Plasticity of Leaf Anatomy of Two Rain Forest Shrubs in Relation to Photosynthetic Light Acclimation

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Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation

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Summary

1. We examined changes in leaf structure and photosynthetic characteristics in two congeneric shrub species growing across natural gap transects in a tropical rain forest.

2. *Piper sancti-felicis*, a shrub characteristic of disturbed clearings and large gaps, exhibits a high capacity to alter photosynthetic capacity in response to spatial variation in light availability compared to the shade-tolerant species *Piper arieianum*.

3. Rooted cuttings were placed in pots along transects in each of two recent, naturally formed canopy gaps in old-growth forest. After 13 months of growth, a subsample of 14 plants per species (seven pairs per gap) was chosen for detailed analyses of leaf light environment and photosynthetic light responses. For 10 of these pairs, leaf tissue was sampled for subsequent anatomical characteristics.

4. Photosynthetic capacity per unit leaf area of *Piper sancti-felicis* showed a strong dependence on all measures of light availability, whereas *Piper arieianum* showed a weak or insignificant light acclimation response. Photosynthetic capacity and dark respiration per unit leaf mass increased significantly with light availability only for *Piper sancti-felicis*. Both species exhibited significant light-dependent variation in leaf mass per area and thickness of mesophyll layers, although only *Piper arieianum* showed light-related variation in total leaf thickness. Photosynthetic capacity of *Piper arieianum* was poorly correlated with leaf anatomical features, whereas leaves of *Piper sancti-felicis* showed significant, positive correlations between photosynthetic capacity and mesophyll tissue thickness. The limited potential for light acclimation in the shade species *Piper arieianum* reflects an inability to adjust metabolic processes at the cellular level, despite extensive anatomical adjustments. Light acclimation in the high-light species *Piper sancti-felicis*, in contrast, is effected by biochemical adjustments at the cellular level as well as by changes in leaf anatomy.

Key-words: Canopy gaps, leaf structure, *Piper*, tropical rain forest

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Introduction

Photosynthetic acclimation to changing light conditions is usually associated with anatomical as well as physiological changes within leaves (Boardman 1977; Björkman 1981). Within a species, leaves developed under high-light conditions tend to be thicker, with increased mesophyll tissue per unit area and higher leaf mass per area (Nobel, Zaragoza & Smith 1975; Chabot & Chabot 1977; Patterson, Duke & Hoagland 1978; Lichtenthaler *et al.* 1981; Fetcher, Strain & Oberbauer 1983). Changes in photosynthetic capacity per unit leaf area may result from physiological and biochemical adjustments at the cellular level and/or changes in anatomy at the leaf level. In assessing the extent and nature of physiological

plasticity with respect to variation in light conditions, both anatomical and physiological adjustments must be considered (Nobel, Zaragoza & Smith 1975). For the herbaceous shade-tolerant species *Alocasia macrorrhiza*, *Fragaria virginiana* and *Plectranthus parviflorus*, photosynthetic light acclimation occurs entirely through anatomical changes in leaf thickness that increase the volume of photosynthetic tissue per unit leaf area (Nobel *et al.* 1975; Chabot, Jurik & Chabot 1979; Sims & Pearcy 1992). Seedlings of early-successional rain forest trees, on the other hand, exhibit increases in photosynthetic capacity per unit volume of mesophyll tissue during light acclimation (Turnbull 1991). These findings suggest that light acclimation may be mediated by two

distinct mechanisms — anatomically based acclimation and cell-based acclimation. The former mechanism involves primarily structural changes, whereas the latter primarily involves physiological and biochemical changes within individual cells and chloroplasts.

