

The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance

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Summary

1. Light-saturated rate of photosynthesis (A_{\max}), nitrogen (N), chlorophyll (Chl) content and leaf mass per unit area (LMA) were measured in leaves of trees of different heights along a natural light gradient in a French Guiana rain forest. The following four species, arranged in order from most shade-tolerant to pioneer, were studied: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*. Light availability of trees was estimated using hemispherical photography.

2. The pioneer species *Goupia* had the lowest LMA and leaf N on both an area and mass basis, whereas *Duguetia* had the highest values. In general, leaf variables of *Vouacapoua* and *Dicorynia* tended to be intermediates. Because A_{\max} /area was similar among species, *Goupia* showed both a much higher light-saturated photosynthetic nitrogen-use efficiency (PNUE_{\max}) and A_{\max} /mass. Leaves of *Vouacapoua* demonstrated the greatest plasticity in A_{\max} /area, particularly in small saplings.

3. A distinction could be made between the effect of tree height and light availability on the structural, i.e. LMA, and photosynthetic leaf characteristics of all four species. The direction and magnitude of the variation in variables were similar among species.

4. LMA was the key variable that mainly determined variation in the other leaf variables along tree height and light availability gradients, with the exception of changes in chlorophyll concentration. A_{\max} /area, N/area, LMA and stomatal conductance to water vapour (g_s) increased, whereas Chl/mass decreased, with both increasing tree height and canopy openness. A_{\max} /mass, PNUE_{\max} and A_{\max} /Chl increased with increasing openness only. N/mass and Chl/area were independent of tree height and openness, except for small saplings of *Goupia* which had a much lower Chl/area.

Key-words: Canopy openness, French Guiana, leaf morphology, tree size, photosynthesis, rain forest

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Introduction

In tropical forests events such as the formation and closure of canopy openings of different dimensions create a light environment which is highly heterogeneous (Chazdon & Fetcher 1984; Kira & Yoda 1989). Consequently, a tree may experience several alternate phases of high and low light conditions in the course of its life span. Because light is a major determinant for survival and growth, it is expected that plants are able to adjust their leaf and/or whole-plant traits to changing light availability.

Leaf responses to different light environments vary widely within and among species. In general, within

species, shade-growing leaves are thinner, have lower mass per unit area and have higher mass-based chlorophyll content than do sun-growing leaves (Bongers & Popma 1988; Kitajima 1994). In addition, shade leaves usually have low area-based rates of photosynthesis and dark respiration and low light saturation points (Boardman 1977). As a result, leaf adjustments to low light increase the capacity of light absorption at the expense of photosynthetic capacity and minimize carbon losses through respiration (e.g. Evans 1989; Pons & Pearcy 1994). In contrast, sun leaves can make more efficient use of the prevailing high light intensities for carbon gain while avoiding a possible reduction in photosynthetic performance as a result of

photoinhibition. Therefore, acclimation to a high light condition often results in thicker leaves with more chloroplasts per unit area and in greater investments in leaf compounds related to the carboxylation and electron-transport processes needed to increase photosynthetic capacity (e.g. Boardman 1977).

Increasingly more evidence indicates that both light-demanding and shade-tolerant species are capable of phenotypic plasticity, indicating adjustments are not necessarily related to the successional status of species (Turnbull 1991; Popma, Bongers & Werger 1992). However, when comparing species, leaves of shade-tolerant species often have both lower rates of photosynthesis (Koike 1988; Raaimakers *et al.* 1995) and a higher leaf mass per unit area (Kitajima 1994; but see Popma *et al.* 1992) than do light-demanding species in both shade and high light conditions.

Independent of the light-environment, leaf and/or whole-plant traits may also change with plant size (Coleman, McConnaughay & Ackerly 1994). For example, Hoflacher & Bauer (1982) found a greater plasticity in leaves of adult plants of *Hedera helix* as in high light these plants were able to develop thicker leaves with higher area-based rates of photosynthesis than did juvenile plants. For several woody species, seedlings showed a decrease in mass-based rates of photosynthesis (Walters, Kruger & Reich 1993a), an increase in photosynthetic saturation irradiance (Bormann 1958) and an increase in leaf mass per unit area (Veneklaas & Poorter 1998) as the plant grew. Studies on mature canopy trees are scarce, however, and mainly focusing on the distribution patterns of leaf level traits within a crown (e.g. Ellsworth & Reich 1993). However, in temperate forests it was found that an increase in tree height of some conifer species was accompanied by an increase in leaf mass per unit area and a decrease in mass-based leaf nitrogen content (Schoettle 1994, Niinemets & Kull 1995; Niinemets 1997a) and a decrease in mass and area based rates of photosynthesis (Kull & Koppel 1987; Schoettle 1994).

Several factors may explain physiological and morphological alterations in leaf characteristics as tree height increases. For instance, because the ratio of total leaf area to total non-photosynthetic, living tissue decreases with increasing tree height less available foliage has to provide the tree with assimilates for growth and maintenance. Also, increasing tree height necessitates extra carbon investments to construct and maintain support tissues such as twigs and branches (Givnish 1986) or to lessen the risk of windthrow (Telewski 1995). Furthermore, because tall trees are likely to have a steeper water-potential gradient from soil to leaf, their leaves must have a sufficiently negative water potential to take water from the soil to the leaf (Friend 1993; Niinemets 1997a).

