberbauer and Strain 1985). However, in a study of growth and physiology of tropical forest climax species *Pentaclethra macroloba*, the seedlings which were experimentally switched from full sun and 25% sun to 1% sun had a negative photosynthetic rate and suffered leaf abscission resulting in negative growth in the shade (Oberbauer and Strain 1985).

In this study, the potential of fully developed high-light leaves of gap species *Bischofia javanica* Blume for adaptation to shadelight is reported. The *Bischofia* seedlings were grown under simulated daylight in a growth cabinet. After transfer of the high-light grown seedlings to simulated shadelight, changes in the photosynthetic capacity, leaf morphology and leaf chlorophylls were examined in fully developed high-light leaves.

## **6.2 Materials and Methods**

### **6.2.1 Plant materials**

The materials and methods for raising seedlings were similar to those described in Sections 2.2.1 and 5.2.1. When the seedlings were of 3 to 4 cm tall and their first true leaf was fully expanded, they were transferred to growth tubes, and placed in a random design on the bench of a growth cabinet.

### **6.2.2 Nutrition**

The materials and methods of nutrition were the same as those described in Section 5.2.2.

### **6.2.3 Growth cabinet and growth conditions**

The light climate and other conditions in the growth cabinets were the same as described in Section 5.2.3.

#### **6.2.4 Growth period**

After 6 weeks of growth in the high-light growth cabinet (simulated daylight,  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), the seedlings were transferred to the low-light cabinet (simulated shadelight,  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). At this point, most of the seedlings had produced at least 10 fully expanded leaves. These transferred seedlings were designated as the HL seedlings, and the seedlings which were grown in the same light regime throughout the experimental period were defined as the controls.

#### **6.2.5 Measurements of photosynthesis and stomatal conductance**

Fully expanded leaves near the top of 5 seedlings were selected and tagged for the measurements of net photosynthetic rate ( $P_n$ ) and stomatal conductance ( $g_s$ ).  $P_n$  was measured by following methods described in Section 5.2.5. Leaf conductance to water vapour was also measured by the ADC gas analyser (the algorithm used by the microprocessor involves calculating leaf temperature from the photon flux density. As the calibration is for sunlight, some error will occur when operating in artificial light). Photosynthetic rate and stomatal conductance were calculated by using formulae described by Long and Hallgren (1985).

#### **6.2.6 Determination of chlorophylls and leaf weight per unit area**

The leaf chlorophylls and leaf weight per unit leaf area ( $L_w$ ) were determined by following methods described in Section 5.2.6.

#### **6.2.7 Measurements of photosynthetic light response**

Seedlings were brought from the growth cabinet to the laboratory, and the leaves were irradiated with a beam from a slide projector, to determine steady state responses to photon flux density. The gas analyser used and the methods of determination of photosynthetic rates were the same as described in Section 5.2.5. The air was drawn from the outside, passed through two 10-litre bottles, and humidified to 2.0-2.2 kPa by passing through bubblers in a water bath. The air temperature in the leaf chamber was  $26 \pm 2 \text{ }^\circ\text{C}$ . Photosynthetic photon flux density was adjusted by interposing neutral

glass filters (Frew-Smith Ltd., Irvine, UK) between the leaf and the projector. These filters transmit, but do not scatter the radiation.

Calibration of the gas analyser was done by using a set of precision mixing pumps (Wosthoff, Bochum, FRG). The quantum sensor was checked against a standard quantum sensor (Li 190SB, Li-Cor, Lincoln, Nebraska, USA). The humidity sensor of the ADC system was calibrated every day using a controlled temperature water bath as a reference.

The leaves (9th from the base), which were fully developed prior to the transfer from the high-light cabinet, were used for the measurements after 8 weeks of exposure to the low light. At the same time, measurements were also taken for leaves, of same age group, belonging to the H light and the L light control seedlings. In all cases, the replicate number was 3.

A rectangular hyperbola was fitted to the measured data to describe the relationship between light and photosynthesis (Long and Hallgren 1985):

$$P = \frac{P_{\max} \cdot Q}{K + Q} - R_d$$

where  $P$  = photosynthetic rate,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $P_{\max}$  = maximum photosynthetic rate,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $Q$  = photosynthetic photon flux density,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $K$  = photon flux density at which  $P$  is half of the  $P_{\max}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $R_d$  = rate of dark respiration,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

This model was fitted to the data from each leaf by using the parameter optimisation program PAR (BMDP Statistical Software, Los Angeles, California 90025, USA). Apparent maximum quantum yield ( $\alpha$ ) was the measure of the initial slope ( $P_{\max}/K$ ). Variation in each photosynthetic parameter was explored by analysis of variance using parameter values from the individual leaves.

## 6.3 Results

### 6.3.1 Changes in photosynthetic characteristics and leaf weight per unit area

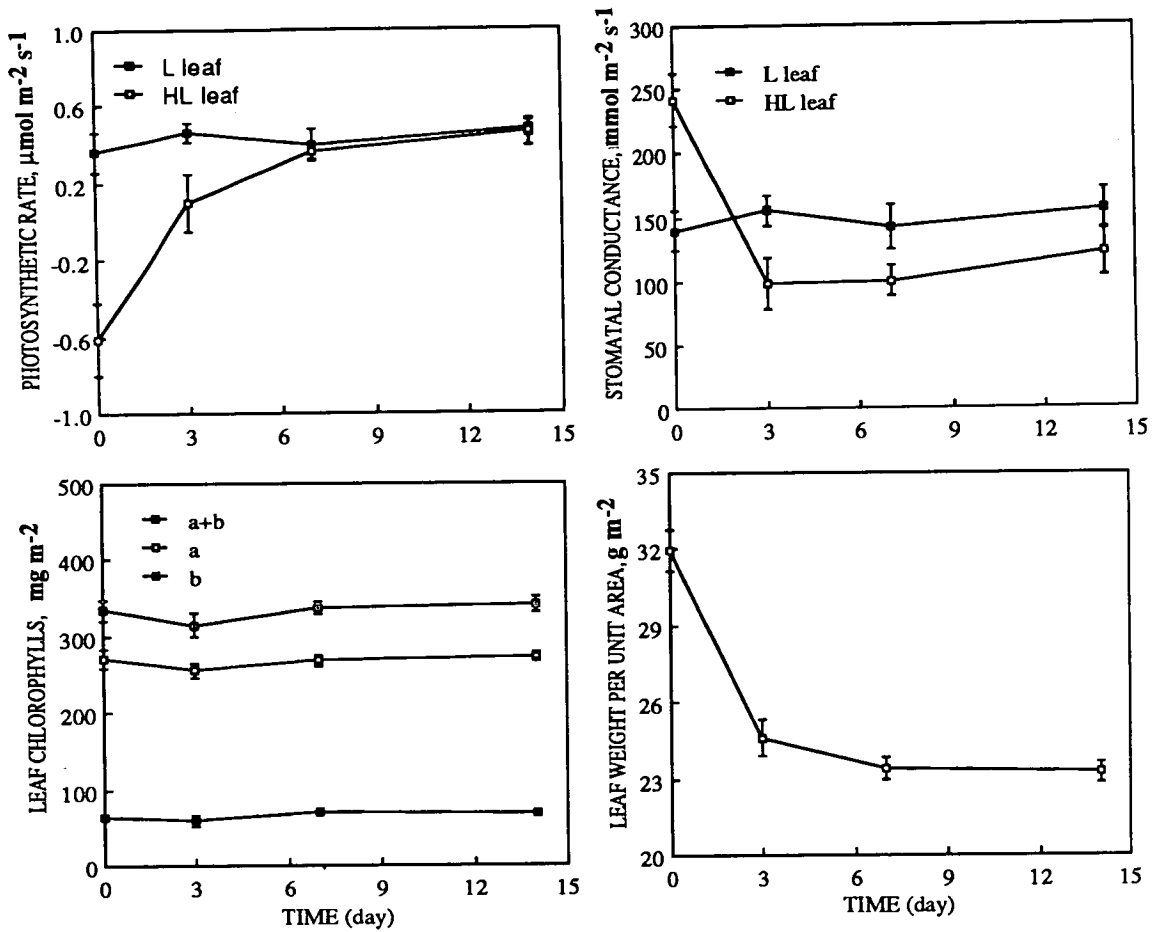
The changes in net photosynthetic rate ( $P_n$ ) and stomatal conductance ( $g_s$ ) in the HL leaf are presented in Figure 6.1. The leaves displayed a negative  $P_n$  on the day of transfer though they showed a quite high value of  $g_s$  after 1 h of transfer. Within 3 days of transfer, the  $P_n$  started to increase and tended to level off in such a manner that on the day 7, the value was equivalent to that of the L light leaf. The  $g_s$ , on the other hand, decreased to a value significantly lower than that of the L light leaf within 3 days of transfer, and increased thereafter more slowly and towards day 14, approached the  $g_s$  for the L light leaf.

No significant change in leaf chlorophylls per unit leaf area was observed within 14 days of exposure (Figure 6.1). Leaf weight per unit area ( $L_w$ ) drastically decreased within 3 days of exposure and continued to decrease further over several days to reach a steady state, but not at the same magnitude as observed over the first 3 days of exposure.

### 6.3.2 Changes in leaf chlorophylls after 8 weeks of exposure

Exposure to the L light brought about changes in leaf chlorophylls (Table 6.1). When expressed on a leaf area basis, no significant difference in total chlorophylls (chlorophyll a and b) was observed between the HL leaf and the H light leaf, but the chlorophyll a significantly decreased in the the former. In these leaf types, chlorophyll b was almost same. However, leaf chlorophylls were significantly higher in the HL leaf than the L light leaf.

This scenario became different when expressed on the leaf weight basis. Total chlorophylls (chlorophyll a and b) were higher in the HL leaf than the H light leaf. This difference between these leaf types was due to the compensating variation in the  $L_w$  (Table 6.1). Because of the same reason, the chlorophyll contents in the H light leaf were lower than the L light leaf. In respect of the  $L_w$ , the HL leaf did not differ significantly from the L light leaf. Total chlorophylls (chlorophyll a and b) per unit leaf weight were significantly higher in the HL leaf than the L light leaf.



**Figure 6.1:** Net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), leaf chlorophylls, and leaf weight per unit leaf area ( $L_w$ ) of the high-light leaf transferred to the low light (HL leaf). Mean of  $5 \pm \text{SE}$ ; the  $P_n$  was measured at  $35 \pm 1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; the same leaves of 5 seedlings were measured over the period; on the day of transfer, the measurements were taken after 1 h of the transfer; for the determination of the leaf chlorophylls and the  $L_w$ , leaves of the same age-group from different seedlings were harvested over the period.

Table 6.1: Leaf characteristics of low-light leaf (L), high-light leaf (H), and high-light leaf after 8 weeks of exposure to low light (HL). Mean of 5 seedlings ( $\pm$ SE); means preceded by the same letter are not significantly different from each other at  $P < 0.05$  (ANOVA and Duncan's multiple range test ).

variables	L	H	HL	<i>F</i>	<i>P</i>
leaf weight per unit leaf area, $L_w$ , ( $\text{g m}^{-2}$ )	b 23.55 (0.29)	a 47.20 (1.58)	b 25.56 (1.18)	129.7	0.0001
leaf chlorophylls: a+b ( $\text{mg m}^{-2}$ )	b 296.96 (14.80)	a 456.51 (11.50)	a 419.46 (17.50)	31.8	0.0001
a "	c 229.08 (12.41)	a 362.18 (9.46)	b 324.40 (13.82)	32.5	0.0001
b "	67.88 (2.59)	a 94.26 (2.20)	a 95.06 (3.88)	27.0	0.0001
a+b ( $\text{mg g}^{-1}$ )	b 12.63 (0.70)	c 9.69 (0.23)	a 16.48 (0.66)	35.2	0.0001
a "	b 9.75 (0.59)	c 7.69 (0.17)	a 12.75 (0.54)	28.9	0.0001
b "	b 2.89 (0.12)	c 2.00 (0.06)	a 3.73 (0.13)	95.9	0.0001
a : b ratio	b 3.37 (0.094)	a 3.84 (0.033)	b 3.41 (0.056)	15.7	0.0001

Chlorophyll a to b ratio (chlorophyll a:b) was significantly lower in the HL leaf than the H light leaf. This ratio in the former was close to the value for the L light leaf.