In this study, we examine changes in leaf structure and photosynthetic characteristics in two species of rain forest shrubs growing across natural gap transects. In natural forest habitats, the light acclimation response differs quantitatively and qualitatively between these species (Chazdon 1992). *Piper sancti-felicis* Trel., a shrub characteristic of disturbed clearings and large gaps, exhibits a high capacity to alter photosynthetic capacity in response to spatial variation in light availability compared to *Piper arieianum* C. DC. (Chazdon 1992). Unlike *Piper sancti-felicis*, *Piper arieianum* fails to increase its photosynthetic nitrogen-use efficiency under conditions of a high-light availability. Yet chlorophyll *a:b* ratios are far more responsive to light availability in *Piper arieianum* (Chazdon 1992).

The objective of this study is to assess the role of plasticity in leaf anatomy in the light acclimation responses of these two species. First, we examine the extent to which photosynthetic and anatomical characteristics of leaves vary with changes in light availability across natural gap transects. We then examine directly relationships between anatomical features and photosynthetic light responses. In this way, we hope to elucidate the relative importance of acclimatory responses at the cellular *vs* leaf level in these two contrasting rain forest species.

Materials and methods

This study was conducted at La Selva Biological Station in the Atlantic lowlands of Costa Rica (10° 26'N, 83° 59'W). This tropical wet forest receives an average of 4000 mm of rain annually. Rooted cuttings of the two *Piper* species were prepared from branch fragments placed in sand under a mister as described by Chazdon (1992). After 6 months, rooted cuttings were potted in 4·5-litre plastic pots in a homogenous mixture of 1:1 sand and sifted alluvial soil and fertilized monthly with 50 ml of a complete nutrient solution. Pots were then placed along transects in each of two recent, naturally formed canopy gaps in old-growth forest — a small gap created by a branch fall (projected area 54 m^2) and a large gap created by a tree-fall (projected area 300 m^2). Transects were laid out facing east–west, and were 15 m and 18 m long in the small and large gaps, respectively. A total of 20 pots per species was located in each gap, spaced 0·5–1·0 m apart along the transect, in pairs (1 plant per species). A 3-m strip of vegetation centred along the transects was routinely cleared to facilitate location of pots and to remove shading vegetation. Pots were fertilized once a month with 50 ml of complete

fertilizer solution and were watered as needed during dry spells lasting more than 1 week.

After 13 months of growth, a subsample of 14 plants per species (seven pairs per gap) was chosen for detailed analyses of leaf light environment, photosynthetic light responses, leaf anatomy and related leaf characteristics. Pairs of plants were sampled from microsites along the gap edges as well as the gap interior to ensure a broad range of light conditions. Photon flux density (PFD) at the leaf surface was measured using leaf-mounted photodiodes monitored at 5-s intervals for several days prior to photosynthesis measurements as described by Chazdon (1992). Light conditions were monitored simultaneously in each of the two gaps. Due to equipment problems caused by lightning and stormy weather, only 1 full day of PFD measurements was collected for both gaps on a relatively sunny day. Hemispherical photographs were also used to describe light availability for each plant using the technique of Chazdon & Field (1987b). These photographs were used to compute weighted canopy openness (a measure of diffuse site factor), estimated daily PFD corresponding to the same day as measured PFD and minutes of direct radiation (direct site factor) for this day. In addition, averages of mean daily PFD and daily minutes of direct radiation were calculated for the 10-day period prior to photosynthetic measurements. Yearly mean daily PFD and daily minutes of direct radiation were computed as described by Chazdon & Field (1987b).

Photosynthetic light–response curves were obtained using a portable photosynthesis system (Li-Cor model 6200; Lincoln, Nebraska, USA) and a generator-powered light source (Solidex, 150 W Halogen lamp) with a 45° cold mirror to reduce cuvette and leaf temperatures. Neutral-density wire screens were used to control light levels at the leaf surface. Field measurements were made on potted plants beneath a tent canopy set up adjacent to the transects to protect equipment from rain and to control light levels. Measurements of photosynthetic capacity were made on the same leaf used for PFD measurements and canopy photographs; in fact, the same region of the leaf was used. Plants were brought under the canopy each morning to avoid potentially injurious effects of high-light exposure prior to photosynthetic measurements. To restrict variation in CO_2 concentration within and among days, an external air supply was created by pumping air from a height of 1–2 m in the forest understorey into a large waterbed mattress. Photosynthesis was measured following stepwise increments in light level to ensure saturation while avoiding photoinhibition. During measurements of photosynthetic capacity, chamber CO_2 concentration was maintained within the range of 344–366 p.p.m. (mean of 356 p.p.m.), leaf temperatures ranged from 27·5 to 34·7°C (mean of 30·5°C), and relative humidity ranged from 57 to