Beside tree-size dependent changes, leaf traits may also change in response to the environment, as an increase in height in the forest is often accompanied

by an increase in air temperature, vapour pressure deficit, irradiance and/or wind speed (e.g. Kira & Yoda 1989). Increasing wind speed, for example, may cause mechanical stress for leaves, inducing morphological and anatomical changes in leaves (e.g. Jaffe & Forbes 1993).

To date studies investigating variations in leaf characteristics as related to tree size are scarce. Our objective in this study was to discriminate between tree height and light availability effects on leaf physiology and morphology of four tree species differing in shade tolerance. Arranged in order of decreasing shade tolerance these species were: *Duguetia surinamensis* R.E.Fr. (Annonaceae), *Vouacapoua americana* Aubl., *Dicorynia guianensis* Amshoff (Caesalpiniaceae) and *Goupia glabra* Aubl. (Celastraceae). We selected individuals of different heights growing in a range of natural light conditions in a French Guiana rain forest. Measurements of light-saturated rate of photosynthesis, leaf nitrogen and chlorophyll content, and leaf mass per unit area were performed on exposed leaves in the upper part of the tree crown.

Materials and methods

STUDY SITE AND SPECIES

The research was carried out in an area of 12 ha at the biological field station Les Nouragues in a low-land tropical rain forest in French Guiana (4°05' N; 52°40' W) in the period from June through November during three consecutive years (1995–1997). The area has well-drained, clayey to sandy-clayey ferrallitic soils on weathered granite parent material. Mean annual rainfall is *c.* 3000 mm with dry periods from September to November and February to March. The maximum height of the forest canopy is *c.* 50 m with some emergent trees reaching 60 m in height.

Duguetia surinamensis is considered to be the most shade-tolerant because it is a subcanopy species reaching 30 m at maturity. *Goupia glabra* is the least shade-tolerant. It is a long-lived pioneer species which needs large gaps to germinate and establish, but may survive under the forest canopy (Schulz 1960). The large canopy trees *Dicorynia guianensis* and *Vouacapoua americana* are of intermediate shade tolerance, and the latter one is considered the most shade-tolerant of the two (Favrichon 1994). Both species can establish themselves in the forest understorey and in small gaps. For brevity, species will hereafter be referred to by genus only. All species except *Goupia* are common in the area studied. Maximum tree height for *Vouacapoua* is about 45 m, and for *Dicorynia* and *Goupia* about 55 m. *Dicorynia* and *Vouacapoua* have compound leaves with five to 15 leaflets. *Goupia* and *Duguetia* produce plagiotropic branches with simple leaves from the seedling stage. In this study, the leaflets of compound leaves are considered to be functionally similar to simple leaves.

SELECTION OF TREES AND LEAF SAMPLING

The total height of the selected trees ranged from 0.7 to 21 m. Individuals in or near gap sites of different sizes and under a closed canopy were chosen. They were selected on the basis of their height at that moment: (1) small saplings (range 0.7–2 m), (2) small juvenile trees (range 4–9 m) and (3) tall juvenile trees (12–21 m). For similar-sized trees both shade and light growing individuals were sampled. In the upper, peripheric part of the tree crown, six to 20 young, fully expanded leaves were harvested to determine leaf mass per unit area (LMA). Access to leaves was obtained by using climbing poles or rope techniques. Prior harvesting, three or four leaves from this sample were used to measure the area-based light-saturated rate of photosynthesis (A_{\max}/area), chlorophyll per unit leaf area (Chl/area) and nitrogen per unit leaf mass (N/mass). All the measurements were averaged per tree. Gas-exchange measurements were made before the leaves were harvested. In addition, for *Duguetia*, *Vouacapoua* and *Dicorynia* mature canopy trees ($n = 1, 3$ and 3 , respectively) were sampled, ranging in tree height from 26 m for *Duguetia* and 34–55 m for the other two species. All canopy trees were exposed to full sun, except the *Duguetia* tree. Owing to the small sample of these individuals, they were not included in the statistical analyses. For *Goupia* no canopy trees were accessible.

GAS-EXCHANGE MEASUREMENTS

Light-saturated rate of photosynthesis at ambient CO_2 partial pressure was measured using a portable infra-red gas-exchange system (CIRAS-1, PP-system, Hitchin, UK) with a Parkinson leaf chamber (2.5 cm^2). Leaves were brought to photosynthetic light saturation under a quartz halogen bulb (12 V, 20 W) at a photon flux density (PFD) of $735 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Preliminary measurements showed that this light intensity was well above the light-saturated point for these leaves. The time needed for photosynthetic induction was about 15 min. During all measurements mean ($\pm \text{SD}$) air temperature in the leaf chamber was $28 \pm 1^\circ\text{C}$, CO_2 concentration was 355 ± 4 p.p.m. and relative air humidity was $66 \pm 8\%$. Gas-exchange measurements were always carried out in the morning between 09.00 and 12.00 h. The rate of photosynthesis and stomatal conductance to water vapour (g_s) at light saturation were calculated according to Von Caemmerer & Farquhar (1981).