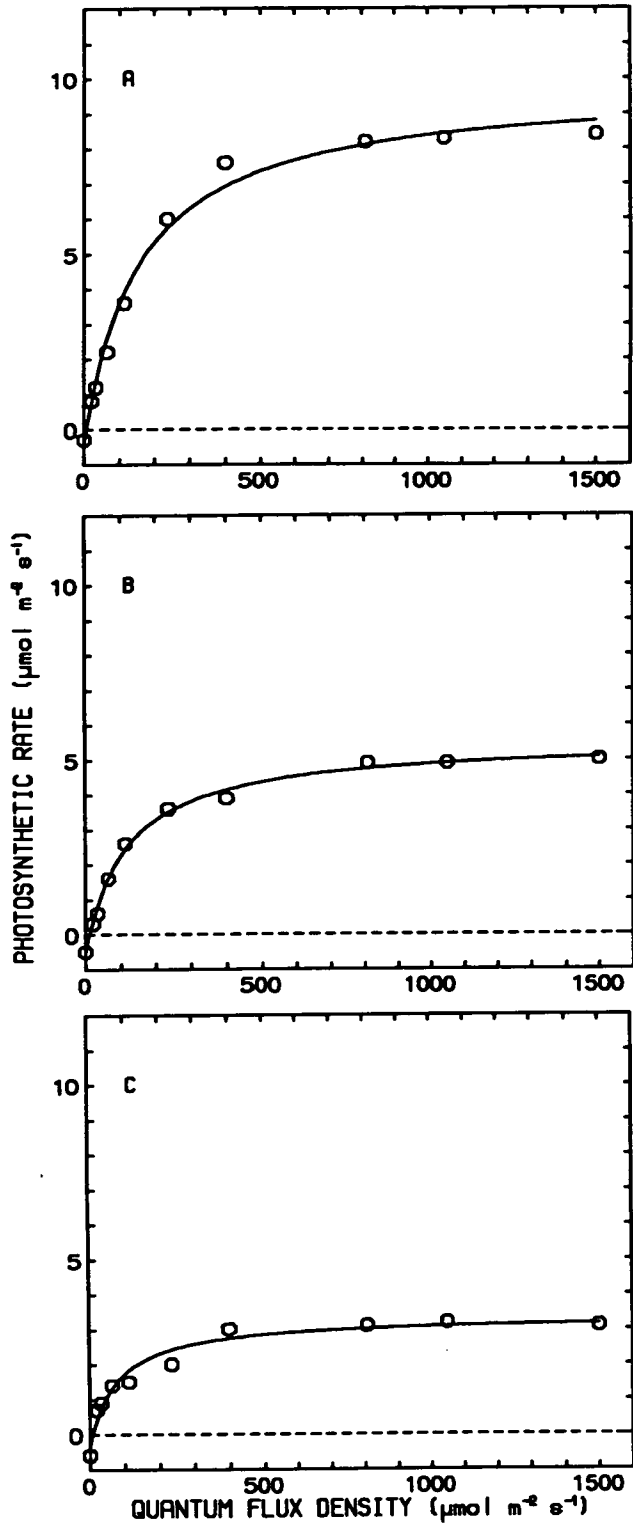
### 6.3.3 Photosynthetic light-response curves

The light-response curve, expressed on leaf area basis, of the H light leaf was clearly different from that for the L light leaf (Figure 6.2). Light saturation in the former was reached at about  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whilst in the latter it was about  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The irradiance required to saturate the photosynthesis to one-half of the maximum value ( $K$ ) was also largely higher in the H light leaf ( $198 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than the L light leaf ( $81 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Table 6.2). Apparent quantum yield ( $\alpha$ ) was not significantly higher in the H light leaf than the L light leaf. Dark respiration ( $R_d$ ) was less precisely determined because the precision of the measurements was limited by the resolution of the gas analyser (see Kwesiga *et al.* 1986; Ramos and Grace 1990). However, apparently the  $R_d$  was higher in the H light leaf than the L light leaf, but not significant at  $P < 0.05$  level. Maximum photosynthesis ( $P_{\text{max}}$ ) was also significantly higher in the H light leaf than the L light leaf (Table 6.2).

The light-response curve showed very pronounced effects of exposure of the high-light grown seedlings to the simulated shadelight (Figure 6.2).  $P_{\text{max}}$  in the HL leaf decreased by about 40% compared to the H light leaf, but was almost at the same extent higher than the L light leaf (Table 6.2). Light saturation in the HL leaf reached at a irradiance in between those for the H light leaf and the L light leaf. The changes in respect of  $K$  also followed the same pattern of response.

## 6.4 Discussion

The high-light leaves, expanded fully prior to the transfer, (HL leaf) exhibited a large negative  $P_n$  after transfer to the low (L) light (Figure 6.1). This decrease was immediate after transfer, and then tended to level off in such a way that on the day 7, the value was equivalent to the  $P_n$  of the L light control leaves. This depression in the  $P_n$  did not follow the pattern of changes in the  $g_s$  though there was a large decrease in the  $g_s$ . The  $g_s$  was significantly lower than that for the L light control leaf even on day 7, when the  $P_n$  in the HL leaf reached the  $P_n$  of the L light control leaf. This indicates that the changes in the  $P_n$  of the HL leaf was not directly related to those in the  $g_s$  after the transfer. Such changes in  $P_n$  of the fully-expanded high-light leaf



**Figure 6.2:** Light response curves of photosynthesis for the high-light control leaf (A), the high-light leaf after 8 weeks of exposure to the low light (B), and the low-light control leaf (C). Each line is the rectangular hyperbola obtained from the model using the observed values, and the data points are from one seedling, in which the maximum photosynthesis ( $P_{\text{max}}$ ) was close to the mean.

**Table 6.2:** Photosynthetic parameters of low-light leaf (L), high-light leaf (H), and high-light leaf after 8 weeks of exposure to low light (HL).  $P_{\max}$  = maximum photosynthetic rate, ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $K$ =photon flux density at which  $P = P_{\max}/2$ , ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $R_d$  = rate of dark respiration, ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); and  $\alpha$  = apparent quantum efficiency, (initial slope,  $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$ ). Mean of 3 seedlings ( $\pm\text{SE}$ ). Other particulars are the same as Table 6.1.

variables	L	H	HL	$F$	$P$
$P_{\max}$	c 3.7 (0.22)	a 10.4 (0.67)	b 6.2 (0.31)	57.3	0.0001
$K$	b 81 (8)	a 198 (39)	ab 121 (14)	6.1	0.036
$R_d$	a 0.369 (0.010)	a 0.439 (0.062)	a 0.532 (0.062)	2.5	0.159
$\alpha$	a 0.047	a 0.055	a 0.052	0.4	0.688

after transfer to the low light have also been reported for *Phaseolus vulgaris* (Louwerse and Zweerde 1977), *Lolium multiflorum* (Sebaa *et al.* 1987) and tropical forest tree species *Pentaclethra macroloba* (Oberbauer and Strain 1985).

Transfer to the L light regime brought about a dramatic decrease in the  $L_w$  of the HL leaf, and most of the decreases occurred within 3 days of transfer (Figure 6.1), when the  $P_n$  of the HL leaf was well below the  $P_n$  of the L light leaf. The large decreases in the  $L_w$  might be due to the translocation of starch and other cell components from the leaves to the other parts of the seedling, an exactly opposite to the effect of transfer of the seedlings of this species to the H light from the L light (see Chapter 5). This decrease in the  $L_w$  did not improve even after 8 weeks of transfer (Table 6.1). Such a decrease in the  $L_w$  has also been observed in the high-light leaf of *Glycine max* after transfer to the low light (Bunce *et al.* 1977).

Though there was no immediate significant effect of the transfer to the L light on leaf chlorophylls per unit leaf area (Figure 6.1), the leaf chlorophylls were significantly changed after 8 weeks of exposure (Table 6.1). A significant decrease in chlorophyll a in the HL leaf without an appreciable change in chlorophyll b resulted in a large decrease in chlorophyll a:b ratio, which was significantly lower than that for the H light control leaf, and very close to the value for the L light control leaf.

It is well known that the high chlorophyll a:b ratio is the characteristic of sun leaf, while a low ratio is an adaptive character of shade leaf (e.g. Patterson *et al.* 1978; Hoflacher and Bauer 1982; Kwesiga 1984; Sims and Percy 1989). The chlorophyll ratios found in the present experiment are comparable to the ratios for high- and low-light leaves of tropical forest tree seedlings (Kwesiga 1984), and higher than those found for species, which typically grow in vegetational shade (Chow *et al.* 1990). The total chlorophyll contents per unit leaf area or per unit dry matter are also comparable to those recorded for the shrub species of forests (Masarovičová and Eliáš 1981), and the tree seedlings from the tropical forests (Kwesiga 1984).

Though the chlorophyll a:b ratio largely decreased, there was no appreciable change in the total chlorophyll contents per unit area in the HL leaf compared to the H light leaf (Table 6.1). There is evidence that a decrease in chlorophyll a:b ratio as a result of canopy closure can be achieved by increasing both chlorophyll a and b at different proportions in herbaceous species (Eliáš and Čiamporová 1986). The increased number of photosynthetic units per unit leaf area with an extensive grana formation in

the shade plants are correlated with an increase in the leaf chlorophyll contents on an area basis (Bunce *et al.* 1977; Eliáš and Čiamporová 1986). Bunce *et al.* (1977) had, however, observed no increase in the number of photosynthetic units per unit leaf area and hence no increase in leaf chlorophylls in fully expanded leaf of *Glycine max* as a result of transfer from the high light to the low light, though size of the photosynthetic units was increased significantly during adaptation to the low light. As the photosynthetic unit size increases, the chlorophyll a:b ratio decreases (Patterson *et al.* 1978). This decrease in chlorophyll a:b ratio is associated with changes in the light-harvesting chlorophyll a and b proteins-complexes (Chow *et al.* 1988).

It is known that the changes in the chlorophyll a:b ratio are correlated with changes in PSII/PSI (photosystem II/photosystem I) reaction centre ratio in the thylakoid membrane of chloroplasts (review by Glazer and Melis 1987; Evans 1987; Chow *et al.* 1990). Typically, the PSII/PSI reaction centre ratio is greater in the high-light leaf than in the low-light leaf (Chow *et al.* 1990). However, both light quantity and light quality can regulate the amounts of PSII and PSI reaction centres. Chow *et al.* (1988) have shown that a growth light regime with a low R:FR ratio results in an additional drop in the chlorophyll a:b ratio below values obtained in a low light alone. Sims and Pearcy (1989) have found a lower chlorophyll a:b ratio in low growth irradiance without lowering the R:FR ratio. In a simulated canopy shadelight, the lower irradiance tends to decrease the amount of PSII reaction centres on a chlorophyll basis (Chow *et al.* 1990), and the concomitant increase in far-red radiation, absorbed preferentially by PSI tends to increase the amount of PSII reaction centres on a chlorophyll basis (Glick *et al.* 1986; Chow *et al.* 1990) in order to balance the input of excitation energy in both the photosystems. Thus, it is the opposing interplay of both low irradiance and low R:FR ratio that modulates the photosystem reaction centre ratio so as to establish an overall balanced absorption of light by PSII and PSI.

The higher  $P_{\max}$  and  $K$  in the H light leaf compared to the L light leaf are well known responses of photon irradiance during growth (e.g. Bazzaz and Carlson 1982; Langenheim *et al.* 1984; Kwesiga *et al.* 1986; Riddoch *et al.* 1991a). The non-significant  $\alpha$  found in the present experiment is in accordance with the responses found in several other studies (e.g. Thompson *et al.* 1988; Sims and Pearcy 1989; Ramos and Grace 1990; Riddoch *et al.* 1991a). The photosynthetic rates found for the H light leaf and the L light leaf are similar to those for many tropical forest tree species (e.g. Langenheim *et al.* 1984; Kwesiga *et al.* 1986; Ramos and Grace 1990; Riddoch *et al.* 1991a,b).

