

Leaf photosynthetic capacity and nitrogen content adjustment to canopy openness in tropical forest tree seedlings

GRÉGOIRE VINCENT¹

ICRAF South East Asia Program, PO Box 161, 16001 Bogor Indonesia

(Accepted 21st November 2000)

ABSTRACT. Maximum assimilation rate under saturating light level, leaf nitrogen and chlorophyll content and specific leaf mass were measured in forest plants grown under a range of canopy openness. Seedlings from three tree species co-occurring in damar agroforest (Sumatra) were examined: *Shorea javanica*, *Lansium domesticum* and *Cinnamomum porrectum*. A shrub species, *Piper hispidum*, growing in a different location was also investigated. All species showed pronounced differences in maximum photosynthetic potential when grown under different canopy openness. All tree seedlings showed an increase in maximum assimilation rate (A_{\max}) with canopy openness (CO) until a certain threshold was reached. This saturation threshold varied between species. A steady increase in the maximum assimilation rate over the entire range of canopy openness explored was found only for *Piper*. Correlation between leaf nitrogen content and A_{\max} was usually highest when expressed on a per unit area basis. However the overall correlation was poor whether expressed per unit mass or per unit area when all species were pooled together questioning the universality of the relationship between both quantities. Potential photosynthetic nitrogen use efficiency, defined as the amount of CO_2 uptake per unit leaf nitrogen under saturating light level, was highest in *Cinnamomum*, supposedly the most light-demanding species, and lowest in *Lansium*, the understorey specialist.

KEY WORDS: acclimation, canopy openness, leaf nitrogen content, light environment

INTRODUCTION

Damar agroforests are cultivated dipterocarp forests (Michon *et al.* 1995). These uneven-aged multi-species forests show relatively high species richness (c. 50 species of trees more than 5 cm diameter at breast height in our permanent 1-ha sample plots). One major species usually accounts for the bulk of the

¹ Grégoire Vincent is a visiting scientist from Institut de Recherche pour le Développement (IRD) at International Center for Research in Agroforestry (ICRAF). Email: G. Vincent@cgiar.org



total basal area (50 to 80%). This species, locally known as damar (*Shorea javanica* Koord. & Valetton, Dipterocarpaceae) is cultivated for the resin extracted from the trunk and is also used as a source of timber. The other species are either planted or selected from natural regeneration. They include mostly native species of fruit trees, legume trees, palm trees and timber trees.

Management of these agroforests consists mainly in favouring natural regeneration of desired species by special tending of selected individuals and slashing undesired regrowth. Special care is directed mostly towards seedlings or young saplings, and consists of mechanical weeding, seedling transplantation, and enrichment planting associated to nursery practices.

The practical concern that initiated this study is whether such systems are susceptible to significant improvement of their biological productivity. The extent to which light requirements differ between tree species is directly related to the available scope for potential improvement in these systems through light management. Since the regeneration is largely controlled by man, is it possible to derive better strategies to shorten the non-productive phase by hastening the growth of the juveniles? We focus here on light as the controlling variable for two reasons. First, adaptation to different light environments is an important factor for niche differentiation in natural tropical moist forest, from the early successional light-demanding pioneer species to the very shade-tolerant understorey specialists (Chazdon 1996, Oldeman & van Dijk 1991). It may be stressed though that the extent to which light requirements contribute to niche definition at a finer scale is debatable (Field 1988, Press *et al.* 1996, Whitmore & Brown 1996). Second, light is the main resource that can be manipulated by the farmers through selective thinning, planting/transplanting species to particular locations, and other practices.

Species may differ both in their optimal light requirement and the extent to which they can acclimate to different light conditions. Determining the optimal conditions for growth would imply measuring actual carbon gain under different conditions. The most robust method probably would be to measure individual tree growth. This is not an easily amenable task when many different species are involved. Determining the range of conditions to which a given species is able to adapt may be more tractable, especially if proxies to photosynthetic performance are available. In this study we measured photosynthetic plasticity vis-à-vis different light environments by measuring changes in maximum assimilation rates under saturating light (A_{\max}) associated with growth under different canopy openness. Photosynthetic capacity is probably only weakly correlated to growth performance in nature as strategies of carbon allocation or water use efficiency may prove at least as important as photosynthetic efficiency at leaf level (Kuppers 1994, Whitmore 1996). A_{\max} alone is by no means a measure of performance, but it can be argued that if A_{\max} does not increase over a certain threshold of canopy openness then the optimal light level lies below that threshold. Indeed, higher light levels may then only have

negative effects, through associated increased temperature, increased evaporative demand (which may lead to stomatal closure or leaf shedding), or photo-inhibition, which will not be compensated for by further increase in photosynthetic potential.

The main objective of the present study is thus to characterize the extent to which tree species with different autecology may adapt at the leaf level to different levels of canopy openness, and to relate this plasticity to a set of morpho-chemical leaf traits in an attempt to derive a proxy for photosynthetic potential in future ecological studies. Such a proxy would indeed prove useful to screen the numerous species present in damar agroforest, and would also permit data acquisition from leaves of mature crowns for which direct gas measurements are difficult to carry out.