LEAF MORPHOLOGY, NITROGEN AND CHLOROPHYLL ANALYSES

The circumference of each harvested leaf was first drawn on paper; these drawings were used, in the Netherlands, to calculate leaf area with a video area-meter (DIAS, Delta-T Image Analyse System,

Eijkelkamp BV, Giesbeek, the Netherlands). The leaves were then dried in a field oven at 50°C for 3 days and afterwards dry-stored. In the Netherlands leaves were oven-dried at 70°C for 48 h before leaf dry mass was measured. From these data LMA was calculated for each leaf. Leaf chlorophyll and nitrogen content were measured for the leaves used for gas-exchange measurements. Chlorophyll was extracted from one leaf disk (1.23 cm^2) per fresh leaf with 3 ml N,N-Dimethylformamide (Inskeep & Bloom 1985) and stored in the dark at 4°C for 2.5 weeks. Chlorophyll content was analysed with a spectrophotometer in the Netherlands. The leaf N-analysis was carried out for a pooled sample of three to four leaves per tree; these leaves were also used for the gas-exchange measurements. In the Netherlands, leaf N was analysed with either an elemental analyser (Carlo Erba, model 1106, Milano, Italy) or, after extraction with hot water, spectrophotometrically with a segmented flow analyser (Skalar San⁺ System, Breda, the Netherlands).

ESTIMATION OF LIGHT AVAILABILITY

Hemispherical photography was used to estimate the light availability for a tree. A photograph was taken above the centre of each individual, using a camera (Canon Ti-70) with a fish-eye lens (Canon, 7.5 mm f5.6), except for tall juvenile trees (12–21 m) where photographs were taken above and close to the position of the harvested leaves. For small saplings (< 2 m) the camera was mounted on a tripod, whereas for all other trees the camera was fixed in an aluminium leveller and mounted on a telescopic aluminium pole (maximum range 6 m). For each photograph canopy openness was calculated as the percentage of unobstructed sky, weighted for angle of incidence, according to Ter Steege (1994); we used a radiation distribution for a standard overcast sky. Percentage canopy openness was used as an estimation of the light availability per tree.

In order to verify if canopy openness was a good estimator of light availability, we measured the daily PFD above 16 trees, differing in height, of *Dicorynia* and *Vouacapoua*, which were also used in this study. Five gallium-arsenide phosphide photo-diodes (Model G-1118, Hamamatsu Photonics, Hamamatsu, Japan), calibrated against a Li-Cor quantum sensor under full sunlight, were attached to petioles of leaves at the top of the tree crown, and close to the position where the hemispherical photograph was taken. The light sensors were orientated horizontally. They monitored PFD every 5 s, and 1 min means were stored with a Li-Cor 1000 data logger from 07.30 until 17.00 h, over five to eight consecutive days during the period from September to November in 1996 and 1997. Simultaneously, the daily PFD above the forest canopy was measured at a fixed site in the research area using a Li-Cor quantum sensor. Daily integrated PFD was

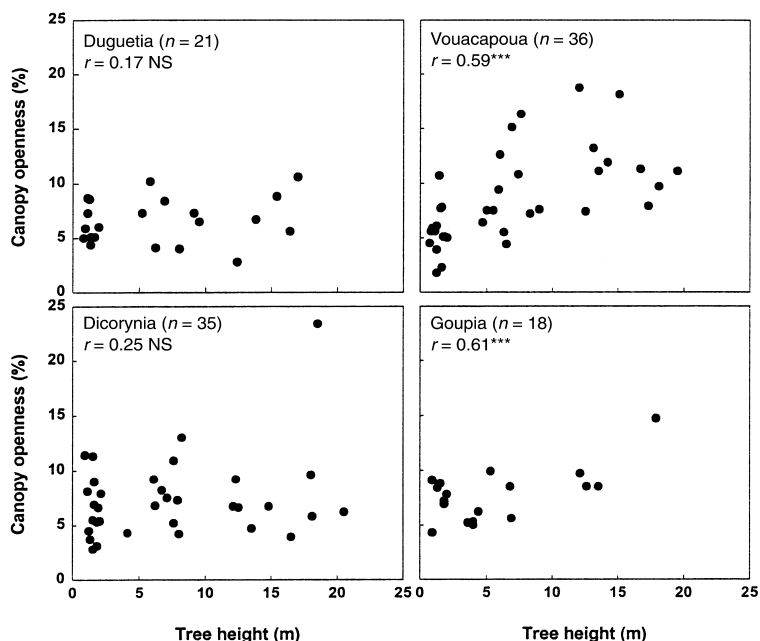


Fig. 1. Light availability, expressed as percentage canopy openness derived from hemispherical photographs, plotted against tree height (m) for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia*. Pearson's correlation coefficient (r), number of individuals (n) and level of significance (NS, $P > 0.05$; ***, $P < 0.001$) are given.

calculated for each sensor and expressed as a percentage of the daily integrated PFD measured above the forest canopy. Above the forest canopy the PFD ranged from 30 to 45 mol m⁻² day⁻¹.

STATISTICAL ANALYSES

The effects of light availability, tree height and species on leaf variables were analysed with an analysis of covariance (ANCOVA), with species as main factor and tree height and percentage canopy openness as covariables. We tested the assumption of homogeneity of regression slopes of leaf variable on both tree height and canopy openness for all four species; if no significant differences were found then the interaction term was removed from the model (Sokal & Rohlf 1995). Within-species scatterplots showed linear relationships between all leaf variables and both tree height and canopy openness, except for Chl/area, as for tree height, no linear relationship was found for trees of *Goupia*. Therefore, Chl/area was excluded from the ANCOVA. The interspecific differences in leaf variables were tested using leaf characteristics for a common tree with an average height and canopy openness; the so-called test for adjusted means. Box plots were used to show the intraspecific variation in leaf variables. For all species pooled, trends of LMA, A_{\max} /area and Chl/area with tree height and canopy openness were analysed by linear regression. Data were log-transformed if variances were not equal among species.