The differences in the  $P_{\max}$  between the H light and the L light leaves are associated with the differences in mesophyll conductance, which increases with growth irradiance and is correlated positively with mesophyll thickness or volume per unit leaf area, chlorophyll content per unit area, and photosynthetic unit density per unit area (Patterson *et al.* 1978; Kwesiga *et al.* 1986; Ramos and Grace 1990). Low irradiance during growth is known to bring about a reduction in the components of electron transport and photophosphorylation. At a low irradiance, plants need to maximise light absorption and can reduce the maximum Hill activity (Evans 1987). Calvin cycle enzymes are also reduced (Björkman 1981). Hoflacher and Bauer (1982) found that the photosynthetic capacity, the Hill reaction, and the activity of RuBP carboxylase, when expressed per chloroplast, were almost identical in the low-light and the high-light grown plants. They concluded that the different photosynthetic capacity per unit leaf area was mainly the result of the different photosynthetically active mass per unit leaf area.

A decrease in leaf thickness and tissue density, and an increase in air space per unit leaf volume after exposure of the high-light grown seedlings to relatively lower light levels have been observed in *Citrus* (Syvertsen and Smith 1984). Decrease in leaf thickness resulting from altered thickness of both the palisade and spongy mesophyll tissue has also been reported for fully expanded leaf prior to transfer to the low light in *Glycine max* (Bunce *et al.* 1977). However, in both the cases, the leaf thickness and the tissue density were higher in the low-light acclimated leaf than the low-light control leaf.

It seems that a large decrease in light-saturated photosynthetic capacity in the HL leaf compared to the H light leaf might be due to the changes in physical, biochemical and possibly anatomical characteristics during adaptation to the low light. The significantly higher photosynthetic rates at the light-saturation point in the HL leaf relative to the L light leaf might not be attributed to the partial adaptation to the low light. The close proximity of chlorophyll a:b ratio of the HL leaf to that of the L light leaf indicates that the adaptation of the HL leaf to the low light was complete. The leaf chlorophylls per unit leaf area were higher in the HL leaf than the L light leaf (Table 6.1). Hence, the higher rates of photosynthesis at light saturation level might be attributed to the higher amount of photosynthetically active mass per unit leaf area in the HL leaf.

The changes in the photosynthetic machinery reported in this chapter suggest a re-organisation over periods of days and weeks. In nature, a decline in PPFD over this time would occur with the closing of small gaps by the growth of the lateral branches or even with the gradual decline in solar elevation and the canopy closure due to the seasonal changes.

## CHAPTER 7

# Growth and Photosynthesis of *Bischofia javanica* Seedlings as influenced by a Changing Light Availability

### 7.1 Introduction

Light conditions in the forest understorey are highly variable. Formation of gaps in the canopy by the falling of a tree or branch and subsequent closure of those gaps by the growth of the lateral branches in the canopy result in a sudden increase in light on one extreme and typically a gradual decrease on the other (Orians 1982; Chazdon and Fetcher 1984; Lee 1989). On the forest floor, a sudden decrease in light availability on the scale of weeks to months is less likely. However, it may happen, for example, with unfolding of a palm over a small gap (Fetcher *et al.* 1983) or when a canopy tree leans over a gap. The light regime on the forest floor also greatly varies due to the seasonal changes in the forest canopy cover and the changes in solar elevation (review by Chazdon 1988). This seasonal variation is more pronounced in the deciduous and the semi-evergreen forests (Lee 1989), while in the evergreen forests, the seasonal variation is primarily due to the changes in solar elevation rather than to the changes in forest canopy cover (Chazdon 1988). Thus, a given seedling growing in the forest understorey may be exposed to a temporally and spatially variable light environment including increases as well as decreases.

It is known that plants living in exposed habitats exhibit different photosynthetic properties than those living in shaded conditions (Boardman 1977; Björkman 1981). The variation in light availability over the scale of weeks to months can lead to differences in photosynthetic acclimation, plant morphology and whole-plant growth (Chazdon 1988). Herbaceous plants in the forests are known to show a variety of growth patterns in relation to the seasonal changes in light availability (review by Chazdon 1988). The photosynthetic light responses of these plants strongly reflect the conditions prevailing during leaf development (Taylor and Pearcy 1976; Masarovičová and Eliáš 1986). Thus the acclimating responses in a direction that leads to a positive whole-plant carbon balance under a new light environment may be critical for survival and growth of a given seedling in a temporally and spatially variable light environment in the forest.

There have been very few studies on acclimation potential for tropical forest tree seedlings (Fetcher *et al.* 1983; Oberbauer and Strain 1985; Pompa and Bongers 1991). However, Fletcher *et al.* (1983) compared the acclimation to the light environment of the pioneer species *Heliocarpus appendiculatus* with the non-pioneer species *Dipteryx panamensis*. Seedlings were grown in full sun, partial shade (20% sun), and full shade (2% sun) and were experimentally switched between environments. Growth of the pioneer species was more plastic than that of the non-pioneer species in responses to changes in the light environment. In a similar study (Oberbauer and Strain 1985) on seedlings of a climax species *Pentaclethra macroloba*, plants which were experimentally switched from full and 25% sun to 1% sun, had a negative CO<sub>2</sub> exchange rate and suffered leaf abscission resulting in negative growth; and the seedlings switched from 1% sun to full sun showed severe photoinhibition and leaf damage. There was little acclimation of photosynthesis to changes in the light environment.

In this study, the potential of gap species *Bischofia javanica* Blume for acclimation to contrasting light regimes was examined. The seedlings, grown under simulated daylight and simulated shadelight, were switched between the contrasting light regimes. The seedling growth in response to the changed light environment was determined. The photosynthetic capacity, leaf chlorophylls, and leaf anatomy of the newly-formed leaves in the new light environment were also examined.

## **7.2 Materials and Methods**

### **7.2.1 Plant materials**

The materials and methods for raising seedlings were similar to those described in Sections 2.2.1 and 5.2.1. When the seedlings were of 3 to 4 cm tall and their first true leaf was fully expanded, they were transferred to growth tubes, and placed in a random design on the bench of growth cabinets of contrasting light regimes.

### **7.2.2 Nutrition**

The materials and methods of nutrition were the same as described in Section 5.2.2. The nutrient solution applied contained 60 mg of N l<sup>-1</sup> and the application of the nutrient solution was regular- twice a day.

### **7.2.3 Growth cabinet and growth conditions**

The light climate and other conditions in the growth cabinets were the same as described in Section 5.2.3.

### **7.2.4 Growth period**

After 7 weeks of growth in the high-light growth cabinet (simulated daylight, 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the high-light seedlings were transferred to the low-light cabinet (simulated shadelight, 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and at the end of 10 weeks growth in the low-light cabinet, the low-light seedlings were transferred to the high-light cabinet. At this point, the height of the high-light seedlings were in between 11 and 17 cm and most of them had produced 10 fully expanded leaves. The low-light grown seedlings attained a height of  $7 \pm 1$  cm and produced 6 or 7 fully expanded leaves. The seedlings of the same age group were maintained as controls.

### **7.2.5 Leaf anatomy**

The stomatal density, and the thickness of leaf and of palisade and spongy mesophyll were determined following the methods described in Section 5.2.7.

### **7.2.6 Determination of chlorophylls**

The leaf chlorophylls were determined by following methods described in Section 5.2.6.

### **7.2.7 Measurements of photosynthetic light response**

The seedlings were brought from the growth cabinet to the laboratory, and the measurements of photosynthetic light response of leaves were determined following methods described in Section 6.2.7. Fully expanded newly-formed leaves close to the

top of the seedlings were used for the measurements. At the same time, the measurements were also taken for the leaves of the high-light and the low-light controls. In all cases the replicate number was 3. Data were analysed by using the methods described in Section 6.2.7.

### **7.2.8 Growth Data**

On the day of the transfer to the contrasting light regimes, the high-light and the low-light grown seedlings, 7 from each group, were harvested ( $t_1 = 0$ ). Finally, after 9 weeks of growth under the changed light regimes, the seedlings were harvested ( $t_2 = 9$  wk). At the same time, the seedlings serving as controls were also harvested. Leaf area and dry weight of the component parts of each seedling were determined as described in Section 2.2.6.

From the raw data, the following parameters were derived by using the methods and the equations described in Section 2.2.6: relative biomass growth rate, *RGR*; net assimilation rate, *NAR*; leaf area ratio, *LAR*; specific leaf area, *SLA*; and leaf weight ratio, *LWR*.

In the same way, the growth data were recorded for the first phase of growth (before the transfer). From these primary data, the growth parameters for the previous light environments were determined. Using these parameters, the effects of the previous and the present environments on the seedling growth were determined.

## **7.3 Results**

### **7.3.1 Seedling growth after transfer to the contrasting light regimes**

The low-light grown seedlings transferred to the high light were designated as the LH seedlings, and the high-light grown seedlings transferred to the low light as the HL seedlings. The seedlings which were grown in the same light regime throughout the experimental period were defined as the controls. The light regime after reciprocal transfer was defined as the present environment, and the light regime in which the seedlings were growing before transfer was defined as the previous environment.

In general, leaves produced in the high light were bigger than those formed in the low light, and the number of leaves were also higher in the high-light grown seedlings than the low-light grown ones. Leaf production rate and the size of the newly-formed leaves were decreased after transfer to the low light. The HL seedlings produced on an average 7 leaves per seedling over 9 weeks of growth, while the LH seedlings produced 14 leaves per seedling over the same period of time. The leaves of the latter were almost double in size (based on leaf area of 5 newly-formed fully expanded leaves) than those of the former. No leaf-shedding was observed till the end of the experiment.

Reciprocal transfer of the seedlings between the high and the low growth light regimes had profoundly affected the seedling growth (Table 7.1). *RGR* was increased in the LH seedlings, whilst it was decreased in the HL seedlings. It was influenced by both the *NAR* and the *LAR*. In the LH seedlings, the *NAR* was increased significantly, but the *LAR* was decreased. This indicates that the increased *RGR* was due to a large increase in the *NAR*. The decrease in *LAR* was due to a large decrease in the *SLA* and a slight decrease in the *LWR*. The reverse situation was observed for the HL seedlings.

Compared to the controls, the transferred seedlings showed different patterns of growth response. *RGR* was significantly higher in the LH seedlings than the high-light control seedlings. Both the *NAR* and the *LAR* were higher in the former than the latter. Same patterns of response were observed in respects of *SLA* and *LWR*. The HL seedlings had significantly lower *RGR* than the low-light control seedlings. This difference was due to the significant difference in the *LAR* since the *NAR* was not significantly different between these two groups of seedlings. The HL seedlings had significantly lower *SLA* and *LWR* compared to the low-light control seedlings.

Two-way analysis of variance was used to distinguish the effects of the present environment from the previous one. The analysis shows that the effects of the present environment on the growth parameters were more strong than those of the previous environment (Table 7.2). All the growth parameters studied displayed significant previous and present environment interactions. *LWR* was the only parameter, which was less affected by the previous or the present environment.

**Table 7.1:** Growth characteristics of the high-light control seedlings (H), the low-light control seedlings (L), the low-light seedlings transferred to the high light (LH), and the high-light seedlings transferred to the low light (HL) for *Bischofia javanica*. Seedlings were harvested after 9 weeks of transfer to the contrasting light regimes. Mean of 7 seedlings. Means preceded by the same letter are not significantly different from each other at  $P < 0.05$  (ANOVA and Duncan's multiple range test).

variables	H	L	LH	HL	F	P
relative growth rate, <i>RGR</i> ( $\text{g g}^{-1} \text{wk}^{-1}$ )	b 0.322	c 0.175	a 0.495	d 0.084	1906.0	0.0001
net assimilation rate, <i>NAR</i> ( $\text{g m}^{-2} \text{wk}^{-1}$ )	b 29.97	c 5.91	a 33.82	c 4.61	280.8	0.0001
leaf area ratio, <i>LAR</i> ( $\text{m}^2 \text{g}^{-1}$ )	d 0.0088	a 0.0305	c 0.0123	b 0.0198	139.9	0.0001
specific leaf area, <i>SLA</i> ( $\text{m}^2 \text{g}^{-1}$ )	d 0.0202	a 0.0509	c 0.0245	b 0.0408	121.3	0.0001
leaf weight ratio, <i>LWR</i>	c 0.436	a 0.599	b 0.500	b 0.484	32.8	0.0001

**Table 7.2:** The effects of previous and present light environments on the growth of seedlings. Two-way ANOVA with previous and present environments as main factors; number of seedlings 7 in all cases; \*\*\*, 0.0001; \*,  $P < 0.01$ ; ns, not significant at  $P < 0.10$ .