91% (mean of 79%). Vapour pressure within the chamber averaged 3·2 MPa ranging from 2·45 to 3·69 MPa. The species did not differ significantly with respect to any of these measurement conditions ($P > 0.05$). Leaves were equilibrated in darkness for at least 20 min before measuring dark respiration. Apparent quantum yields were calculated as the slope of the linear regression of photosynthesis as a function of incident PFD from 0 to 40 μmol m^{-2} s^{-1}, based on three to four data points.

Following photosynthetic measurements, samples of leaf tissue in the vicinity of the light sensor were removed from each leaf for measurements of leaf mass per area, nitrogen content and chlorophyll content. Per cent nitrogen (dry weight basis) was analysed for approximately 5 mg of ground leaf tissue using a C/N analyser. Chlorophyll was extracted using the technique of Moran & Porath (1980). Chlorophyll a and b concentrations were calculated according to the equations developed by Inskeep & Bloom (1985).

For a sample of 10 plants per species (five pairs per gap), small strips of leaf tissue approximately 2 × 15 mm were removed parallel and close to the mid-rib and fixed in 3% glutaraldehyde in 0·2 M sodium cacodylate buffer. Secondary fixation was performed using 1% osmium tetroxide. Following fixation, sections were gradually dehydrated in ethanol and embedded in Epoxy Resin 812. Thick sections (1 μm) were cut using a LKB Ultratome III (Bromma, Sweden) and stained with 0·5% toluidine blue and 0·5% sodium carbonate. Total leaf thickness and thickness of individual tissue layers were quantified using an image analysis program (CUE-4, Olympus Corp., Cherry Hill, New Jersey, USA) on a Compaq Deskpro 286. The video image

was acquired using a video camera attached to a Zeiss microscope. For each of six randomly chosen sections per leaf, five sets of measurements were taken that were equally spaced across the viewing screen. The thickness of the non-uniform epidermal layers was determined by measuring the middle of a cell, approximately the widest area, and the conjunctive region of two neighbouring cells which was generally the thinnest region. Six consecutive cells were used in each of six sections. Thickness averages were computed using an even number of both measurements.

Sections were divided into the following cell layers: upper epidermis, palisade mesophyll, spongy mesophyll, hypodermis and lower epidermis. Airspace was computed as a per cent of the total tissue cross-sectional area. Chloroplast density of the mesophyll tissues was determined by using a threshold level of brightness and contrast to darken the chloroplasts within all mesophyll cell layers. Chloroplast density was then computed as the per cent of total mesophyll cross-sectional area.

Results

Across both transects, the species did not differ significantly with respect to mean photosynthetic capacity per unit area ($P = 0.065$), but *Piper sancti-felicis* showed significantly greater mean photosynthetic capacity per leaf mass and per leaf nitrogen content compared to *Piper arieianum*, as well as greater apparent quantum yield ($P < 0.02$; Table 1). The species did not differ significantly in mean leaf anatomical characteristics, except for chloroplast density, which was marginally significantly greater in *Piper sancti-felicis* ($P = 0.051$; Table 1).