Results

ESTIMATION OF LIGHT AVAILABILITY

A significant positive relationship was found between direct measurements of PFD and percentage canopy openness above 16 trees of *Dicorynia* and *Vouacapoua* ($r^2 = 0.60$, $P < 0.001$). We therefore concluded that, in this study, canopy openness could be used as an estimator for light availability and that the hemispherical photographs sufficiently represent the light environment of the leaves on which gas-exchange measurements were made.

For all species the range of canopy openness was moderately low, as almost 95% of the trees had an openness of less than 15% (Fig. 1). This rather low range of canopy openness resulted from the lack of large gaps in the research area. There was no correlation between canopy openness and tree height for individuals of *Dicorynia* and *Duguetia* (Fig. 1). This was expected because, for similar-sized trees, individuals were selected both in gap sites and under a closed forest. However, trees of *Vouacapoua* and *Goupia* showed a positive correlation between openness and height (Pearson's correlation coefficients of 0.59 and 0.61, respectively). When the canopy openness value of the tallest tree of *Goupia* was excluded, no correlation was found for this species (data not shown). In general, we found a fairly uniform distribution of percentage canopy openness over the different tree heights and species; this is a prerequisite of the data analysis.

VARIATION IN LEAF VARIABLES AMONG SPECIES

Species differed in their expression of leaf variables, and these differences were greatest for LMA, N/area and PNUE_{\max} as indicated by their high F -values (Table 1). Interspecific differences in leaf variables, derived from the test for adjusted means, are shown in Fig. 2. Differences in leaf variables were most pronounced between the pioneer species *Goupia* and the most shade-tolerant species *Duguetia*, whereas leaf traits of *Dicorynia* and *Vouacapoua* tended to be intermediates (Fig. 2). *Duguetia* had the highest LMA, followed in decreasing order by *Vouacapoua*, *Dicorynia*, with *Goupia* having the lowest LMA. Because *Goupia* had the lowest LMA and N/mass this resulted in a much lower N/area and a much higher A_{\max} /mass as compared to the other species. Among the three shade-tolerant species PNUE_{\max} was significantly higher for leaves of *Vouacapoua* than for *Duguetia*, whereas PNUE_{\max} was almost twice as high for *Goupia* than for the other species (Fig. 2). Both *Duguetia* and *Vouacapoua* had a significantly lower Chl/mass than did the two other species. Although A_{\max} /area was similar among the four species (Fig. 2), *Vouacapoua* tended to have a greater plasticity in photosynthetic capacity, which is

Table 1. Results of an one-way ANCOVA with species ($n = 4$) as main factor, and tree height (m) and canopy openness (%) as covariables. The following leaf variables are given: light-saturated rate of photosynthesis per unit leaf area (A_{\max}/area) and per unit leaf dry mass (A_{\max}/mass); nitrogen content per unit leaf area (N/area) and per unit leaf dry mass (N/mass); chlorophyll content per unit leaf dry mass (Chl/mass); leaf mass per unit leaf area (LMA); light-saturated rate of photosynthesis per unit leaf nitrogen (PNUE_{max}) and per unit chlorophyll (A_{\max}/Chl) and stomatal conductance to water vapour (g_s). Units for the variables are given in parentheses. F -values, level of significance (P), intercept, common regression slope of tree height (b1) and canopy openness (b2), coefficient of determination (r^2) and number of individuals (n) per variable are given. A_{\max}/mass , N/mass, PNUE_{max}, A_{\max}/Chl and g_s were log transformed prior to analysis. Significant levels: NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. F -values for intercepts are not shown ($P < 0.001$). Regression model: variable = intercept + (b1 \times tree height) + (b2 \times canopy openness) + species effect + error

Variable	Species		Intercept	Tree height			Canopy openness			Model	
	F	P		F	P	b1	F	P	b2	r^2	n
A_{\max}/area ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	3	*	3.0	31	***	0.11	37	***	0.21	0.55	112
A_{\max}/mass ($\text{nmol g}^{-1} \text{s}^{-1}$)	23	***	1.9	0.3	NS	-0.001	14	***	0.01	0.47	110
N/area (mmol m^{-2})	45	***	67	34	***	1.77	20	***	2.23	0.69	112
N/mass (mmol g^{-1})	19	***	0.2	0.4	NS	-0.001	1	NS	0.002	0.36	112
Chl/mass (mmol mg^{-1})	23	***	13	13	***	-0.14	11	**	-0.22	0.53	105
LMA (g m^{-2})	48	***	42	97	***	1.18	26	***	1.01	0.77	112
PNUE _{max} ($\mu\text{mol mol}^{-1} \text{s}^{-1}$)	40	***	1.7	0.1	NS	0.001	6	*	0.01	0.57	112
A_{\max}/Chl ($\mu\text{mol mmol}^{-1} \text{s}^{-1}$)	6	***	0.8	3	NS	0.004	23	***	0.020	0.38	105
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.2	NS	1.9	3	*	0.005	14	***	0.017	0.23	112