<i>Probability</i> :	<i>P</i>		
Light environments:	previous	present	previous*present
relative growth rate, <i>RGR</i>	***	***	***
net assimilation rate, <i>NAR</i>	*	***	***
leaf area ratio, <i>LAR</i>	*	***	***
specific leaf area, <i>SLA</i>	*	***	***
leaf weight ratio, <i>LWR</i>	ns	ns	***

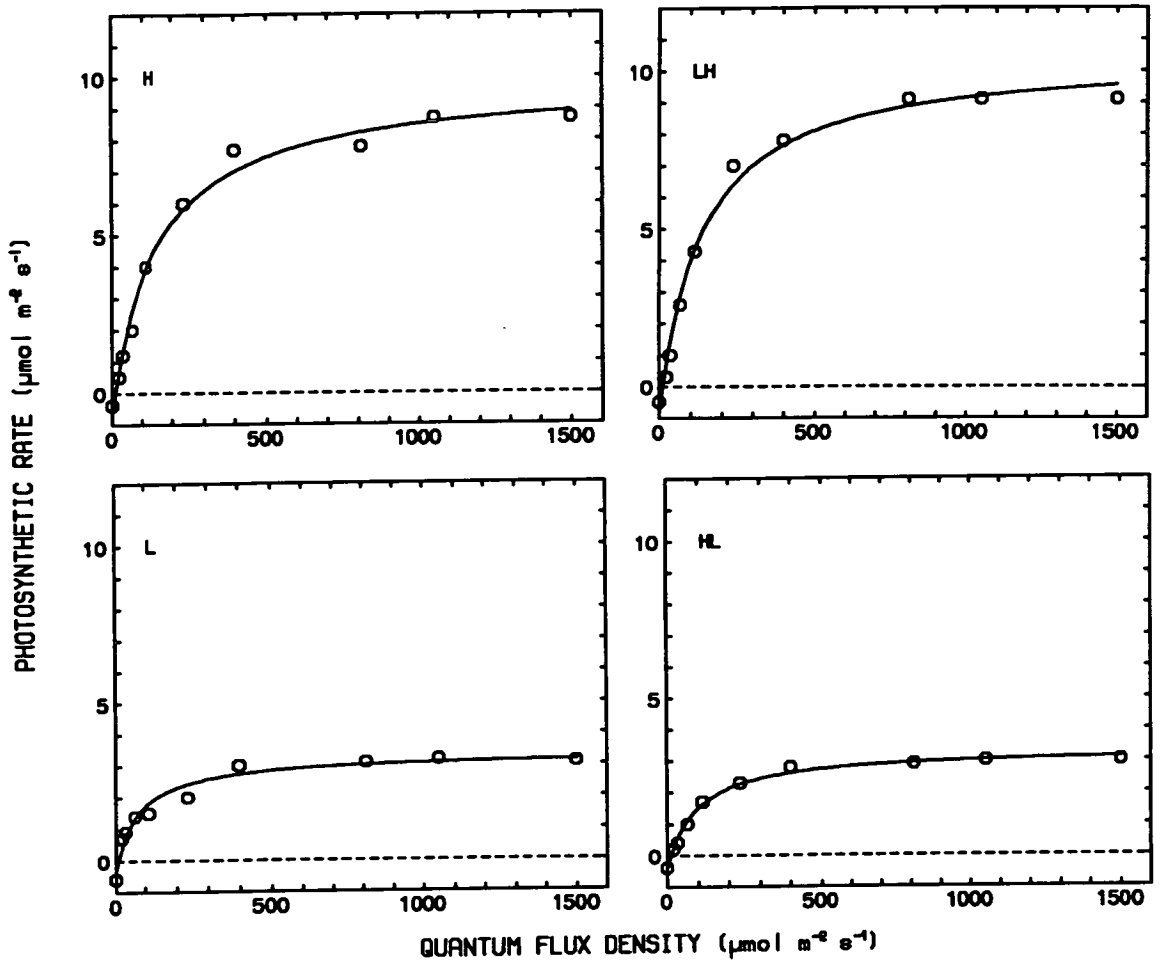
### 7.3.2 Photosynthetic light response curves

The photosynthetic light response curves were determined on the fully developed newly-formed leaf of the LH and the HL seedlings as well as for the leaves of the same age group in the control seedlings (Figure 7.1 and Table 7.3). The observed values and the predicted values from the model were well fitted in all the cases. Substantial changes occurred in the relationship between photosynthesis and the quantum flux density. Mean  $P_{\max}$  ranged from only  $3.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for the HL leaf to  $11.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for the LH leaf. Light saturation in the former was reached at about  $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , whilst in the latter, it was about  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Mean irradiance required to saturate the photosynthesis to one-half of the maximum value ( $K$ ) was  $154 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for the LH leaf, whilst it was only  $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for the HL leaf. Apparent quantum yield ( $\alpha$ ) was not significantly affected by the growth irradiance. Apparently, dark respiration ( $R_d$ ) was higher in the LH leaf than the HL leaf, but not significant at  $P < 0.05$  level. It may be mentioned here that the  $R_d$  was less precisely determined because of the limitation of the resolution of the gas analyser (see Section 6.3.3). In respect of  $P_{\max}$ ,  $K$ ,  $R_d$ ,  $\alpha$ , and the light saturation point, the newly-formed leaves of the LH and the HL seedlings did not significantly differ from the high-light and the low-light leaves respectively.

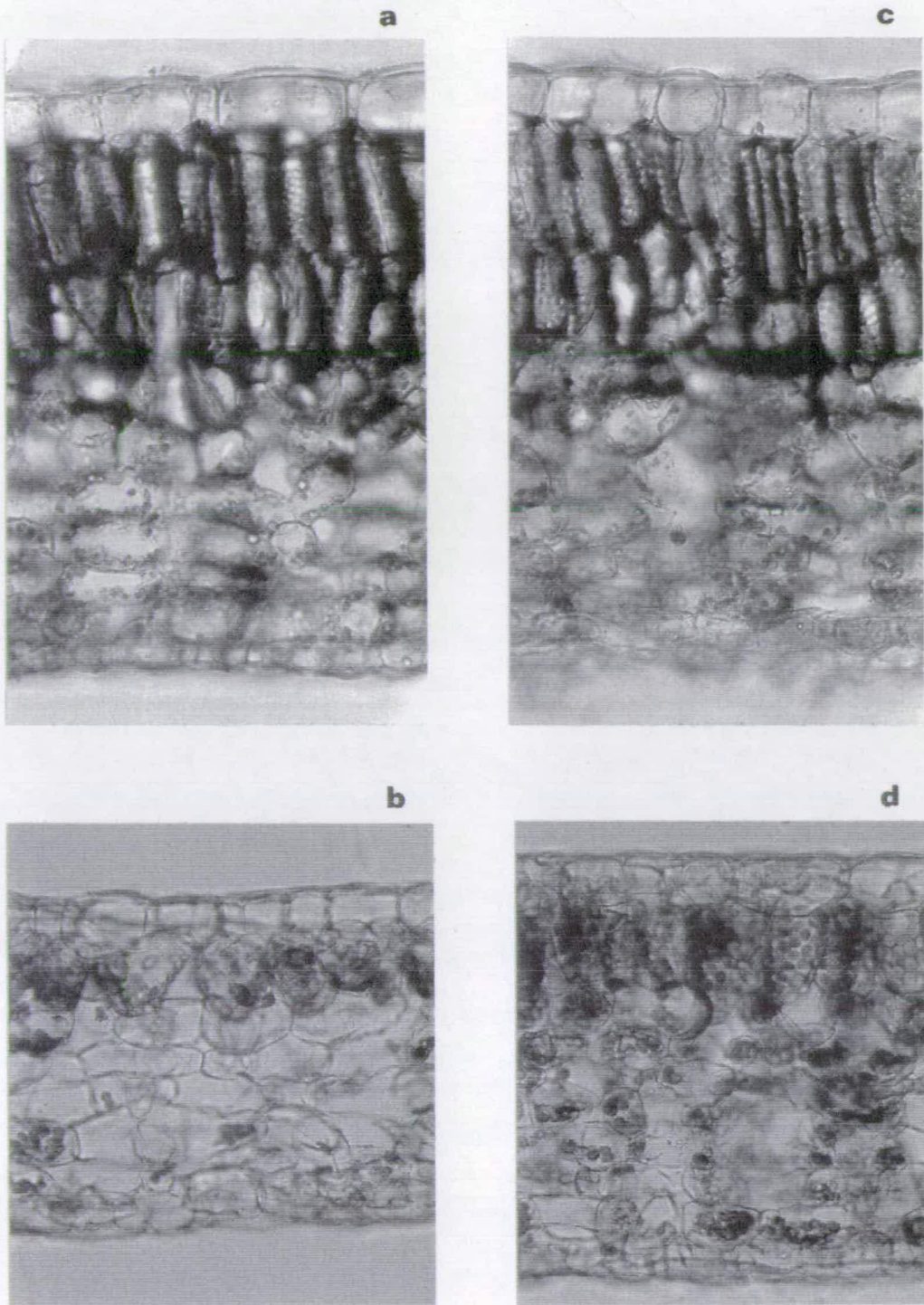
### 7.3.3 Leaf anatomy and leaf chlorophylls

The photosynthetic differences were accompanied by differences in leaf anatomy and leaf chlorophylls (Table 7.4). LH leaf was significantly thicker than the HL leaf (Plate 7.1). Thickness of palisade tissue was significantly higher in the LH leaf than the HL leaf, but the thickness of spongy tissue was not significantly different. This difference in the palisade thickness resulted in a higher palisade/spongy ratio in the LH leaf relative to the HL leaf. Stomatal density was also significantly higher in the former than the latter.

LH leaf contained higher amount of chlorophylls (a and b) than the HL leaf. This higher amount of total chlorophylls was due to the significantly higher amount of chlorophyll a in the LH leaf since the chlorophyll b content was not significantly different. Chlorophyll a:b ratio was also higher in the LH leaf than the HL leaf.



**Figure 7.1:** Light response curves of photosynthesis for the high-light control leaf (H), the newly-formed leaf in the low-light seedlings transferred to the high light (LH), the low-light control leaf (L), and the newly-formed leaf in the high-light seedlings transferred to the low light (HL). Each line is the rectangular hyperbola obtained from the model using the observed values, and the data points are from one seedling, in which the maximum photosynthesis ( $P_{\text{max}}$ ) was close to the mean.



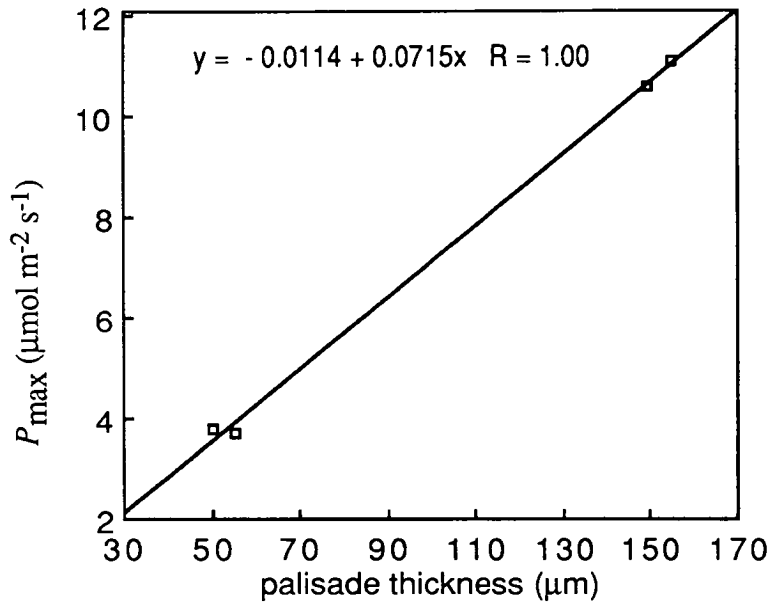
**Plate 7.1:** Transverse sections of leaves from *Bischofia javanica* seedlings grown in the growth cabinets. Magnification,  $\times 312$ . **a:** high-light control leaf, H; **b:** low-light control leaf, L; **c:** newly-formed leaf in the high light, LH; **d:** newly-formed leaf in the low light, HL.