Table 1. Photosynthetic and anatomical characteristics of leaves of two rain forest shrubs growing across gap transects. Photosynthesis data are means ± SE for 14 plants per species, whereas anatomical data are means ± SE for a subset of 10 plants per species

Characteristic	*Piper arieianum*	*Piper sancti-felicis*	P
A_{max} area^{-1} (μmol CO$_2$ m^{-2} s^{-1})	3·75 ± 0·26	5·57 ± 0·90	0·065
A_{max} mass^{-1} (nmol CO$_2$ g^{-1} s^{-1})	119·81 ± 9·26	173·27 ± 17·62	0·012
A_{max} Chl^{-1} (μmol CO$_2$ nmol^{-1} Chl s^{-1})	9·63 ± 0·71	12·74 ± 6·37	0·105
A_{max} N^{-1} (mol CO$_2$ mol^{-1} N s^{-1})	49·91 ± 2·80	72·58 ± 8·73	0·020
Dark respiration (μmol CO$_2$ m^{-2} s^{-1})	−0·19 ± 0·02	−0·24 ± 0·03	0·192
Apparent quantum yield (mol CO$_2$ photon^{-1})	0·03 ± 0·00	0·04 ± 0·00	0·002
Chl area^{-1} (μmol Chl m^{-2})	401·27 ± 23·93	418·81 ± 16·46	0·551
Chl mass^{-1} (μmol Chl g^{-1})	13·26 ± 1·17	14·57 ± 0·73	0·350
Chlorophyll a:b ratio (mol mol^{-1})	2·10 ± 0·16	2·17 ± 0·16	0·739
N area^{-1} (mmol N m^{-2})	75·55 ± 4·05	72·20 ± 3·47	0·536
N mass^{-1} (mmol N g^{-1})	2·42 ± 0·15	2·47 ± 0·82	0·774
Leaf mass area^{-1} (g m^{-2})	32·60 ± 2·32	30·04 ±2·25	0·435
Leaf thickness (μm)	142·04 ± 14·65	125·72 ± 8·04	0·342
Palisade mesophyll (μm)	26·82 ± 3·46	24·08 ± 1·79	0·491
Spongy mesophyll (μm)	36·87 ± 5·82	24·57 ± 2·83	0·073
Upper epidermis (μm)	27·04 ± 4·84	28·27 ± 2·47	0·824
Lower epidermis (μm)	12·65 ± 0·68	13·77 ± 1·14	0·411
Hypodermis (μm)	39·01 ± 2·07	29·60 ± 4·88	0·093
Chloroplast density (% mesophyll area)	18·18 ± 3·26	25·27 ± 0·93	0·051
Airspace (% cross-sectional area)	21·51 ± 2·97	16·88 ± 2·38	0·239

Photosynthetic capacity per unit leaf area (A_{max} area^{-1}) of *Piper sancti-felicis* showed a strong dependence on all measures of light availability, whereas *Piper arieianum* showed a weak response or no significant acclimation, depending on the light measurement used (Fig. 1A; Table 2). Moreover, the slopes of these regression equations were significantly higher for *Piper sancti-felicis* in all but one case (Table 2). The species also differed dramatically in their plasticity of photosynthetic capacity per unit leaf mass across the gap transects (Fig. 1B). In this case, *Piper arieianum* showed no significant regression ($P = 0.524$), whereas *Piper sancti-felicis* leaves exhibited a strong relationship, with light availability explaining 78.6% of the variation in A_{max} mass^{-1} ($P < 0.001$).

Because chamber conditions were not precisely controlled during photosynthesis measurements, we accounted for the influence of chamber vapour pressure on light acclimation responses by using vapour pressure as a second independent variable in regression analyses of A_{max} *vs* canopy openness. This analysis showed that the effect of chamber vapour pressure on A_{max} was highly significant for *Piper arieianum* ($P < 0.000$), but the effect of canopy openness remained insignificant ($P = 0.063$). In contrast, chamber vapour pressure had no significant effect on A_{max} for *Piper sancti-felicis*, whereas canopy openness had a highly significant effect ($P < 0.000$).