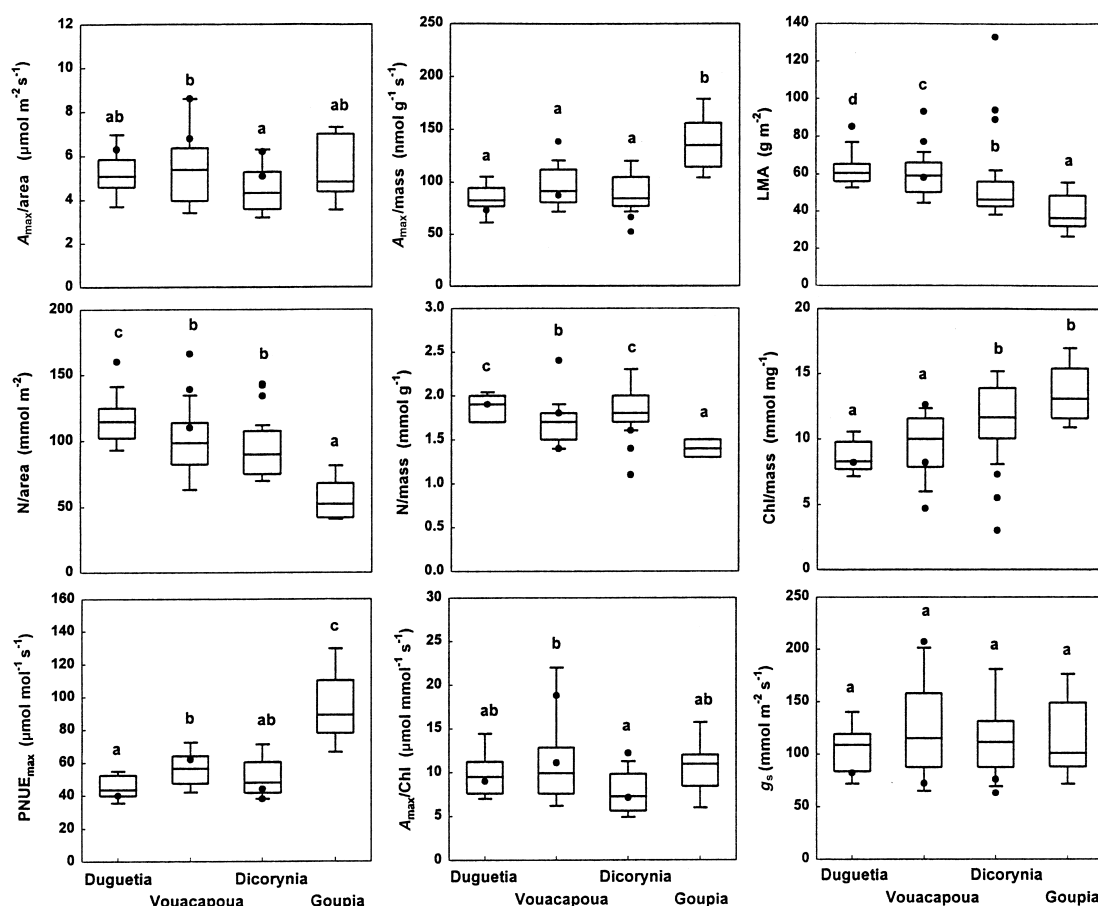


Fig. 2. Box plots of A_{\max}/area , A_{\max}/mass , N/area, N/mass, LMA, Chl/mass, PNUE_{max}, A_{\max}/Chl and g_s for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia* (abbreviations as in Table 1). The upper and lower border of the box are the 75th and 25th percentiles, respectively, the black horizontal line within the box is the median and the error bars are the 10th and 90th percentiles. For each leaf variable species with the same letter were not significantly different ($P > 0.05$). Interspecific differences in leaf variables were analysed using the test for adjusted means. The closed circles represent values for the leaf variables of the large, mature canopy trees of *Duguetia* ($n = 1$), *Vouacapoua* ($n = 3$) and *Dicorynia* ($n = 3$).