Previous work (e.g. Field & Mooney 1986) has shown that there is a consistent relationship between leaf nitrogen content and maximum assimilation rate (at saturating light level) in naturally occurring vegetation that seems to hold across species and across habitats. Similar positive correlation has also been reported by Chazdon & Field (1987), Chazdon (1992), Chazdon & Kaufmann (1993) for different *Piper* species across a range of light environments and 23 Amazonian tree species (Reich *et al.*). This relationship is believed to reflect the fact that the proteins of the Calvin-Benson cycle and the thylakoids represent the majority of the leaf nitrogen (Evans 1989). Because variation in leaf nitrogen explains much of the variation in photosynthetic capacity, across a wide variety of plant communities, leaf nitrogen content was selected as a candidate predictor for A_{\max} . The specific hypothesis tested here is that the nitrogen-photosynthesis relationship can be used for preliminary ranking of photosynthetic capacity of co-occurring species.

METHODS

Study site

The study site was located in Lampung, the southern province of Sumatra (Indonesia) where most of the damar forests are found. The area was once covered with lowland primary forest, of which very little is left today. Average annual rainfall is 3500 mm, with a peak in October/November (about 400 mm mo^{-1}) and a minimum in June/July (about 200 mm mo^{-1}). Soils at the study site are deep loamy soils developed on uplifted corals. *Piper* samples were collected from a different site – the arboretum of the Centre for International Forestry Research (CIFOR) in Bogor, West Java, Indonesia with moderately acid soils and similar climatic conditions. Soil analyses were carried out in both sites on the uppermost 20 cm. Similar pH values ($\text{pH-H}_2\text{O} = 5.3$, $\text{pH-KCl} = 4.3$), similar nitrogen content (0.2%) and comparable C/N ratios (nine in Damar forest and seven in CIFOR arboretum) were found. There was no indication of differences in nitrogen availability between sites.

Species selection

Three tree species were selected amongst the most abundant found in the area. Damar (*Shorea javanica*) is the major constituent of the upper canopy strata. *Lansium domesticum* Correa (Meliaceae) is a widely grown fruit tree from the intermediate strata. *Cinnamomum porrectum* (Roxb.) Kosterm. (Lauraceae) (also referred to as *Cinnamomum parthenoxylon*) is a timber tree. *Piper hispidum* Sw. (Piperaceae), a shrub naturally occurring in a wide range of light environments was added as a reference to a highly plastic species (Fredeen & Field 1996). *Shorea javanica* has been reported in Java and Sumatra (Ashton 1982). It commonly reaches a height of more than 40 m. *Lansium domesticum* is a widespread understorey tree of lowland primary forest, which is widely cultivated throughout the range of the genus (Ng 1989). It is a relatively small tree that can reach more than 20 m in height. *Cinnamomum porrectum* is valued locally and internationally for its timber. It can reach 45 m in height and 300 cm in girth (Ng 1989). The autecology of this species is not clearly established. It has been, most likely mistakenly, classified as a shade type of plant on the basis of its relatively low maximum assimilation rate estimated on cut twigs ($6\text{--}10\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$) (Koyama 1981). However average yearly girth increment is around 3 cm in permanent plots compared to 1.5 cm for *Lansium* and 2 cm for *Shorea* (pers. obs.). Local farmers consider it to be a light-demanding species.

Methods

Nine to 17 seedlings of each tree species 1–3 m tall were selected to cover the whole range of light environments found in several damar gardens close to each other. Three leaves from each seedling were selected from the upper part of the crown avoiding the youngest, not fully mature, leaves. Most care was taken in selecting leaves with a similar position on equivalent twigs as a mean of controlling leaf age, a factor which affects nitrogen content and photosynthetic capacity (Kitajima *et al.* 1997). For each leaf (or leaflet in the case of *Lansium*) the following were measured: chlorophyll content, maximum assimilation rate, nitrogen content, leaf area and specific leaf mass (ratio of dry mass to leaf or leaflet area). *Piper* leaves were sampled on a different site. Six erect twigs were selected from naturally occurring plants growing in three contrasting light environments. On each twig the second or third fully expanded leaf (numbered from apex to base) was selected.

Chlorophyll content was measured using a portable chlorophyllmeter (SPAD, Minolta, Japan). Six to 12 readings were taken per leaf depending on leaf size, and values were averaged. The readings given by the SPAD chlorophyll meter are based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different (Minolta 1989). SPAD values were previously shown to be in good agreement with chlorophyll content estimated after extraction, with linear regression coefficient (r^2) superior to 0.9 in all 12 species tested (Marquard & Tipton 1987).

Assimilation rates were measured using a portable infrared gas analyser (CIRAS 1, PPSsystem, UK). An external lamp unit fitted to the leaf chamber

provided white light with photon flux density (PFD) of *c.* 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Maximum assimilation rate was most often measured under 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD (the reduction in PFD being achieved by means of a neutral filter), at which level CO_2 assimilation appeared to be light saturated in most cases. Natural light levels recorded at the time of measurement were usually much lower and it commonly took 20–30 min for the assimilation rate to stabilize. Maximum assimilation rate was considered to be reached when assimilation rate would not increase further for 3 min. The leaf chamber was equipped with Peltier elements fitted with a heat sink and a fan that allowed increase in leaf temperature computed using the energy balance equations (Parkinson *et al.* 1980) to remain below 3 °C in all cases. *Lansium* however showed a distinct pattern of assimilation rate evolution. Assimilation rate would initially increase and then sharply decrease after 5–10 min and stabilize at a lower value. The maximum rate registered in this study was the highest rate measured during the early phase of fast CO_2 assimilation. The reason for this decrease in assimilation rate is not clear as no correlation with temperature or water vapour deficit inside the leaf chamber was found but it was always paralleled by a decrease in stomatal conductance. No oscillation pattern in stomatal conductance was detected after light induction contrary to what was found, for example, by Zipperlen & Press (1997) studying *Shorea leprosula* Miq. which also showed transient peaks in assimilation rates.