Rates of daytime dark respiration (area basis) increased significantly with light availability in both species, although regressions for *Piper sancti-felicis* were more highly significant (Table 3). Except for the two measures of yearly diffuse and direct site factors, slopes of the regression lines did not differ between the species (Table 3). Daytime dark respiration per unit leaf biomass did not vary significantly with any measures of light availability for *Piper arieianum*. For *Piper sancti-felicis*, however, the rate of dark respiration per unit mass increased significantly with light availability ($P < 0.03$; Fig. 2). Apparent quantum
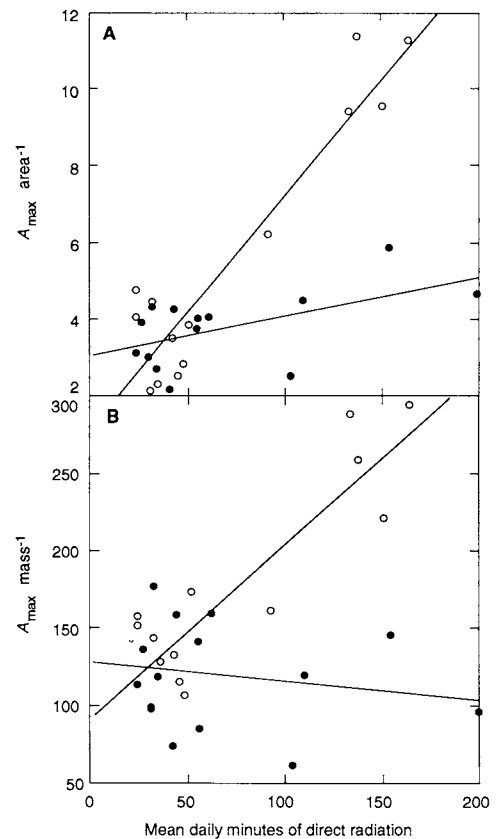

Fig. 1. Photosynthetic capacity on an area basis (μmol CO_2 m^{-2} s^{-1}) and on a dry mass basis (nmol CO_2 g^{-1} s^{-1}) as a function of mean minutes of direct radiation per day for a 10-day period prior to photosynthesis measurements predicted from hemispherical photographs: *Piper sancti-felicis* (○); *Piper arieianum* (●). Statistics for area-based regressions are given in Table 2. For mass-based regression, $r^2 = 0.786$ for *Piper sancti-felicis* ($P < 0.001$) and 0.035 for *Piper arieianum* ($P = 0.524$).

yields of *Piper arieianum* remained constant across the gap transects, whereas leaves of *Piper sancti-felicis* showed a highly significant dependence of apparent quantum yield on all measures of light availability (Table 4).

Light compensation points did not vary significantly with light availability in either species

Table 2. Regression statistics describing relationships between photosynthetic capacity (μmol CO_2 m^{-2} s^{-1}) and measures of the light environment at the leaf. $n = 14$ for all regressions, except for mean daily PFD for *Piper sancti-felicis*, where $n = 12$. Different letters indicate that slopes are significantly different between species at $P < 0.05$

	Piper arieianum		*Piper sancti-felicis*	
Independent variable	Slope	r^2	Slope	r^2
Mean daily PFD (mol m^{-2} day^{-1})	0.106[a]	0.104	0.624[a]	0.728***
Weighted canopy openness (%)	0.082[a]	0.188	0.637[b]	0.907***
Estimated daily PFD (mol m^{-2} day^{-1})	0.140[a]	0.323*	0.754[b]	0.866***
Minutes of direct radiation	0.012[a]	0.412*	0.066[b]	0.821***
10-day mean daily PFD (mol m^{-2} day^{-1})	0.118[a]	0.240	0.712[b]	0.885***
10-day mean minutes of direct radiation	0.010[a]	0.308*	0.061[b]	0.876***
Yearly mean estimated PFD (mol m^{-2} day^{-1})	0.125[a]	0.163	1.002[b]	0.905***
Yearly mean minutes of direct radiation	0.012[a]	0.178	0.086[b]	0.857***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$(P > 0.05;$ data not shown). Neither did the species differ significantly in mean light compensation points across the gap transects $(x = 6.00$ for *Piper arieianum* and 5.56 for *Piper sancti-felicis*).