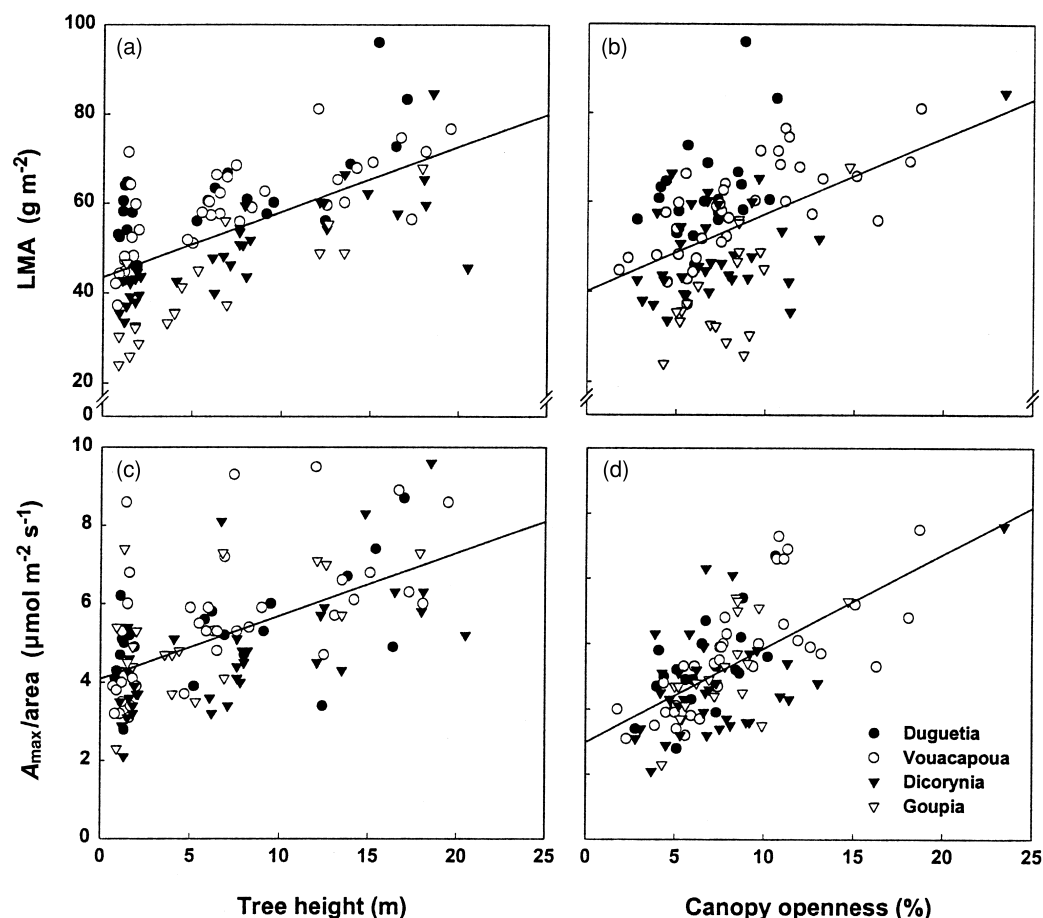


Fig. 3. Leaf mass per unit area (LMA) and light-saturated rate of photosynthesis per unit area (A_{\max}/area) plotted against (A,C) tree height and (B,D) canopy openness for *Duguetia* (●), *Vouacapoua* (○), *Dicorynia* (▼) and *Goupia* (▽). Solid lines represent linear regression for all species pooled. Regression equations: $43.52 + 1.46 \times \text{Height}$ ($r^2 = 0.40$, $P < 0.001$, LMA), $40.12 + 1.72 \times \text{Openness}$ ($r^2 = 0.21$, $P < 0.001$, LMA); $4.08 + 0.16 \times \text{Height}$ ($r^2 = 0.32$, $P < 0.001$, A_{\max}/area); $2.98 + 0.29 \times \text{Openness}$ ($r^2 = 0.39$, $P < 0.001$, A_{\max}/area).

particularly evident in small saplings having a great variation in A_{\max}/area (Fig. 3). A_{\max}/Chl was similar among species, except for *Dicorynia* which had a significantly lower value than had *Vouacapoua*. Stomatal conductance to water vapour (g_s) did not differ among the four species.

COMPARISON OF TREE HEIGHT AND LIGHT AVAILABILITY EFFECTS

The measured leaf variables LMA and A_{\max}/area were used to illustrate the single effect of tree height and canopy openness on leaf traits (Fig. 3); N/mass was not included because it was unaffected by height and openness (Table 1). For all species, both LMA and A_{\max}/area increased significantly with increasing height and openness (Fig. 3).

Table 1 summarizes the results of the ANCOVA, which discriminates between tree height and light availability effects on leaf variables. For most of the dependent variables the explained variation of the model was moderate to fairly high (range 23–77%). For A_{\max}/mass , N/mass , A_{\max}/Chl and g_s this variation was less than 50%.

A_{\max}/area , N/area and LMA were strongly positively affected by both tree height and canopy openness as indicated by their high F -values. A_{\max} and N on a mass basis, however, were constant across different heights and openness, except for A_{\max}/mass , which slightly increased with increasing canopy openness. Furthermore, PNUE_{\max} and A_{\max}/Chl were unaffected by tree height, whereas N/mass was unaffected by canopy openness. Chl/mass was the only variable which decreased with both increasing height and openness as indicated by the sign of the regression slopes (b1 and b2, respectively). Stomatal conductance moderately increased with increasing canopy openness and, to a lesser extent, with increasing tree height.

The variable Chl/area was excluded from the ANCOVA (see Statistical Analyses). Because LMA and Chl/mass showed opposite patterns Chl/area was independent of tree height in all species, except for leaves of *Goupia*, which showed a positive logarithmic relationship (Fig. 4). This non-linear relationship for *Goupia* was the result of a much lower leaf chlorophyll content found in small saplings compared to the content in the large trees. Chl/area was independent of canopy openness for all species (data not shown).

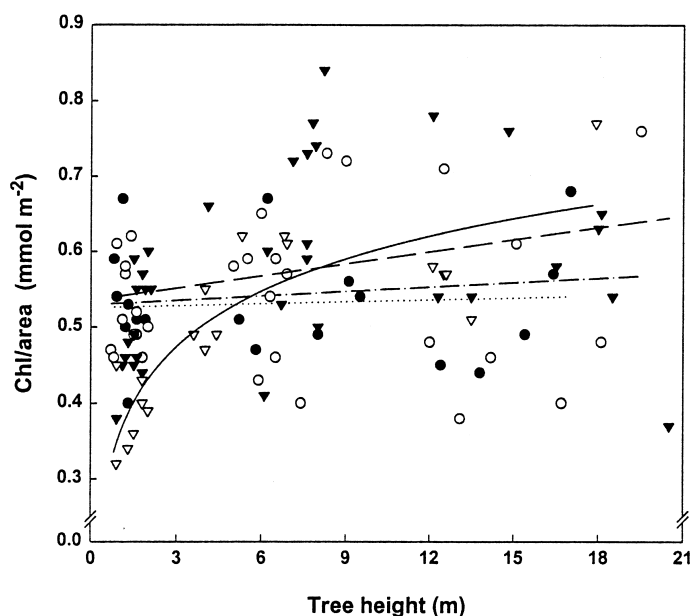


Fig. 4. Chlorophyll content per unit area (Chl/area) plotted against tree height (m) for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia*. Species symbols are the same as in Fig. 3. Mean values of Chl/area for three to four leaves per tree are shown. The solid line and broken lines represent non-linear and linear regression, respectively. Regression equations: $0.53 + 8.85 \times 10^{-4} \times \text{Height}$ ($r^2 = 0.005$, NS, Chl/area *Duguetia*, dotted line); $0.53 + 1.89 \times 10^{-3} \times \text{Height}$ ($r^2 = 0.012$, NS, Chl/area *Vouacapoua*, dash-dot line); $0.54 + 5.39 \times 10^{-3} \times \text{Height}$ ($r^2 = 0.074$, NS, Chl/area *Dicorynia*, long-dash line); $0.36 + 0.24 \times \log(\text{Height})$ ($r^2 = 0.72$, $P < 0.001$, Chl/area *Goupia*, solid line).