Leaf area was measured using a flatbed scanner. Leaf dry mass was measured after drying for 72 h in an oven at 70 °C. Nitrogen content was measured following the DUMAS method (Horwitz 1998). Nitrogen, freed by pyrolysis and subsequent combustion, is swept by a CO_2 carrier. CO_2 is absorbed in KOH and residual N is measured.

The light environment was assessed by taking hemispherical photographs vertically oriented towards the sky (Sigma 8-mm fish-eye lens). A single view was taken above each seedling using either a tripod or a hand-held monopod with self-levelling mount (Delta T, UK) when necessary. Photographs were then scanned and analysed using Hemiview 2.0 software (Delta T, UK) to determine canopy openness defined as the proportion of the hemispherical sky vault not obscured by vegetation above the point of interest (Jennings *et al.* 1999). Hemispherical photographs have been widely used in ecology to characterize light environment (Becker *et al.* 1989, Bellingham *et al.* 1996, Chazdon & Field 1987, Whitmore *et al.* 1993). At a particular site, and for the same reference period (same latitude and climate characteristics), canopy openness is a convenient index of the amount of light received at any given location and thus may be used to compare different locations.

RESULTS

Adaptation to different canopy openness

Leaf morphological traits such as individual leaf area and specific leaf mass appeared to be more variable than the biochemical traits measured, namely chlorophyll content and nitrogen content (Table 1).

Table 1. Range of variation, mean and coefficient of variation of some leaf characteristics of four tropical forest tree species grown under a wide range of canopy openness.

		Chlorophyll content (SPAD value)	Leaf (or leaflet) area (cm ²)	Specific leaf mass (g m ⁻²)	Nitrogen content (% dry mass)	Max. assim. rate ($\mu\text{mol CO}_2$ s ⁻¹ m ⁻²)	Stomatal conductance (mmol H ₂ O s ⁻¹ m ⁻²)
<i>Shorea javanica</i> (n=52)	min	35.5	75	42	1.54	5.2	127
	max	56.3	311	112	2.61	15.4	635
	mean	42.4	162	68	2.16	10.1	341
	CV	0.11	0.37	0.26	0.09	0.26	0.36
<i>Lansium domesticum</i> (n=23)	min	39.2	95	58	1.86	3.3	86
	max	69.6	224	95	2.82	11.2	418
	mean	56.4	156	73	2.39	6.64	234
	CV	0.11	0.23	0.17	0.11	0.36	0.46
<i>Cinnamomum porrectum</i> (n=27)	min	34.4	20	39	1.17	6.1	225
	max	53.7	110	117	2.36	13.9	555
	mean	44.3	61	67	1.83	9.65	359
	CV	0.14	0.32	0.34	0.19	0.24	0.25
<i>Piper hispidum</i> (n=18)	min	45.5	36	18	3.58	2.8	97
	max	58.7	88	46	4.72	15.2	439
	mean	51.8	63	31	4.09	7.85	215
	CV	0.08	0.21	0.33	0.09	0.54	0.54

The leaves of all species showed a substantial degree of adaptation to light environment as can be seen from Table 2, although not all parameters seemed to be as responsive. The tightest and most consistent correlation with CO was found for SLM ($r = 0.73$ – 0.92). Numerous studies (see for example: Niinemets 1997a, b; Syvertsen & Smith 1984; Wooge & Barden 1987) have reported such correlation between light environment and SLM which is widespread but not universal (Givnish 1988).

Chlorophyll content (SPAD value) and nitrogen per unit dry mass tended to be negatively correlated to CO but the correlation was rather weak and not systematic. Chlorophyll content expressed per unit area was previously reported to be negatively correlated with light level in seven out of eight *Acer* species (Lei & Lechowicz 1997). In a shading experiment, it was found that citrus leaves acclimated to the deepest shade showed the highest chlorophyll content per unit area (Syvertsen & Smith 1984). In contrast Niinemets (1997b)

Table 2. Pearson correlation coefficient between canopy openness above seedling and a set of structural and physiological features of leaves.

	<i>Shorea</i> (n=52)	<i>Lansium</i> (n=23)	<i>Cinnamomum</i> (n=27)	<i>Piper</i> (n=18)
Chlorophyll content – SPAD (CC)	0.43 (NS)	-0.45 (*)	-0.57 (**)	0.45 (NS)
N content per unit mass (N_m)	-0.25 (NS)	0.25 (NS)	-0.66 (**)	-0.68 (**)
Leaf (or leaflet) area	0.03 (NS)	0.03 (NS)	-0.53 (**)	0.39 (NS)
SLM	0.86 (**)	0.73 (**)	0.75 (**)	0.92 (**)
N content per unit area (N_a)	0.75 (**)	0.76 (**)	0.51 (**)	0.91 (**)
Max. stomatal conductance (G)	0.73 (**)	0.15 (NS)	0.20 (NS)	0.85 (**)
Max. assimilation rate (A_{max})	0.72 (**)	0.12 (NS)	0.64 (**)	0.96 (**)

**, highly significant ($P < 0.01$); *, significant ($P < 0.05$); NS, not significant (Bartlett chi-square statistic).

found no correlation between canopy openness and chlorophyll content per unit area in four temperate deciduous woody species. Nitrogen per unit dry mass was found to decrease with lower position in canopy and thus decreasing light intensity in one study (Hollinger 1989). In other cases no clear relation was found between light level and leaf nitrogen content expressed per unit dry mass (Ellsworth & Reich 1992, Niinemets 1997b, Straus-Debenedetti & Bazzaz 1991).