**Table 7.3:** Photosynthetic parameters of the high-light control leaf (H), the low-light control leaf (L), the newly-formed leaf in the high light (LH) and the newly-formed leaf in the low light (HL) for *B. javanica*.  $P_{\max}$  = maximum photosynthetic rate, ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $K$  = photon flux density at which  $P$  is half of the  $P_{\max}$ , ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $R_d$  = rate of dark respiration, ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); and  $\alpha$  = apparent quantum efficiency, (initial slope,  $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{photon}$ ). Mean of 3 seedlings ( $\pm\text{SE}$ ). Other particulars are the same as Table 7.1.

variables	H	L	LH	HL	$F$	$P$
$P_{\max}$	a 10.6 (0.35)	b 3.7 (0.22)	a 11.1 (0.63)	b 3.8 (0.12)	116.5	0.0001
$K$	a 172 (21)	b 81 (8)	a 154 (14)	b 80 (10)	11.8	0.003
$R_d$	a 0.500 (0.096)	a 0.369 (0.010)	a 0.645 (0.154)	a 0.463 (0.053)	1.5	0.293
$\alpha$	a 0.063	a 0.047	a 0.073	a 0.049	2.6	0.129

**Table 7.4:** Leaf anatomy and leaf chlorophylls of the high-light control leaf (H), the low-light control leaf (L), the newly-formed leaf in the high light (LH) and the newly-formed leaf in the low light (HL) for *B. javanica*. Mean of 5 seedlings ( $\pm$ SE). Other particulars are the same as Table 7.1.

variables	H	L	LH	HL	F	P
<b>Leaf anatomy:</b>						
leaf thickness ( $\mu\text{m}$ )	a 298.2 (16.8)	b 197.8 (8.6)	a 312.8 (15.6)	b 198.0 (5.6)	24.7	0.0001
palisade thickness ( $\mu\text{m}$ )	a 149.2 (11.2)	b 55.4 (6.7)	a 154.6 (7.8)	b 50.0 (2.6)	55.2	0.0001
spongy thickness ( $\mu\text{m}$ )	a 103.0 (8.0)	a 100.2 (5.2)	a 113.4 (8.2)	a 101.6 (3.4)	0.9	0.489
palisade/spongy ratio	a 1.48 (0.17)	b 0.57 (0.10)	a 1.37 (0.05)	b 0.49 (0.02)	26.3	0.0001
stomatal density ( $\text{mm}^{-2}$ )	a 266 (12)	b 163 (9)	a 277 (12)	b 166 (9)	34.8	0.0001
<b>Leaf chlorophylls:</b>						
a+b ( $\text{mg m}^{-2}$ )	a 420.61 (25.39)	b 296.96 (14.80)	a 416.37 (22.10)	b 327.44 (28.24)	7.3	0.003
a "	a 340.49 (18.77)	b 229.08 (12.41)	a 344.59 (18.51)	b 252.04 (20.16)	11.3	0.0001
b "	a 80.13 (6.94)	a 67.88 (2.59)	a 71.78 (3.96)	a 75.41 (8.27)	0.8	0.519
chlorophyll a:b	b 4.30 (0.16)	c 3.37 (0.09)	a 4.81 (0.13)	c 3.39 (0.12)	31.9	0.0001



**Figure 7.2:** Relationship between leaf palisade thickness and maximum rates of photosynthesis ( $P_{\max}$ ). Data from Tables 7.3 and 7.4.

The newly-formed leaves in the LH and the HL seedlings were not significantly different from those of the high-light and the low-light control seedlings respectively, in respect of leaf anatomy and leaf chlorophylls. Curiously, the chlorophyll a:b ratio was significantly higher in the LH leaf than the high-light control leaf.

#### 7.4 Discussion

Seedlings after transfer to the contrasting light regimes tended to acclimate to the prevailing light environment through both physiological and morphological adjustments. Seedling growth was enhanced in the high light. The seedlings growing in the high light had higher *RGR* than those growing in the low light. The differences in *RGR* were due to the differences in *NAR* and/or *LAR*. As shown in the Table 7.1, light regimes had opposing effects on *NAR* and *LAR*. The high light regime resulted in an increase in *NAR*, but a decrease in *LAR*. The low light regime, on the other hand, brought about an increase in *LAR* and a decrease in *NAR*. The increase in *LAR* contributed significantly in maintaining a positive carbon gain at the low irradiance. Although seedlings growing in the high light had a lower *LAR*, their *RGRs* were relatively high, due to the relatively high *NAR*. This indicates that the higher *RGR* in the high-light seedlings was the result of the physiological adjustment (*NAR*) rather than the morphological adjustment (*LAR*).

The differences in *NAR* between the high-light and the low-light seedlings was found to be related with the differences in the single-leaf photosynthetic capacity. The leaves produced under the high light regime displayed higher  $P_{\max}$  than those developed at the low growth irradiance. The  $P_{\max}$  per unit leaf area in high-light leaf is associated with increases in mesophyll conductance (Patterson *et al.* 1978). The increases in mesophyll conductance are in turn related to increases in the amount of photosynthetic tissue per unit leaf area (Patterson *et al.* 1978; Kwesiga *et al.* 1986; Ramos and Grace 1990). These increases in the amount of photosynthetic tissue include increases in mesophyll thickness, chlorophylls per unit leaf area, and photosynthetic unit density per unit area (Patterson *et al.* 1978). The most conspicuous difference between the high-light and the low-light leaf was the difference in the anatomical characteristics (Table 7.4 and Plate 7.1). The high-light leaf was thicker resulting from thicker palisade tissue, which consisted of two well-developed layers of cylindrical cells. The mesophyll tissues were also relatively denser.

The higher photosynthetic capacity per unit leaf area in the high-light leaves is generally associated with greater amounts of RuBP carboxylating enzymes (review by Boardman 1977). The number of chloroplasts per unit leaf area has been found to increase almost proportionately with leaf thickness (Louwerse and Zweerde 1977) and the amount carboxylation enzyme per unit surface is increased accordingly (Sinclair *et al.* 1977). Thus, it seems that the increased photosynthetic capacity of the high-light leaves is directly related to their increase in leaf thickness, particularly the palisade thickness, and hence more photosynthetically active mass per unit leaf area. It was found that the maximum photosynthesis was highly correlated with palisade thickness (Figure 7.2). High light during growth is also known to bring about an increased capacity for light-saturated photosynthetic electron transport, which is associated with greater amounts of the electron carriers per unit leaf area (review by Boardman 1977; Evans 1987).

Compared to the high-light leaf, the low-light leaf was thinner with only a rudimentary palisade layer. Along with this structural difference, the internal biochemical activity also limits the photosynthetic capacity of the low-light leaf. The low irradiance during growth is known to bring about a reduction in the components of electron transport and photophosphorylation (Evans 1987). Calvin cycle enzymes are also reduced (review by Boardman 1977; Björkman 1981).

The growth parameters together with the anatomical and physiological characteristics of leaves produced after transfer to the contrasting light regimes indicate how the seedlings had acclimated to the changed light environments. The data on photosynthetic parameters, leaf anatomy, and chlorophylls per unit leaf area suggest that the newly-formed leaves of the LH and the HL seedlings were almost identical to those of the high-light and the low-light control seedlings respectively. Still there were significant differences in the growth parameters between the transferred and the control seedlings.

It may be mentioned here that the leaves produced in the transferred seedlings prior to transfer to the new light environments were morphologically and physiologically different from those developed after the transfer. There is evidence that the leaves of this species have the capacity for acclimation to a new light environment through reorganisation of the photosynthetic machinery and/or leaf anatomy even after the leaves are fully mature prior to transfer, but at the same time they remain morphologically and physiologically significantly different from the newly-formed

leaves in the new light environment (see Chapter 5 and 6). In other words, the leaves produced before transfer retain the effects of the previous environment. The differences between the transferred and the control seedlings in respect of growth parameters might be attributed, at least partly, to the carry-over effects of the previous environment. Two-way analysis of variance on previous and present environment showed significant interactions of present-previous environment (Table 7.2). Oberbauer and Strain (1985) have found significant present-previous environment interactions for specific leaf area, leaf weight ratio and root:shoot ratio in tropical forest tree seedlings of *Pentaclethra maculosa*, when the seedlings were experimentally exchanged between two contrasting light environments. Significant present-previous environment interaction effects have also been reported for total plant dry weight, leaf area ratio and leaf weight per unit leaf area in tropical forest tree seedlings of *Cordia megalantha* even at 183 days of transfer (Bongers *et al.* 1988). Fetcher *et al.* (1983) have analysed the effects of previous and present environments for growth characteristics such as height, total dry weight, leaf, stem, and root dry weight, root:shoot ratio, leaf area ratio, and leaf weight ratio for tropical forest tree seedlings of *Heliocarpus appendiculatus* and *Dipteryx panamensis*, and found significant interactions between present and previous environment only for total plant dry weight, and dry weight of leaves and roots for *Dipteryx* and for leaf area ratio of *Heliocarpus*.

HL seedlings displayed significantly lower *RGR* in comparison to the low-light control seedlings. Clearly, it was mostly because of the lower *LAR*, which was the result of carry-over effects of the previous environment. The larger *RGR* in the LH seedlings compared to the high-light control ones was the result of higher *LAR* and higher *NAR*. Significantly higher *LAR* might be the consequence of the effects of previous environment, but the causes of higher *NAR* in the LH seedlings were rather complicated. The high-light control seedlings were bigger, and produced more leaves per seedling than the LH seedlings. As shown in Figure 7.1 and Table 7.3, the newly-formed leaves in the LH seedlings and the leaves of the high-light control seedlings were almost identical in respect of their photosynthetic capacity per unit leaf area. Thus, the lower *NAR* in the high-light control seedlings might be attributed, at least partly, to the effects of self-shading, and not to the differences in the photosynthetic capacity.

These results are largely consistent with the the results of the previous studies on tropical forest tree seedlings. Oberbauer and Strain (1985) have found higher *RGR* in

the full shade plants (1% daily PPFD) experimentally moved to the partial shade (25% daily PPFD) compared to the partial shade controls for *Pentaclethra macroleoba*. Also, they have reported lower *RGR* values in the seedlings transferred from the partial shade to the full shade in comparison with the full shade controls. Popma and Bongers (1991) have also observed higher *RGR* in the forest understorey or the small gap seedlings experimentally moved to the large gap than the large gap plants which stayed in the same environment, and smaller *RGR* values in the large gap seedlings experimentally transferred to the forest understorey compared to the understorey control seedlings for *Cordia megalantha*, *Lonchocarpus guatemalensis* and *Omphalea oleifera*.

The results reported in this chapter suggest that the species has a wide acclimation potential, which seems to be determined by both morphological and physiological plasticity. The high-light acclimation potential, that was largely determined by the physiological plasticity, will make its seedlings more competitive following a disturbance, while the shade adaptation capacity will help to maintain a positive carbon balance with the decrease in irradiance following canopy closure.

# CHAPTER 8

## General Discussion

### 8.1 Response to shade

In this thesis, the term 'low irradiance' is used for simulated shadelight with low photosynthetic photon flux density (PPFD) and low R:FR ratio, and the term 'high irradiance' for simulated daylight with high PPFD and high R:FR ratio.

Leaf photosynthesis is directly and dramatically influenced by growth light regime. Comparative studies of the photosynthetic response and leaf characteristics of plants grown in high and low levels of irradiance provide crucial insights into the significance of several leaf-level traits found in plants adapted to high vs. low light conditions. These insights come from the study of photosynthetic light response curves of leaves. In this study, it has been shown for gap species *Bischofia javanica* (Chapter 7) that leaves grown at the low irradiance had a lower maximum photosynthetic rate, lower rates of respiration and their photosynthesis saturated at the lower level of irradiance compared to the high-light leaves. Further, quantum yield of photosynthesis of the low-light leaves was not significantly different from that of the high-light leaves. Some authors have shown higher quantum yield of photosynthesis in shade plants compared to sun plants (e.g. Langenheim *et al.* 1984). However, several other studies on tropical species have demonstrated that apparent quantum efficiency is insensitive to growing conditions (e.g. Thompson *et al.* 1988; Sims and Pearcy 1989; Riddoch *et al.* 1991a).