Leaf characteristics of the additional mature canopy trees of *Duguetia*, *Vouacapoua* and *Dicorynia* were added in the box plots as closed circles (Fig. 2). It seemed that traits changed when trees reach their maximum height. In all three species, LMA and N/area tended to be higher for canopy trees than for the smaller individuals. Mass-based A_{\max} and leaf N tended to be lower in canopy trees of *Dicorynia*, resulting from a higher LMA in these large individuals (Fig. 2).

Discussion

DIFFERENCES IN LEAF VARIABLES AMONG SPECIES

Considerable variation in leaf variables existed among the species, especially between the pioneer *Goupia* and the three shade-tolerant species (Fig. 2). Rather low rates of A_{\max} /area were found for light growing individuals of *Goupia*, whereas higher rates were expected for a pioneer species. Higher rates of A_{\max} /area for *Goupia* have been reported, however, for individuals growing in much brighter light conditions (Huc, Ferhi & Guehl 1994; Raaimakers *et al.* 1995; Reich, Ellsworth & Uhl 1995). Moreover, Huc *et al.* (1994) found a higher A_{\max} /area ($8.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) for trees of *Dicorynia* growing in artificial stands than for similar-sized trees in our study. These findings suggest that, in our study, light availability might not be enough for *Goupia* and *Dicorynia* to express their full photosynthetic capacity.

LMA was lowest for the pioneer species *Goupia* and increased with increasing shade tolerance of the other species (Fig. 2), which is consistent with other studies (e.g. Kitajima 1994; Reich *et al.* 1995). However, Popma *et al.* (1992) investigated 68 tropical tree species and found no differences in LMA between obligate gap species and gap-independent species. In general, interspecific variation in LMA is caused by differences in leaf anatomy, morphology (e.g. leaf hairs, thorns) and/or chemical composition (e.g. starch, secondary compounds) (Lambers & Poorter 1992; Van Arendonk & Poorter 1994). A higher LMA, typically found for shade-tolerant species, is often owing to extra investment in compounds, e.g. lignin, or in support tissue which reduce the palatability of leaves (Lambers & Poorter 1992).

The greater efficient use of nitrogen in terms of photosynthetic capacity, i.e. PNUE_{\max} , in leaves of *Goupia* was probably owing to its very low N/mass (Fig. 2). In general, high values of mass-based leaf N are observed in pioneer species because they usually occupy resource-rich habitats such as large gaps. Reich *et al.* (1995), however, found that only the earliest successional species, e.g. *Cecropia*, had a high N/mass, and that other species along the successional gradient, such as *Goupia*, had low to moderate N/mass. The low PNUE_{\max} found for the three shade-tolerant species, despite their higher N/mass, may have resulted from a large investment of N in leaf components that are not associated with photosynthesis (Lambers & Poorter 1992; Pons, Van der Werf & Lambers 1994). It may also be a consequence of a suboptimal distribution of N within the photosynthetic apparatus (Pons *et al.* 1994).

LEAF VARIABLES AS DEPENDENT ON TREE HEIGHT AND LIGHT AVAILABILITY

Both PNUE_{\max} and A_{\max}/mass only marginally increased with increasing light availability, with the exception of changes in chlorophyll content (Table 1), suggesting that the species were hardly capable of physiological light acclimation. Mass-based chlorophyll content increased with both decreasing tree height and canopy openness (Table 1). The increase of Chl/mass with decreasing light often results from enhanced investments of resources, especially nitrogen, in chlorophyll (Chazdon 1992; Poorter, Oberbauer & Clark 1995; Niinemets 1997b). Furthermore, for all species, A_{\max}/Chl was only affected by canopy openness (Table 1). The ratio of photosynthetic capacity to chlorophyll content is an important aspect of shade adjustment in leaves. The higher A_{\max}/Chl at high light availability indicates that in high light conditions species were able to invest more of their resources in carbon-assimilation, rather than in light-harvesting which, in turn, should be more advantageous in low light conditions (Seeman *et al.* 1987; Evans 1989). Moreover, Chl/mass also decreased with

increasing tree height, which may be a consequence of an increased investment in cell-wall material (e.g. Niinemets 1997a). LMA and Chl/mass showed opposite patterns, resulting in Chl/area to be unaffected by tree height and light availability. This suggests that, within species, leaves of small and/or shade-growing individuals could capture a similar amount of light as do large and/or bright-growing individuals (Poorter *et al.* 1995; Niinemets 1997b). Small individuals of *Goupia*, however, showed a contrasting pattern, as their leaves had a much lower Chl/area compared to those of saplings of the other three species (Fig. 4).