Stomatal conductance showed positive correlation with canopy openness but this correlation was significant only for two species out of four. Increase in maximum stomatal conductance under higher light levels seems to be a common adaptive feature of tropical species (Straus-Debenedetti & Bazzaz 1991, Wiebel *et al.* 1993).

Maximum assimilation rate against canopy openness curves (Figure 1) were drawn using the distance weighted least squares smoothing technique provided in Systat 9.0 statistical package (Wilkinson *et al.* 1999). All species showed a substantial degree of responsiveness to light environment in terms of the maximum assimilation rate achieved. The curves showed the same curvilinear trend for the three tree species. Starting at the lowest values of canopy openness, a few percentage points increase in CO determined an important increase in A_{\max} whereas above a certain threshold of CO which seemed to be species dependent a further increase in CO determined no further increase (if not a decrease) in A_{\max} . The threshold can be estimated to be about 15% for *Lansium* and 30% for *Shorea* and somewhere in between 20% and 40% for *Cinnamomum*. It is difficult to be more precise for *Cinnamomum* as no observations were available between 20 and 40% canopy openness. In any case it should be stressed that canopy openness above 30% is uncommon in a damar forest environment. Noticeably the relationship for *Piper* did not show such a tendency to saturate with increasing canopy openness. Here, again, the conclusions were conditional on the sampling of light environment studied (only three levels for *Piper*) and thus should be considered as tentative. An increase in canopy openness from 15% to 50% was paralleled in *Piper* by a twofold increase in A_{\max} whereas this increase was only about 50% for the two tree species measured under similar change in canopy openness (Figure 1).

Relative contribution of diffusion vs. carboxylation limitation of A_{\max} was assessed through multiple linear regression. A_{\max} was modelled as a linear function of stomatal conductance (G) and nitrogen per unit area (N_a). The relative importance of each contributor can be estimated by comparing the full model with the model restricted to one predictor. Results shown in Table 3 indicate that *Shorea* and *Piper* showed significant positive correlation between A_{\max} and both predictors, whereas this was not the case for *Lansium* and *Cinnamomum*. For the latter species one predictor alone (N_a) contributed to most of the reduction in variance measured by the adjusted r^2 . For *Lansium*, only G showed a significant effect on A_{\max} .

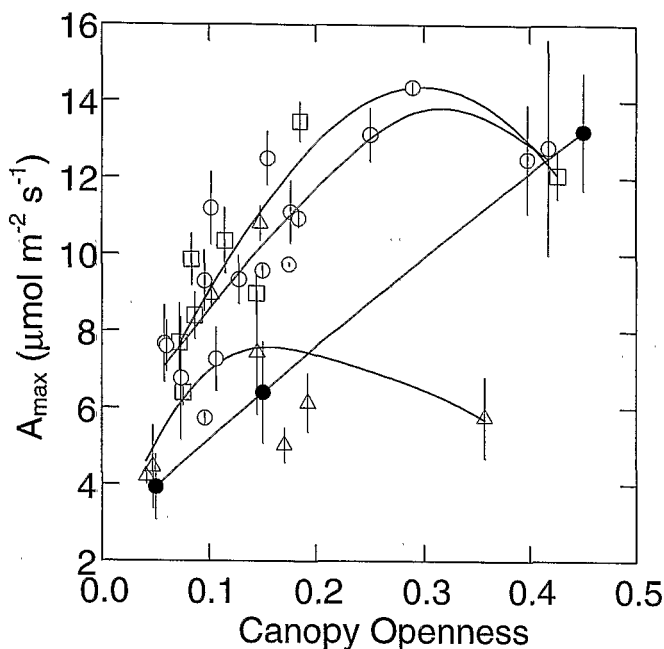


Figure 1. Maximum assimilation rate at saturating light level measured on plants growing under different canopy openness. Error bar represents \pm SD. Each point is the average value obtained for three leaves (*Cinnamomum porrectum* \square , *Shorea javanica* \circ , and *Lansium domesticum* \triangle) or six leaves (*Piper hispidum* \bullet) from a single plant.

Table 3. Multiple linear regression showing relative importance of stomatal conductance (G) and nitrogen content per unit chlorophyll (N_a) in determining assimilation rate achieved under saturating light conditions (A_{max}) for four species monitored under a range of canopy openness. All predictors have significant effect ($P < 0.05$) unless otherwise mentioned as not significant (NS).

	Adjusted r^2 of linear regression			Pearson correlation coefficient (N_a, G)
	full model	G alone	N_a alone	
<i>Shorea</i> (n=52)	0.86	0.73	0.73	0.70 (**)
<i>Lansium</i> (n=23)	0.77 (N_a NS)	0.74	0.03 (NS)	0.06 (NS)
<i>Cinnamomum</i> (n=27)	0.59	0.10 (NS)	0.45	-0.03 (NS)
<i>Piper</i> (n=18)	0.97	0.81	0.81	0.70 (**)

Nitrogen content- A_{max} relationship

Correlation coefficients between A_{max} and nitrogen content (Table 4) appeared to be higher when expressed per unit area than per unit dry mass for every species that showed a significant correlation between A_{max} and nitrogen content (Figures 2 and 3). Only *Lansium* did not show any significant correlation.

When expressed per unit mass, the correlation by species was lower but the correlation for the pooled data set was higher. *Piper* leaves that were both thinner and richer in nitrogen showed much higher assimilation rate per unit mass and tended to reinforce the correlation.