Typically, leaves with higher amounts of Rubisco (RuBP carboxylase-oxygenase) have higher photosynthetic rates at high irradiance where carboxylation is likely to limit photosynthesis (Björkman 1981). This can be achieved by a higher number of chloroplasts per unit leaf area. The number of chloroplasts per unit area has been found to increase almost proportionately with leaf thickness (Louwerse and Zweerde 1977) and the amounts of Rubisco per unit surface are increased accordingly (Sinclair *et al.* 1977). Leaf anatomy showed that leaves produced under low irradiance were thinner and had only a rudimentary palisade layer (Table 7.4). Their mesophyll tissues were loosely arranged (Plate 7.1) resulting in lower amounts of photosynthetically active mass per unit leaf area. Further it was found that the maximum photosynthesis was highly correlated with palisade thickness (Figure 7.2). Low irradiance during growth

is known to bring about a reduction in the components of electron transport and photophosphorylation. At a low irradiance, plants need to maximise light absorption and can reduce the maximum Hill activity (Evans 1987). Calvin cycle enzymes are also reduced (Björkman 1981). Hoflacher and Bauer (1982) have shown for woody climber *Hedera helix* that the photosynthetic capacity, the Hill reaction, and the activity of RuBP carboxylase, when expressed per chloroplast, are almost identical in plants grown in low or high irradiance. They have concluded that different photosynthetic capacity per unit leaf area is mainly the result of the different photosynthetically active mass per unit leaf area resulting from a change in leaf thickness. Lower maximum photosynthetic rates in thinner low-light leaves have also been observed for non-pioneer and pioneer tropical forest tree seedlings of *Flindersia brayleyana* (Thompson *et al.* 1988) and *Nauclea diderrichii* (Riddoch *et al.* 1991a) respectively.

In *Bischofia*, there was a large increase in specific leaf area (*SLA*) in seedlings growing at the low irradiance (Chapter 2). An increased *SLA* combined with an almost equal leaf weight ratio (*LWR*) led to an increased leaf area ratio (*LAR*), and this relative increase in leaf area compensated, at least partially, for a lower photosynthesis per unit leaf area under low irradiance. In this way, *LAR* made a significant contribution in maintaining a positive relative growth rate (*RGR*) under low irradiance. In *Hopea*, on the other hand, leaf thickness was not appreciably changed under low irradiance as is evident from a slight change in specific leaf area. As a result, denser mesophyll tissue in the low-light leaf enabled the leaf to maintain relatively higher amounts of photosynthetically active mass per unit area and hence possibly higher rate of net photosynthesis under the low irradiance. With this ability, the seedlings of *Hopea* displayed a significantly higher net assimilation rate (*NAR*) than those of *Bischofia* at the low irradiance. This was an important difference between these two contrasting species.

Shade tolerant species are able to grow in the deep shade because they are able to maintain a positive *NAR*, and hence a positive *RGR* under light-limiting understorey habitats. Seedlings of both *Bischofia* and *Hopea* had maintained a positive *NAR* under low irradiance. These results are in accordance with most other studies on non-pioneer tropical forest tree seedlings (e.g. Oberbauer and Strain 1985; Popma and Bongers 1988). Seedlings of pioneer tropical forest tree species like *Terminalia ivorensis* (Kwesiga 1984) and *Cecropia obtusifolia* (Popma and Bongers 1988) had failed to maintain a positive *NAR* under low irradiance. Though the seedlings of both pioneer and non-pioneer species show an increase in *LAR* in response to shade, the

magnitude of this increase is generally much higher in the seedlings of pioneers (e.g. Popma and Bongers 1988). In this respect, the seedlings of *Bischofia* behaved like pioneer species. Relatively higher *NAR* in *Hopea* seedlings growing under low irradiance indicates that *Hopea* was more shade tolerant than *Bischofia*.

## 8.2 Response to shadelight quality

Pioneer tree species *Anthocephalus chinensis* and climax species *Hopea odorata* showed differential responses to R:FR ratio. Seedlings showed several growth responses to low R:FR ratio (Chapter 3). The main effect of a reduction in R:FR ratio was a large increase in stem extension growth in *Anthocephalus*, not in *Hopea*, when the seedlings of these species were grown under shadelight with contrasting R:FR ratios for several weeks. This long-term effect of a low R:FR ratio on stem extension rate was in accordance with short-term observations. Significantly higher stem extension rates in *Anthocephalus* seedlings were also observed (a) when the internodes were subjected to a low R:FR ratio as well as (b) when the terminal internode was irradiated with supplementary far-red radiation to lower the R:FR ratio at the internode level. Morgan and Smith (1976) have shown that higher stem extension rate as a result of a low R:FR ratio is an inverse linear function of Pfr/P of photoreceptor phytochrome. This linearity has been found for a wide range of herbaceous species (review by Smith 1986) and for *Pinus radiata* (Warrington *et al.* 1989).

With the increase in stem extension growth, dry matter allocation to stem was significantly increased resulting in a higher stem weight ratio (*SWR*) with simultaneous reduction in leaf area ratio (*LAR*). This indicates that the increased stem extension growth was at the expense of the development of leaf area. Further, the increased stem extension growth under a low R:FR ratio was more the result of change in dry matter allocation between plant organs than that of a reduction in stem thickness as is evident from a non-significant specific stem length. These results are consistent with those reported for herbaceous plants (review by Smith 1986), and confirm that pioneer tree seedlings are not different from herbaceous species from open habitats in response to a low R:FR ratio. Increased *SWR* with concomitant increase in stem extension rate has also been found for *Pinus radiata* when grown under shadelight with a low R:FR ratio (Warrington *et al.* 1989). In contrast to these results, Kwesiga and Grace (1986) found no increase in stem extension growth in response to low R:FR

ratio even for seedlings of pioneer tree *Terminalia ivorensis*. They had also found differential growth response between pioneer and climax species. In their experiment, specific leaf area was enhanced with low R:FR ratio in pioneer species *T. ivorensis*, whereas it was largely unaffected in climax species *Khaya senegalensis*.

Stem extension rates in seedlings of *Anthocephalus* were not significantly affected when young leaves were subjected to a low R:FR ratio, though there was a large increase in extension rate when the internodes instead of leaves were exposed to a low R:FR ratio. These results indicate that internodes might be the more effective sites of perception and response. These results are in accordance with those reported for *Sinapis alba* and *Datura ferox* (Ballaré *et al.* 1991). Further, the extension rates, when internodes were subjected to low R:FR ratio, were not significantly different from those, where the internodes were exposed simultaneously to a low R:FR ratio and a low proportion of blue light.

This short-term observation was consistent with the results from long-term experiments, where the seedlings were grown for several weeks under shadelight with different amounts of blue light (Chapter 4). In this experiment, the stem extension growth in response to a low R:FR ratio was not affected by blue light. Recently, it has been shown in several studies with herbaceous plants (Mohr 1986; Casal and Smith 1988; Ballaré *et al.* 1991) that the effect of a low R:FR ratio on stem extension growth is independent of low proportions of blue light receiving by the internodes. Interestingly, very small amounts of blue light in the shadelight restricted leaf expansion in *Anthocephalus* and *Bischofia*, and increased specific stem length in the former (Chapter 4). This might be attributed to the limitation of photosynthates under that light regime.

These results on the effects of shadelight quality, a low R:FR ratio as well as a low proportion of blue light, confirm that seedlings of pioneer/gap species are more plastic in their responses than those of climax species.

### **8.3 Photoinhibition and recovery**

Exposure of the low-light grown seedlings to high light results in photoinhibition of photosynthesis. This photoinhibition is manifested by a reduced quantum yield and light saturated capacity of photosynthesis, a reduced electron transport activity and

altered chlorophyll fluorescence characteristics. These effects are generally considered to result from the inactivation of reactions associated with photosystem II (review by Bolhar-Nordenkampf *et al.* 1989). In this study, the low-light grown seedlings of *Bischofia*, when transferred to the high light, showed severe photoinhibition of photosynthesis in leaves fully developed in the low light prior to transfer as indicated by a large depression in chlorophyll fluorescence ratio ( $F_v/F_m$ ) with concomitant decline in pool size of electron acceptors on the reducing side of photosystem II ( $t_{1/2}$ ) and in net photosynthesis ( $P_n$ ) (Chapter 5). The decline in the  $F_v/F_m$  was caused mostly by an increase in initial chlorophyll fluorescence ( $F_0$ ) indicating the inactivation of photosystem II reaction centres. This photoinhibition was followed by a substantial decline in leaf chlorophylls resulting in pigment bleaching. These results on the trends of fluorescence are in agreement with previous studies in which leaves had been exposed to a step-wise increase in light (e.g. Demmig and Björkman 1987).

Though there are many examples of photoinhibition of photosynthesis in the low-light leaves on exposure to the high light, there have been very few studies on the recovery of photoinhibited leaves (Syvertsen and Smith 1984; Bauer and Thöni 1988). In this study, there was a substantial recovery in photoinhibited leaves, which were fully developed before exposure to the high light. This recovery was manifested by an orientation of the leaf to minimise the interception of incident high light, an increase in chlorophyll contents per unit leaf area, and anatomical changes resulting in a thicker palisade layer and denser mesophyll tissue. Consequently, photosynthetically active mass per unit leaf area was increased resulting in a higher photosynthetic rate per unit area by 75% of that at the start of exposure to the high light. However, the final  $P_n$  was less than that of newly-formed high light leaf by more than 30%. These observations on recovery were consistent with those on the  $F_v/F_m$  ratio, the  $t_{1/2}$ , and the leaf chlorophylls per unit leaf area.

#### **8.4 Shade adaptation of high-light leaf**

Fully developed high-light leaves acclimated to the low light through reorganisation of the photosynthetic apparatus (Chapter 6). A conspicuous change, as a result of transfer from the high light to the low light, is known to be an increase in size of the photosynthetic units in fully developed high-light leaf during adaptation to the low-light (Bunce *et al.* 1977). As the photosynthetic unit size increases, the chlorophyll a:b ratio decreases (Patterson *et al.* 1978). In *Bischofia*, there was a significant decrease

in chlorophyll a in the high-light leaf transferred to the low light (HL leaf) without an appreciable change in chlorophyll b resulting in a large decrease of chlorophyll a:b ratio. This ratio was significantly lower than that for the high-light control leaf, and very close to the value for the low-light control leaf. The decrease in chlorophyll a:b ratio is associated with changes in the light harvesting chlorophyll a to b protein (Chow *et al.* 1988). Typically, thylakoids of the low-light leaf have larger, though fewer, photosystem II photosynthetic units with more chlorophyll a/b-proteins (Anderson *et al.* 1988) to maximise light harvesting under low irradiance.

After 8 weeks of transfer to the low light, maximum photosynthetic rate in HL leaf was decreased by 40% compared to that for the high-light control leaf, but was almost at the same extent higher than the low-light control leaf. This decrease in photosynthetic capacity might be attributed, at least partly, to the decrease in the level of constituents of the electron transport chain and those involved in CO<sub>2</sub> fixation (Anderson *et al.* 1988). However, a decrease in leaf thickness and tissue density, and an increase in air space per unit leaf volume after exposure of the high-light grown seedlings to relatively lower light levels have been observed in *Citrus* (Syvertsen and Smith 1984). Decrease in leaf thickness resulting from altered thickness of both the palisade and spongy mesophyll tissue has also been reported for fully expanded leaf prior to transfer to the low light in *Glycine max* (Bunce *et al.* 1977). In both the cases, leaf thickness, and tissue density in low-light adapted leaf were higher than the low-light control leaf. Leaf chlorophylls per unit leaf area were higher in the HL leaf than the low-light control leaf (Table 6.1). So, the higher rates of photosynthesis at light saturation level might be attributed to the higher amounts of photosynthetically active mass per unit leaf area in the HL leaf.