Significant positive area-based A_{\max} -N relationships ($P < 0.001$), with similar slopes (about 0.052), were found for leaves of the four species (data not shown). When expressed on a mass basis regressions were only significant for *Duguetia* and *Vouacapoua*; however, these slopes were almost horizontally orientated (data not shown). Within species, a stronger A_{\max} -N relationship on an area than on a mass basis is caused by a larger variation in LMA than in N/mass across different environmental gradients, and by a strong correlation between LMA and N/area (Ellsworth & Reich 1993; Reich *et al.* 1994; Table 1). In our study the variation of A_{\max} /area and N/area could also be traced back to variation in LMA, as this variable was strongly correlated with both tree height and canopy openness (Table 1). Other studies also found positive relationships between LMA and light availability (Walters, Kruger & Reich 1993b; Kitajima 1994; Raaimakers *et al.* 1995) and between LMA and tree height, independently of light (Niinemets & Kull 1995; Poorter *et al.* 1995; Niinemets 1997a), suggesting a consistent trend within tree species.

An increase in LMA with increasing light availability often is the result of the formation of a thicker mesophyll tissue, especially the development of elongated or more layers of palisade parenchyma (Bongers & Popma 1988; Chazdon & Kaufmann 1993). In addition, leaves with thicker mesophyll typically have more chloroplasts per unit area, resulting in a higher A_{\max} /area (Hoflacher & Bauer 1982; Turnbull 1991; Chazdon & Kaufmann 1993). LMA, however, also increased with increasing tree height, independently of light, suggesting the developmental phase constrained plasticity in LMA; that is, small trees were prevented from developing heavy and thick leaves like in large trees (e.g. Hoflacher & Bauer 1982; Knapp & Fahnestock 1990).

When trees grow taller they often experience an increased water limitation, generally owing to changed environmental conditions, such as increasing vapour pressure deficits and wind speed with height, and an increased hydraulic and gravitational resistance to water flow in the xylem, as water has to travel a longer distance from the soil to a leaf (Tyree & Ewers 1991; Friend 1993; Ryan & Yoder 1997). For leaves in a tree crown to be supplied with water they have to reduce their water potential, e.g. by increasing the

amounts of solutes, to below that in the soil to overcome the effects of gravity and hydraulic resistance in the trunk and branches. In *Picea abies*, Niinemets (1997a) showed that the higher LMA of needles in tall trees compared to those in small trees was owing to larger investments of structural (e.g. cell-wall thickening) and non-structural (e.g. starch) carbon per needle dry mass; in that study an increased water stress in taller trees was given as an explanation for the greater LMA.

An adverse effect of higher carbon investments in leaves of tall trees, however, is the dilution of nitrogen and/or other compounds involving photosynthesis, leading to a lower A_{\max} /mass. In light-grown trees of *Pinus aristata*, Schoettle (1994) found that needles of tall trees had a higher LMA, a lower N/mass and a lower area and mass based light-saturated photosynthesis than those of small trees. Our results did not support this phenomenon, as N/mass and A_{\max} /mass were unaffected by tree height (Table 1), indicating that allocation of nitrogen to the leaves was similar between tall and small trees.

In light-grown trees of two *Pinus* species, Yoder *et al.* (1994) found from diurnal light-saturated rates of photosynthesis that tall trees had a much lower daily mean A_{\max} /area than small trees, despite similar N/mass and peak net photosynthesis rate between tall and small trees. In that study, the authors suggested that the reduced net photosynthesis rate in tall trees was related to the earlier closure of stomata in the day resulting from an increased hydraulic resistance in these tall trees. Limiting transpirational water loss through stomatal closure in tall trees, which have an increased hydraulic resistance, is a necessity to prevent cavitation and embolism in the xylem, i.e. to avoid air entering in the xylem vessels causing a decrease in water transport (e.g. Tyree & Sperry 1988; Sperry, Alder & Eastlack 1993). Fredericksen *et al.* (1996) also suggested that the effect of increased hydraulic resistance could explain the reduced mean diurnal gas-exchange rates in canopy trees of *Prunus serotina* compared to saplings and seedlings. In our study, gas-exchange rates were measured between 09.00 and 12.00 h, as we assumed that environmental conditions in the afternoon might reduce the photosynthetic potential. For trees up to 21 m in height, stomatal conductance was almost unaffected by tree height, whereas A_{\max} /area increased with tree height (Table 1). In addition, leaves of the large, mature canopy trees tended to have a higher A_{\max} /area and LMA than those of the smaller trees, despite their somewhat lower stomatal conductance (Fig. 2). Apparently, photosynthetic capacity was uniformly higher in taller trees than in smaller ones for all species. However, midday depression of gas-exchange rates, owing to increasing air temperature and light, and decreasing humidity along a vertical gradient in the forest, could diminish the observed difference in photosynthesis rates between tall and small trees.

In summary, the results of this study show that tree height and canopy openness had independent effects on the physiological and structural, i.e. LMA, leaf characteristics of all the four species. Moreover, the direction and magnitude of leaf responses to height and openness were similar among the species. Overall, leaves of taller trees had higher LMA, N/area and A_{\max} /area, and a lower Chl/mass than those of small trees, whereas canopy openness had an effect on all leaf variables, except on N/mass. LMA seemed to be a key variable, as the variation in photosynthesis rates, nitrogen and chlorophyll content could be traced back to variation in LMA. Our results show that the developmental phase of trees constrained plasticity in leaf variables, especially LMA. Beside environmental effects, differences in leaf variables between different sized trees might also be explained by changing water relations within a tree caused by increased hydraulic limitations in taller trees.

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