Table 4. Pearson correlation coefficient between A_{\max} and leaf nitrogen content for four plant species.

	<i>Shorea</i> (n=52)	<i>Lansium</i> (n=23)	<i>Cinnamomum</i> (n=27)	<i>Piper</i> (n=18)	All 4 species (n=120)
A_{\max} and nitrogen content expressed per unit area	0.86**	0.27 NS (0.53 *)	0.69 **	0.91 **	0.44 **
A_{\max} and nitrogen content expressed per unit weight	0.55 **	0.26 NS (0.48 *)	0.65 **	-0.17 NS	0.58 **

**, highly significant; *, significant; NS, non significant (Bartlett chi-square statistic). Values between parentheses for *Lansium* refer to a subset of trees excluding one tree showing very low stomatal conductance under very open conditions.

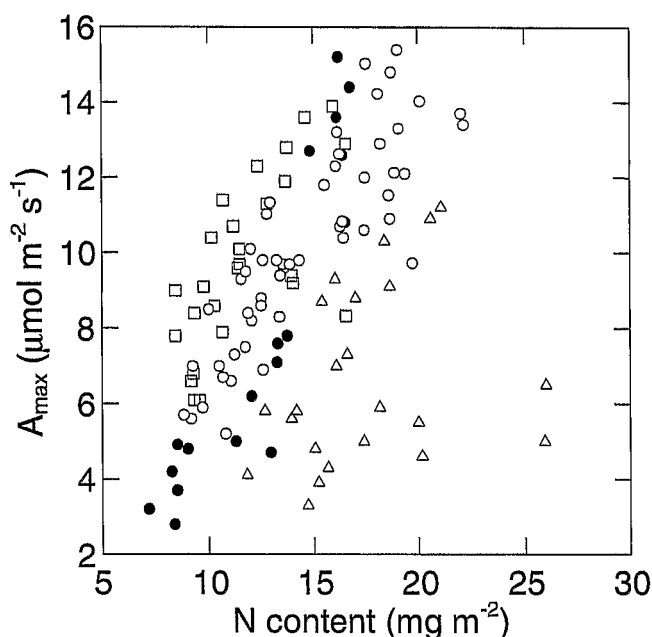


Figure 2. Scatter plot depicting the nitrogen- A_{\max} relationship recorded for seedlings of three tree species (*Cinnamomum porrectum* □, *Shorea javanica* ○, and *Lansium domesticum* △) and one shrub species (*Piper hispidum* ●). Relation is expressed per unit area.

To eliminate the confounding effect of the varying SLM we now consider the potential photosynthetic nitrogen use efficiency (PPNUE) defined as the ratio between maximum assimilation rate and nitrogen content (either expressed per unit dry mass or per unit area). The ranking of the average value of PPNUE for three tree seedlings is the same as the one obtained graphically (Table 5 and Figure 2). *Cinnamomum* showed the highest value of CO_2 uptake per unit nitrogen and *Lansium* the lowest. This ranking seemed to hold irrespective of N_a content sampled (which itself was tightly correlated to the canopy openness as can be seen in Table 2). The average PPNUE for *Piper* appeared to be intermediate. The graphical representation per unit area shows that the PPNUE of *Piper* tended to be higher than, or equal to, the woody species examined when N_a content was high, and lower when N_a content was low. *Piper* was

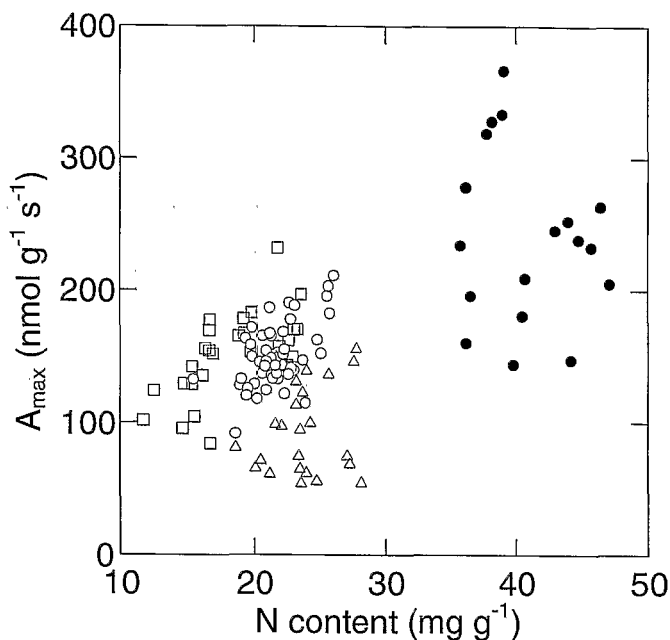


Figure 3. Scatter plot depicting the nitrogen- A_{\max} relationship recorded for seedlings of three tree species (*Cinnamomum porrectum* □, *Shorea javanica* ○, and *Lansium domesticum* △) and one shrub species (*Piper hispidum* ●). Relation is expressed per unit area.

also the only species showing a significant positive relationship between PPNUE and CO (Table 5).

DISCUSSION

Adaptation to different light environments

The tight relationship between canopy openness (CO) and SLM induced an almost as tight relationship between canopy openness and nitrogen per unit area (N_a). This is due to the much higher variability in SLM than N_m (Table 1). This relationship between nitrogen per unit area and light level has been found in almost all cases where it was investigated, reflecting the increase in the carboxylating capacity of sun-adapted leaves mediated through an increase in the Rubisco-associated nitrogen pool (Evans 1989). Plants grown under high irradiance usually also show an increase in their stomatal density resulting in

Table 5. Average potential photosynthetic nitrogen use efficiency measured over a wide range of canopy openness and Pearson correlation coefficient between PPNUE and canopy openness (CO).