### **8.5 Seedling growth after transfer to the contrasting light regimes**

Seedlings after transfer to the contrasting light regimes tended to acclimate to the prevailing light environment through both physiological and morphological adjustments (Chapter 7). Seedling growth was enhanced in the high light. Seedlings growing in the high light had higher relative growth rate (*RGR*) than those growing in the low light. The differences in *RGR* were due to the differences in net assimilation rate (*NAR*) and/or leaf area ratio (*LAR*). The differences in *NAR* between the high-light and the low-light seedlings were found to be related with the differences in the single-leaf photosynthetic capacity. Leaves produced under the high light regime

displayed higher maximum rates of photosynthesis than those developed at the low growth irradiance.

Growth parameters together with the anatomical and physiological characteristics of leaves produced after transfer to the contrasting light regimes indicate how the seedlings had acclimated to the changed light environments. Data on photosynthetic parameters, leaf anatomy and chlorophylls per unit leaf area suggest that the newly-formed leaves of the seedlings transferred to the high light (LH) and of those transferred to the low light (HL) were almost identical to those of the high-light and the low-light control seedlings respectively. Still there were significant differences in the growth parameters between the transferred and the control seedlings. The HL seedlings displayed significantly lower *RGR* in comparison to the low-light control seedlings. Clearly, it was mostly because of the lower *LAR*, which was the result of carry-over effects of the previous environment. The larger *RGR* in the LH seedlings compared to the high-light control seedlings was the result of higher *LAR* and higher *NAR*. The significantly higher *LAR* might be the consequence of the effects of previous environment, and the relatively lower *NAR* in the high-light control seedlings might be attributed, at least partly, to the effects of self-shading, and not to the differences in the photosynthetic capacity. These results on growth parameters are largely consistent with the results of the previous studies on tropical forest tree seedlings (Oberbauer and Strain 1985; Popma and Bongers 1991).

## **8.6 Growth cabinet vs. natural light environment**

Plants in a forest understorey habitat receive less than 2% of the irradiance under full sun (Chazdon and Fetcher 1984). This irradiance on the forest floor comprises two very different radiations: the continuous, diffuse, low flux radiation is markedly enriched in far-red light, and sunflecks, short intermittent bursts of direct radiation. This shadelight is also characterised by a low proportion of blue light (Holmes 1981). This complex spectral distribution is not readily achieved in the growth cabinet. When the purpose of a growth cabinet study is to duplicate the natural environment, the spectral quality of the lighting system is important. The R:FR ratio is one measure of the spectral distribution of daylight, which is approximately 1.10-1.25 under full sun and as little as 0.10 under forest canopies (Chazdon and Fetcher 1984; Lee 1987). In this study, no attempt was taken to simulate sunflecks. However, simulation of a low proportion of blue light under shadelight and R:FR ratios was achieved. The R:FR ratios obtained in the growth cabinet by using 'shade-covers' with appropriate filter

materials, were close to those encountered in nature. It is apparent that some present controlled environment lighting systems do not provide R:FR ratios close to those occurring in nature (Warrington *et al.* 1989). This may be crucial in the interpretation of the results on the effects of R:FR ratios on plant growth and development under controlled environments.

Unlike natural conditions, the photon flux density in the growth cabinet was kept almost constant over the photoperiod. Some workers, however, have tried to compare the results from growth cabinet experiments with the data from field studies. Oberbauer and Strain (1986) have studied the effects of canopy position and irradiance on the leaf physiology and morphology of a tropical rain forest tree *Pentaclethra maculosa*. This study shows that chamber-grown seedlings can provide a fair representation of leaf characteristics of saplings and mature trees in the forest. Chabot *et al.* (1979) and Nobel and Hartsock (1981) have investigated acclimation of morphological and photosynthetic characteristics under different levels of integrated as well as instantaneous photosynthetic photon flux density (PPFD). These studies demonstrate that photosynthesis and leaf structure are determined by the integrated PPFD rather than by the peak PPFD.

## **8.7 Relevance to tropical forest management**

Most tropical foresters have been trying to base forest management practices on a good understanding of the basic biology of trees and their environment. The dynamics of regeneration after exploitation has received particular attention because of the involvement of ecological processes in the regeneration of tropical forests.

Recently, Gómez-Pompa and Burley (1991) have reviewed all the silvicultural systems practised in the tropical forests, focusing the importance of ecological knowledge in the regeneration of tropical forests. Whitmore (1991) has given a useful discussion on the monocyclic and polycyclic systems of management. In monocyclic systems, trees on a particular area are removed at one cut and regeneration, either natural or artificial, is then allowed to occur. These systems tend to create big canopy gaps and so favour regrowth of pioneer or near pioneer species with eminently marketable timber. Polycyclic systems involve selective felling of a few trees at a time and allowing natural regeneration to fill in the gaps created, and maintaining standing volumes of all tree species. It is assumed that the regeneration of the desired species occurs naturally

without further manipulation or enrichment. Selective logging is usually done at 20-30 years cycles. The removal of isolated, often widely spaced, individual trees resembles natural tree-fall gaps and regeneration occurs normally as in undisturbed forests. Where selective felling is at low intensity, a polycyclic system creates small gaps and so favours growth and perpetuation of climax species.

Pioneer seedlings are not usually present at the time when a big canopy gap is created. They develop from seed which was either present in the soil as a seed-bank or which arrived after gap creation as seed-rain. There have been several studies (review by Whitmore 1991), which demonstrate that rain forest soils do have a seed-bank and that germination is commonly triggered by a change in light quality, or by elevated soil surface temperatures. Seeds of pioneer species *Anthocephalus chinensis* are known to persist in the soil under the canopy for several months (Fox 1971). Quintos *et al.* (1975) have shown that seed germination of this species is enhanced with white light or red light. Seedlings generally appear in the open or the logged areas in great abundance, approximately 45000 seedlings per ha (Troup 1921; Fox 1971). In the present study, it has been shown that *Anthocephalus* seedlings appear to have evolved phytochrome-related mechanisms to detect changes in the R:FR ratio of the prevailing light, and alter their growth responses accordingly (Chapter 3). The seedlings of this species showed an increased stem extension growth with concomitant increase in allocation of dry matter to stem in response to the low R:FR ratio of the shadelight. In the seedling stands formed by plants of similar stature, a reduction in R:FR ratio within the stand occurs without an appreciable change in photon flux density. This reduction in R:FR ratio is accounted for by the increased far-red (FR) radiation reflected by leaves of neighbouring plants (Ballaré *et al.* 1989). Stem extension rates of *Anthocephalus* seedlings were shown to be increased by a drop in R:FR ratio at the internodes irradiated with supplementary FR radiation under a background white light of reasonably high photon flux density. In nature, in a competitive situation for light, either in vegetational shadelight or in open sun, the increased stem extension growth in response to low R:FR ratio will result in young leaves of *Anthocephalus* seedlings reaching a better lit stratum within the seedling canopy. A similar response, with respect to petiole growth and dry matter allocation, was found for white clover and was shown as the means by which this species successfully competed against neighbouring grass species in mixed swards (Thompson and Harper 1988).

While seed-banks become more important in larger gaps and/or moderately severe disturbances, the seedling-bank is most important in small gaps. Species with

immediate germination form seedling-bank in understorey habitats, where they grow very slowly until gaps in the canopy are formed. *Bischofia javanica* in the present study is a species of immediate germination and forms seedling-bank under forest understorey (Troup 1921). Seedlings, saplings and juveniles will take advantage of a gap to grow towards the upper canopy of the forest. The high growth rates of these plants will lead to an increased competition for light and nutrients, which will result in higher mortality rates. This competition for resources is usually much more intense immediately after the formation of gaps (Brokaw 1985), which, at least partially, explains a drop in species diversity after the phase of colonisation (Manokaran and Kochummen 1987).

Drastic changes in light and moisture conditions consequent to canopy opening are known to adversely affect the survival and growth of certain species (Nair 1991). Success of species in the filling process will depend on the competitive ability of the component species, and the competition will mostly occur among the successional species with high photosynthetic rates. Photosynthetic acclimation with the changed light conditions will be more crucial for their success. In this study, the seedlings of gap species *Bischofia* showed substantial photosynthetic acclimation potential to the changed light conditions analogous to changes that might occur on the forest floor with the formation of a canopy gap (Chapter 5). The changes occurred in leaf characteristics during light acclimation indicate that the leaves can acclimate even after the leaves are fully developed in shade.

Since leaf-level and whole-plant carbon gain are influenced by leaf life span, the retention of acclimated leaves is of considerable importance. The seedlings of *Bischofia* retained the acclimated leaves in the new light environment, and hence significantly contributed in maintaining a faster growth by improving the photosynthetic capacity of those leaves. These transferred seedlings displayed rather higher relative biomass growth rates compared to the high-light control seedlings (Chapter 7). Thus this ability of photosynthetic light acclimation in *Bischofia* will make its seedlings more competitive following disturbance.

With this ability of photosynthetic light acclimation to the changed light conditions, *Bischofia* seedlings growing under forest understorey habitats will be able to utilize the sunflecks more efficiently. Through utilisation of sunflecks, the species will live for long under light-limiting forest understorey habitats, and wait until a gap in the canopy is formed to resume faster growth.

The mean period of time between consecutive disturbances is small in comparison to the potential life span of trees. This means that an individual tree has a high probability of encountering one or several gaps during its life time. In fact, the mature forest understorey conditions are restored after gap closure. Moreover, light conditions in the forest understorey are strongly affected by seasonal changes in the canopy cover. This seasonal variation is more pronounced in the deciduous and the semi-evergreen forests (Lee 1989), while in the evergreen forests the seasonal variation is primarily due to the changes in solar elevation rather than to the changes in forest canopy cover (Chazdon 1988). Thus a given seedling growing on the forest floor will be exposed to a temporally and spatially variable light environment including increases as well as decreases.

Replacement of leaves developed before canopy closure is slow and difficult in a light-limiting forest understorey habitats. Maintenance of a positive carbon balance will be crucial for a given species under this condition. Hence, retention and function of leaves developed before canopy closure will be of considerable significance for maintenance of carbon balance. This study shows that *Bischofia* seedlings, transferred from the high light to the low light analogous to changes that might occur in nature, showed adaptation to shade in leaves developed prior to the transfer (Chapter 6). Their light saturated photosynthetic rates were higher than those developed under the prevailing shadelight. With this capacity, these leaves will utilize sunflecks more efficiently, and thus the whole-plant carbon gain of these seedlings will be higher than those which will have developed their all leaves under the low irradiance.

Tropical forests occur on soils of different fertility, and the nutrient availability differs even in different areas within a single tree-fall gap (Orians 1982; Vitousek and Denslow 1986). The growth attributes and recruitment patterns of the component species are likely to differ. The results reported here suggest differential growth between gap species *Bischofia* and climax species *Hopea* in response to nutrient supply (Chapter 2). The growth of the gap species was more plastic than that of the climax species. At the high irradiance, the gap species like *Bischofia* will be able to increase the growth rate by a large physiological adjustments rather than by morphological adjustments, and the expression of full potential of them will be limited in the nutrient-poor sites. The growth of the climax species like *Hopea*, on the other hand, will be relatively less plastic in response to the nutrient status of soils even at the high irradiance.

It is now clear that the process of gap creation and filling controls the structure and function of the tropical forest. With the introduction of computer simulation and techniques, the structure and physiological information relevant for determining growth and yield of forest can be incorporated into models. These biologically-based models which include within their structure those underlying biological factors and processes, which control and determine tree and forest growth. A well-constructed simulation model can make predictions on changes in species composition and growth rates following natural or man-induced disturbances in the forest, and also on the consequences of a particular management practice. FORMIX model, for example, is a simulation model developed for the simulation of tropical forest growth dynamics (Bossel and Krieger 1990). This model integrates relevant geometric relationships, empirical photosynthesis data, energy accounting based on the law of energy conservation, conservation of mass, and empirical data from tropical natural forests. Though FORMIX forest dynamics model is judged structurally and behaviourally valid, and also has a reasonable degree of empirical validity, it needs further improvement with the incorporation of more empirical research data.

It is known that a forest canopy drastically modifies the quantity and quality of light reaching the forest floor. Both of these factors have been shown to be significant in influencing the growth, photosynthetic capacity and development of plants under a forest canopy. Accordingly, plant responses to both the factors are related to the processes associated with establishment, management, natural regeneration and succession within a forest. The predictive models of tropical forest dynamics, therefore, will have to take cognizance of effects of light quality on growth and development, if they are to be realistic in their predictions of individual tree growth and developmental responses.