	<i>Shorea</i> (n = 52)	<i>Lansium</i> (n = 23)	<i>Cinnamomum</i> (n = 27)	<i>Piper</i> (n = 18)
Average PPNUE	0.70 (0.09)	0.39 (0.13)	0.84 (0.14)	0.60 (0.18)
Correlation between PPNUE and CO	-0.18 NS	-0.19 NS	0.23 NS	0.88 **

**, highly significant; NS, non significant.

an increase in their maximum stomatal conductance (Bolhar-Nordenkamp & Draxler 1993). However, measured maximum stomatal conductance in *Lansium* did not increase with CO over the whole range of CO explored. It is likely that the seedlings under the most open canopy were suffering sub-optimal water supply at the time of measurement or, that they had previously suffered some water stress with lingering effect. It has been reported indeed that water stress in *Lansium domesticum* may result in reduced stomatal conductance long after water supply has returned to optimal level (Mohd. Razi Ismail *et al.* 1993). Seedlings of both emergent species achieved comparable A_{\max} in similar light environments but showed different ability to adjust their stomatal conductance. Whereas growth under a low-light environment in *Shorea* was associated with a decrease in G , this was not the case for *Cinnamomum*. Stomatal conductance in *Cinnamomum* was on average higher than the other species examined here (Table 1) especially under low light (data not shown) but this did not result in a higher A_{\max} . As a consequence of this low assimilation rate and high stomatal conductance *Cinnamomum* seedlings will show lower water use efficiency (ratio of CO₂ uptake per H₂O transpired) under such conditions. It is not known however to what extent stomatal closure regulation may mitigate this low water use efficiency in a natural climate. Co-adjustment of G and N_a occurred across the whole range of CO sampled in *Piper* leaves confirming its widespread adaptability to light conditions previously reported (Chazdon & Field 1987, Field 1988, Fredeen & Field 1996, Walters & Field 1987).

Nitrogen- A_{\max} relationship

In the present study the variation in nitrogen content was achieved by sampling leaves in different light environments. It can reasonably be assumed that there was no dramatic variation in soil fertility and thus in nitrogen availability between tree seedlings. The nitrogen- A_{\max} relationship appeared tighter and more systematic when expressed per unit area as can be seen from Figures 2 and 3. For one species studied though no correlation was found (*Lansium*) due to low stomatal conductance leading to spurious A_{\max} values under the most open canopy. In fact N_a did scale with canopy openness probably reflecting an adjustment of the photosynthetic potential. Correlation was significant if outlying data from a single tree with abnormally low stomatal conductance under very open canopy were excluded from the analysis.

When considered on a per unit dry mass basis then the correlation between A_{\max} and nitrogen content held for *Shorea* and *Cinnamomum* (upper canopy trees) and also *Lansium* (provided the tree showing abnormally low stomatal conductance was excluded). Correlation did not hold for *Piper* leaves though, in agreement with the results of Walters & Field (1987).

Overall lack of significant correlation between nitrogen and A_{\max} was previously reported by Straus-Debenedetti & Bazzaz (1991) working on fertilized potted plants. Although it might hold in first approximation when comparing

species varying widely in their nitrogen and A_{\max} characteristics, the relationship between A_{\max} and nitrogen seems too crude to systematically rank co-occurring species in terms of their photosynthetic capacity by simply measuring their leaf nitrogen content. As a matter of fact, amongst the three tree seedlings examined in this study, the species with the highest leaf nitrogen content is also the one with lowest A_{\max} , whether expressed on a mass or an area basis.

Seedlings showed different potential photosynthetic nitrogen use efficiency (PPNUE). It has been hypothesized (Field & Mooney 1986) that such differences may reflect different partitioning of N between chlorophyll and the thylakoid pool on the one side and the enzymatic proteins involved in the Calvin-Benson cycle (mainly Rubisco) on the other. Species flourishing in the open tend to allocate relatively less nitrogen to thylakoids and achieve higher photosynthetic capacity per unit nitrogen (Evans 1989). This interpretation is consistent with the a priori ranking of the three tree species. PPNUE was also found to be higher in early successional species than late successional species in a study of 23 Amazonian species of different communities (Reich *et al.* 1994). The case of *Piper* is somewhat peculiar. Its leaf nitrogen content per unit dry mass was much higher and the relative change in leaf nitrogen content was much lower than the other species suggesting that an important part of the nitrogen in the leaf was devoted to non-photosynthetic compounds. If indeed, a large (and constant per unit area) amount of nitrogen is devoted to non-productive compounds, and if moreover, in response to higher light levels only the specific fraction of nitrogen devoted to photosynthetic machinery may increase, then the PPNUE will increase with increasing nitrogen content, as every additional unit of nitrogen is more and more efficient. This interpretation also suggests that, in the other species studied, only a relatively small share of nitrogen is not devoted to photosynthesis, as constant PPNUE indicates a linear relationship between A_{\max} and N_a with close to zero intercept. As a matter of fact *Piper* species often contain alkaloids, the commonest of which (piperine) is insecticidal (Mooney *et al.* 1984), so a possibly important part of the leaf nitrogen in *Piper* may indeed be devoted to an anti-herbivory compound.

CONCLUSION

Our data indicate that both *Shorea javanica* and *Cinnamomum porrectum* adapt to a wide range of canopy openness by altering their maximum photosynthetic potential. Above a certain threshold of canopy openness estimated to be around 30% no further increase in A_{\max} was achieved under more open canopy. This threshold does not necessarily coincide with maximum integrated assimilation over time but it is a likely upper limit for optimal light regime. The threshold for *Lansium* seems to be around 15%. This lower threshold for *Lansium* seedlings seems to be determined more by lower stomatal conductance than incapacity to adapt its carboxylation capacity under more open canopies. The data collected

confirm that the shrub species (*Piper*) shows a high capacity of adaptation to a wide range of light conditions.

From the methodological point of view the following conclusions can be drawn.

A strong A_{\max} -nitrogen relationship was found within species in co-occurring plants growing under a wide range of canopy openness. The correlation was highest when both quantities were expressed per unit leaf area. Nevertheless the relationship did not hold across species and nitrogen may not be used as a proxy to photosynthetic potential when comparing different co-occurring species.

Potential Photosynthetic Nitrogen Use Efficiency (PPNUE) seemed to be related to light requirement, the most light-demanding species showing the highest PPNUE, as conjectured by Field & Mooney (1986). The relationship is blurred however if an important part of the nitrogen is not allocated to photosynthetic compounds as in *Piper* in this study.

More attention towards water use strategy as a potential determinant of performance (and niche segregation) in damar agroforests is also called for. Photosynthetic potential of the shade tolerant species indeed seemed to be constrained by low stomatal conductance under high light. The most light-demanding species showed very little reduction in maximum stomatal conductance under low light, possibly resulting in low water-use efficiency.

ACKNOWLEDGEMENTS

The author is indebted to Dennis Garrity who kindly accepted to revise the English and to Jean-Marc Guehl, and three anonymous reviewers for their valuable comments on earlier drafts of this manuscript. The author also wishes to acknowledge the help of Sulhul Authar during data collection.

LITERATURE CITED

- ASHTON, P. S. 1982. *Dipterocarpaceae*. Pp. 237–552 in *Flora Malesiana*. Volume 9.
- BECKER, P., ERHART, D. W. & SMITH, A. P. 1989. Analysis of forest light environments Part 1. Computerized estimation of solar radiation from hemispherical canopy photographs. *Agricultural and Forest Meteorology* 44:217–232.
- BELLINGHAM, P. J., TANNER, E. V. J., RICH, P. M. & GOODLAND, T. C. R. 1996. Changes in light below the canopy of a Jamaican montane rainforest after a hurricane. *Journal of Tropical Ecology* 12:699–722.
- BOLHAR-NORDENKAMPF, H. R. & DRAXLER, G. 1993. Functional leaf anatomy. Pp. 91–112 in Hall, D. O., Scurlock, J. M. O., Bolhar-Nordenkamp, H. R., Leegood, R. C. & Long, S. P. (eds). *Photosynthesis and production in a changing environment*. Chapman & Hall, London.
- CHAZDON, R. L. 1992. Photosynthetic plasticity of two rain forest shrubs across natural gap transects. *Oecologia* 92:586–595.
- CHAZDON, R. L. 1996. Photosynthetic responses of tropical forest plants to contrasting light environment. Pp. 5–55 in Mulkey, S. S., Chazdon, R. L. & Smith, A. P. (eds). *Tropical forest plant ecophysiology*. Chapman & Hall, New York.
- CHAZDON, R. L. & FIELD, C. B. 1987. Determinants of photosynthetic capacity in six rainforest *Piper* species. *Oecologia* 73:222–230.
- CHAZDON, R. L. & KAUFMANN, S. 1993. Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Functional Ecology* 7:385–394.