In the forest, both sun and shade leaves are likely to be found on the same individual (Oberbauer and Strain 1986). Their carbon gain capacities and contribution to the total carbon budget of that individual are probably different. The present study shows that seedlings, transferred to the contrasting light conditions, had different types of leaves with different structures and photosynthetic capacities. Also the relative growth rates of these transferred seedlings were different from those maintained as controls. So, incorporation of single-leaf photosynthetic attributes in a predictive models will not be enough to explain the whole-plant carbon gain over time. Similarly, differential growth responses in response to nutrient availability are of considerable importance. This

study, and other recent studies on the effect of nutrient supply on leaf morphology and physiology, and photosynthetic capacity and growth (see also Thompson *et al.* 1988; Riddoch *et al.* 1991a) suggest that nutrient status will also play an important role in competition and growth of tree seedlings.

It is now clear that successful management of tropical forests and their continued use as a renewable resource must be based on a good understanding of the basic biology of the component species and their role in the process of regeneration. Data on the influence of light and nutrient supply on the growth and physiology of tree seedlings in the field are required to ascertain whether the large treatment effects observed in this study can be demonstrated in nature. However, light dynamics and nutrient availability are not the only factors controlling the forest regeneration. There are many other factors like seed dispersal, germination, moisture relations, and herbivores and pathogens are also important. Clearly, there is an urgent need for more research relevant to forest management.

## 8.8 Suggestions for further work

There is an obvious requirements to take into account the possible differences in response to R:FR ratio between tree seedlings and competing vegetation. This study shows that the seedlings of climax species *Hopea odorata* were relatively unresponsive to R:FR ratio. In these seedlings, stem elongation was not enhanced in response to low R:FR ratio and hence forest weed species, particularly those which are responsive to low R:FR ratio resulting in an increased stem extension growth, might be expected to be strongly competitive with *Hopea* seedlings. Many temperate forest weeds from open habitats are known to increase their extension growth in response to low R:FR ratio (e.g. Morgan and Smith 1976; review by Smith 1986), and virtually nothing is known about the responses of tropical forest weeds to low R:FR ratio. This is an area where ecological work is badly needed to have an insight of the competition between tree seedlings and forest weeds.

The rates of photosynthesis in individual leaves are only one factor in the growth and production of tropical plants. Allocation of photosynthate to competing structures and functions and the display of these structures, the architecture of plants, play a significant role as well. Architectural configurations which reduce self-shading and allow the development of monolayered canopies will enhance carbon gain capacities

and may be common in pioneer or near pioneer species. Fast growth over a longer period of time may result in sparse branch arrangement and greater leaf exposure. Data presented in this study have shown enhanced extension growth with concomitant increase in allocation of dry matter to stem in response to low R:FR ratio. In addition to these, Warrington *et al.* (1989) found increased apical dominance and decreased fascicle density for older materials of *Pinus radiata* in response to a reduction in R:FR ratio. It is not known whether changes in R:FR ratio within crown of a sapling or a large tree of pioneer species influence branch arrangement through their effects on extension growth of young branches or orientation of leaf by extension growth of petioles.

Recently, Chazdon (1988) has written a useful review on sunflecks and their importance to forest understorey plants. This review demonstrates that many gaps remain in our understanding of sunfleck frequency, duration and intensity within tropical forests as well as of sunfleck utilisation of leaves and whole plants. If irradiance during sunflecks exceeds light saturation for long period, photoinhibition of photosynthesis may occur. The present study shows that exposure of the low-light grown seedlings to the high light brought about photoinhibition of photosynthesis within 1 hr of exposure, and adaptation to this situation was manifested by a range of responses from changes in leaf orientation to modifications of leaf structure. However, such responses of leaves and whole plants to sunflecks have not been investigated in nature. These studies should also take into account the changes in spectral quality, both R:FR ratio and blue part of the spectrum.

Light acclimation to constant light conditions may well affect photosynthetic dynamics, as suggested by growth cabinet experiments in the present study. We do not know whether growth under fluctuating light conditions may lead to changes in dynamics or steady-state photosynthetic responses.

Although many studies and the present one as well have shown that increases in light availability are often associated with increases in plant growth, relatively little is known about constraints on the high-light utilisation by plants in nature. One of the constraints may be the nutrient status of site. The present study showed that at high irradiance, growth of tree seedlings of pioneer species was limited when nutrient supply was low. Water stress during the dry season in tropical forests may also affect the plant growth. Studies of daily courses of light, photosynthesis, stomatal conductance, and leaf temperature within a particular forest are needed during different

times of the year as well as under different weather conditions. Extensive field studies in conjunction with laboratory investigations will provide a detailed understanding of potential and actual constraints on light utilisation.

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## APPENDIX A

### *Bischofia javanica*

#### A.1 Analysis of variance for relative growth rate, *RGR* (g g<sup>-1</sup> wk<sup>-1</sup>)

Source	Degrees of freedom (DF)	Sum of Squares (SS)	Mean of Squares (MS)	Variance Ratio ( <i>F</i> )	Probability ( <i>P</i> )
Position	7	0.003839	0.000548	1.43	0.226
Nutrient	1	0.081345	0.081345	211.73	0.000
Light	2	0.298466	0.149233	388.43	0.000
Nutrient*Light	2	0.051102	0.025551	66.51	0.000
Error	35	0.013447	0.000384		
Total	47	0.448199			

#### A.2 Analysis of variance for net assimilation rate, *NAR* (g m<sup>-2</sup> wk<sup>-1</sup>)

Source	DF	SS	MS	<i>F</i>	<i>P</i>
Position	7	100.74	14.39	1.65	0.154
Nutrient	1	176.33	176.33	20.24	0.000
Light	2	3315.84	1657.92	190.27	0.000
Nutrient*Light	2	251.87	125.94	14.45	0.000
Error	35	304.97	8.71		
Total	47	4149.75			

#### A.3 Analysis of variance for leaf area ratio, *LAR* (m<sup>2</sup> g<sup>-1</sup>)

Source	DF	SS	MS	<i>F</i>	<i>P</i>
Position	7	0.00003146	0.00000449	0.52	0.815
Nutrient	1	0.00018058	0.00018058	20.78	0.000
Light	2	0.00144954	0.00072477	83.39	0.000
Nutrient*Light	2	0.00002357	0.00001178	1.36	0.271
Error	35	0.00030419	0.00000869		
Total	47	0.00198934			

#### A.4 Analysis of variance for specific leaf area, $SLA$ ( $m^{-2} g^{-1}$ )

Source	DF	SS	MS	F	P
Position	7	0.00009700	0.00001386	0.73	0.645
Nutrient	1	0.00008321	0.00008321	4.41	0.043
Light	2	0.00489696	0.00244848	129.69	0.000
Nutrient*Light	2	0.00012936	0.00006468	3.43	0.044
Error	35	0.00066077	0.00001888		
Total	47	0.00586730			

#### A.5 Analysis of variance for leaf weight ratio, $LWR$

Source	DF	SS	MS	F	P
Position	7	0.022503	0.003215	1.07	0.403
Nutrient	1	0.084672	0.084672	28.17	0.000
Light	2	0.019389	0.009694	3.22	0.052
Nutrient*Light	2	0.017724	0.008862	2.95	0.066
Error	35	0.105214	0.003006		
Total	47	0.249502			

#### A.6 Analysis of variance for specific stem length, $SSL$ ( $cm g^{-1}$ )

Source	DF	SS	MS	F	P
Position	7	6768	967	1.14	0.359
Nutrient	1	6188	6188	7.32	0.010
Light	2	221408	110704	131.04	0.000
Nutrient*Light	2	24607	12304	14.56	0.000
Error	35	29569	845		
Total	47	288540			

### A.7 Analysis of variance for stem weight ratio, *SWR*

Source	DF	SS	MS	F	P
Position	7	0.006534	0.000933	0.93	0.496
Nutrient	1	0.011750	0.011750	11.71	0.002
Light	2	0.088814	0.044407	44.25	0.000
Nutrient*Light	2	0.008788	0.004394	4.38	0.020
Error	35	0.035128	0.001004		
Total	47	0.151014			

### A.8 Analysis of variance for root weight ratio, *RWR*

Source	DF	SS	MS	F	P
Position	7	0.018879	0.002697	1.95	0.090
Nutrient	1	0.033075	0.033075	23.95	0.000
Light	2	0.086132	0.043066	31.19	0.000
Nutrient*Light	2	0.001883	0.000942	0.68	0.512
Error	35	0.048333	0.001381		
Total	47	0.188302			

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### A.9 Analysis of variance for relative growth rate, *RGR* ( $\text{g g}^{-1} \text{wk}^{-1}$ )

Source	DF	SS	MS	F	P
Position	7	0.001495	0.000214	1.47	0.209
Nutrient	1	0.010005	0.010005	69.04	0.000
Light	2	0.122012	0.061006	420.97	0.000
Nutrient*Light	2	0.003828	0.001914	13.21	0.000
Error	35	0.005072	0.000145		
Total	47	0.142412			

**A.10 Analysis of variance for net assimilation rate,  $NAR$  ( $\text{g m}^{-2} \text{wk}^{-1}$ )**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	78.92	11.27	1.09	0.389
Nutrient	1	26.24	26.24	2.54	0.120
Light	2	2556.61	1278.31	123.86	0.000
Nutrient*Light	2	15.20	7.60	0.74	0.486
Error	35	361.21	10.32		
Total	47	3038.17			

**A.11 Analysis of variance for leaf area ratio,  $LAR$  ( $\text{m}^2 \text{g}^{-1}$ )**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	1.0461E-05	1.4945E-06	1.16	0.350
Nutrient	1	8.9380E-05	8.9380E-05	69.44	0.000
Light	2	5.8768E-05	2.9384E-05	22.83	0.000
Nutrient*Light	2	9.6279E-06	4.8140E-06	3.74	0.034
Error	35	4.5052E-05	1.2872E-06		
Total	47	2.1329E-04			

**A.12 Analysis of variance for specific leaf area,  $SLA$  ( $\text{m}^2 \text{g}^{-1}$ )**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	1.7605E-05	2.5150E-06	1.11	0.382
Nutrient	1	6.8880E-05	6.8880E-05	30.26	0.000
Light	2	3.7859E-04	1.8929E-04	83.17	0.000
Nutrient*Light	2	7.1129E-06	3.5565E-06	1.56	0.224
Error	35	7.9659E-05	2.2760E-06		
Total	47	5.5184E-04			

### A.13 Analysis of variance for leaf weight ratio, *LWR*

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	0.011066	0.001581	1.23	0.315
Nutrient	1	0.091438	0.091438	70.96	0.000
Light	2	0.013564	0.006782	5.26	0.010
Nutrient*Light	2	0.019667	0.009834	7.63	0.002
Error	35	0.045098	0.001289		
Total	47	0.180833			

### A.14 Analysis of variance for specific stem length, *SSL* (cm g<sup>-1</sup>)

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	3219.3	459.9	0.53	0.806
Nutrient	1	3675.0	3675.0	4.23	0.047
Light	2	81483.3	40741.6	46.89	0.000
Nutrient*Light	2	3393.4	1696.7	1.95	0.157
Error	35	30413.0	868.9		
Total	47	122183.9			

### A.15 Analysis of variance for stem weight ratio, *SWR*

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	0.0052133	0.0007448	0.92	0.500
Nutrient	1	0.0049410	0.0049410	6.14	0.018
Light	2	0.0169622	0.0084811	10.53	0.000
Nutrient*Light	2	0.0019322	0.0009661	1.20	0.313
Error	35	0.0281868	0.0008053		
Total	47	0.0572355			

### A.16 Analysis of variance for root weight ratio, *RWR*

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Position	7	0.0067703	0.0009672	1.10	0.387
Nutrient	1	0.0537341	0.0537341	60.90	0.000
Light	2	0.0154228	0.0077114	8.74	0.001
Nutrient*Light	2	0.0223890	0.0111945	12.69	0.000
Error	35	0.0308804	0.0008823		
Total	47	0.1291967			