- ELLSWORTH, D. S. & REICH, P. B. 1992. Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. *Functional Ecology* 6:423-435.
- EVANS, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78:8-19.
- FIELD, C. B. 1988. On the role of photosynthetic responses in constraining the habitat distribution of rainforest plants. *Australian Journal of Plant Physiology* 15:343-358.
- FIELD, C. B. & MOONEY, H. A. 1986. The photosynthesis-nitrogen relationship in wild plants. Pp. 25-55 in Givnish, T. J. (ed.). *On the economy of plant form and function*. Cambridge University Press, Cambridge.
- FREDEEN, A. L. & FIELD, C. B. 1996. Ecophysiological constraints on the distribution of *Piper* species. Pp. 597-618 in Mulkey, S. S., Chazdon, R. L. & Smith, A. P. (eds). *Tropical forest plant ecophysiology*. Chapman & Hall, New York.
- GIVNISH, T. J. 1988. Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology* 15:63-92.
- HOLLINGER, D. Y. 1989. Canopy organization and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. *Functional Ecology* 3:53-62.
- HORWITZ, W. 1998. *Official methods of analysis of AOAC International*. AOAC International, Gaithersburg, USA.
- JENNINGS, S. B., BROWN, N. D. & SHEIL, D. 1999. Assessing forest canopies and understorey illumination: canopy closure, canopy cover and other measures. *Forestry* 72:59-73.
- KITAJIMA, K., MULKEY, S. S. & WRIGHT, S. J. 1997. Decline of photosynthetic capacity with leaf age in relation to leaf longevities for five tropical canopy tree species. *American Journal of Botany* 84:702-708.
- KOYAMA, H. 1981. Photosynthetic rates in lowland rain forest trees of Peninsular Malaysia. *Japanese Journal of Ecology* 31:361-369.
- KUPPERS, M. 1994. Canopy gaps: competitive light interception and economic space filling - a matter of whole-plant allocation. Pp. 111-144 in Caldwell, M. M. & W. P. R. (eds). *Exploitation of environmental heterogeneity by plants*. Academic Press, San Diego.
- LEI, T. T. & LECHOWICZ, M. J. 1997. Functional responses of *Acer* species to two simulated forest gap environments: leaf-level properties and photosynthesis. *Photosynthetica* 33:277-289.
- MARQUARD, R. D. & TIPTON, J. L. 1987. Relationship between extractable chlorophyll and an in situ method to estimate leaf greenness. *HortScience* 22:1327.
- MICHON, G., DE FORESTA, H. & LEVANG, P. 1995. Stratégies agroforestières paysannes et développement durable: les agroforêts à damar de Sumatra. *Natures Sciences Sociétés* 3:207-221.
- MINOLTA. 1989. *Chlorophyll meter SPAD 502, Instruction manual*, MINOLTA Co Ltd., Osaka.
- MOHD. RAZI ISMAIL, MOHD. IDRIS, Z. A. & RUZIAH SALEH. 1993. Water relations, stomatal responses and physiological changes of *Lansium domesticum*. *Journal of Tropical Agricultural Science* 16:179-185.
- MOONEY, H. A., FIELD, C. & VAQUEZ-YANES, C. 1984. Photosynthetic characteristics of wet tropical forest plants. Pp. 113-128 in Medina, E., Mooney, H. A. & Vazquez-Yanes, C. (eds). *Physiological ecology of plants of the wet tropics*. Dr. W. Junk, The Hague.
- NG, F. S. P. (ed.) 1989. *Tree flora of Malaya*. Longman Malaysia.
- NINEMETS, U. 1997a. Distribution patterns of foliar carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea abies*. *Trees - Structure and Function* 11:144-154.
- NINEMETS, U. 1997b. Role of foliar nitrogen in light harvesting and shade tolerance of four temperate deciduous woody species. *Functional Ecology* 11:518-531.
- OLDEMAN, R. A. A. & VAN DIJK, J. 1991. Diagnosis of the temperament of tropical trees. Pp. 21-63 in Gomez-Pompa, A., Whitmore, T. C. & Hadley, M. (eds). *Rain forest regeneration and management*. Volume 6. UNESCO, Paris.
- PARKINSON, K.J., DAY, W. & LEACH, J. E. 1980. A portable system for measuring the photosynthesis and transpiration of graminaceous leaves. *Journal of Experimental Botany* 31:1441-1453.
- PRESS, M. C., BROWN, N. D., BARKER, M. G. & ZIPPERLEN, S. W. 1996. Photosynthetic responses to light in tropical forest tree seedlings. Pp. 41-58 in Swaine, M. D. (ed.). *The ecology of tropical forest tree seedlings*. UNESCO, Paris.
- REICH, P. B., WALTERS, M. B., ELLSWORTH, D. S. & UHL, C. 1994. Photosynthesis-nitrogen relations in Amazonian tree species. I. Patterns among species and communities. *Oecologia* 97:62-72.
- STRAUS-DEBENEDETTI, S. & BAZZAZ, F. A. 1991. Plasticity and acclimation to light in tropical *Moraceae* of different successional positions. *Oecologia* 87:377-387.
- SYVERTSEN, J. P. & SMITH, M. L. J. 1984. Light acclimation in *Citrus* leaves. I. Changes in physical characteristics, chlorophyll, and nitrogen. *Journal of the American Society of Horticultural Science* 109:807-812.
- WALTERS, M. B. & FIELD, C. B. 1987. Photosynthetic light acclimation in two rainforest *Piper* species with different ecological amplitudes. *Oecologia* 72:449-456.

- WHITMORE, T. G. 1996. A review of some aspects of tropical rain forest seedling ecology with suggestions for further enquiry. Pp. 3-30 in Swaine, M. D. (ed.). *The ecology of tropical forest tree seedlings*. Volume 17. Parthenon Publishing Group Ltd. & UNESCO, Carnforth & Paris.
- WHITMORE, T. G. & BROWN, N. D. 1996. Dipterocarp seedling growth in rain forest canopy gaps during six and a half years. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences* 351(1344):1195-1203.
- WHITMORE, T. G., BROWN, N. D., SWAINE, M. D., KENNEDY, D., GOODWIN-BAILEY, G. I. & GONG, W. K. 1993. Use of hemispherical photographs in forest ecology: measurement of gap size and radiation totals in a Bornean tropical rain forest. *Journal of Tropical Ecology* 9:131-151.
- WIEBEL, J., EAMUS, D., CHACKO, E. K., DOWNTON, W. J. S. & LÜDDERS, P. 1993. Gas exchange characteristics of mangosteen (*Garcinia mangostana* L.) leaves. *Tree Physiology* 13:55-69.
- WILKINSON, L., KHALILEEV, S., ROSS, L., LEUTHNER, T., SIPIORA, S., KROEGER, K., MARCANTONIO, R., WOODS, M., KIRSTEN, K. & HINRICHSEN, D. 1999. SYSTAT. SPSS Inc, USA.
- WOOG, J. D. & BARDEN, J. A. 1987. Seasonal changes in specific leaf weight and leaf anatomy of apple. *HortScience* 22:292-294.
- ZIPPERLEN, S. W. & PRESS, M. C. 1997. Photosynthetic induction and stomatal oscillations in relation to the light environment of two dipterocarp rain forest tree species. *Journal of Ecology* 85:491-503.