PLASTICITY OF LEAF ANATOMY

Leaves of *Piper arieianum* exhibited significant light-dependent variation in total leaf thickness, thickness of the upper epidermis, and thickness of palisade and spongy mesophyll layers along the gap transects (Table 5, Fig. 3A,C). The hypodermis and lower epidermis showed no significant variation due to light availability. In the gap microsites with the highest light levels, leaves of *Piper arieianum* developed a second cell layer in the upper epidermis (Fig. 3C). Photographic measures of light availability proved to be more useful in predicting patterns of anatomical variation than direct sensor measurements for a single day. Of the photographic measures, estimates for a single day and for the 10-day period prior to tissue sampling were better predictors of leaf anatomy than yearly estimates of light availability.

Total leaf thickness of *Piper sancti-felicis* did not vary significantly with light availability across the transects (Table 6, Fig. 3B,D). In this species, both palisade and spongy mesophyll tissues increased in thickness with increases in light availability, while the
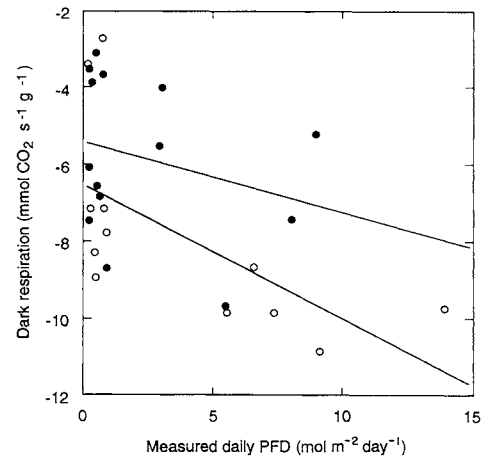

Fig. 2. Rate of dark respiration per unit leaf dry mass as a function of measured daily PFD for *Piper sancti-felicis* (O) and *Piper arieianum* (●) across natural gap transects. For *Piper sancti-felicis*, $r^2 = 0.395$ $(P < 0.03)$; for *Piper arieianum*, $r^2 = 0.073$ $(P = 0.351)$.

thickness of the lower epidermis decreased, maintaining a constant overall leaf thickness (Table 6). The thickness of the upper epidermis and the hypodermal layer remained constant. For all but the yearly estimates of light availability, coefficients of determination for thickness of mesophyll tissues were lower than those observed for *Piper arieianum* (Tables 5 and 6).

Table 3. Regression statistics describing relationships between dark respiration (μmol CO_2 m^{-2} s^{-1}) and measures of the light environment at the leaf. Sample sizes are the same as for Table 2. Different letters indicate that slopes are significantly different between species at $P < 0.05$

	Piper arieianum		*Piper sancti-felicis*	
Independent variable	Slope	r^2	Slope	r^2
Mean daily PFD (mol m^{-2} day^{-1})	−0.018[a]	0.443**	−0.023[a]	0.694***
Weighted canopy openness (%)	−0.011[a]	0.466**	−0.021[a]	0.788***
Estimated daily PFD (mol m^{-2} day^{-1})	−0.015[a]	0.556**	−0.024[a]	0.701***
Minutes of direct radiation	−0.001[a]	0.483**	−0.002[a]	0.623***
10-day mean estimated PFD (mol m^{-2} day^{-1})	−0.015[a]	0.528**	−0.023[a]	0.722***
10-day mean minutes of direct radiation	−0.001[a]	0.461**	−0.063[a]	0.669***
Yearly mean estimated PFD (mol m^{-2} day^{-1})	−0.018[a]	0.479**	−0.034[b]	0.807***
Yearly mean minutes of direct radiation	−0.001[a]	0.322*	−0.003[b]	0.767***

* $P < 0.05;$ ** $P < 0.01;$ *** $P < 0.001.$

Table 4. Regression statistics describing relationships between apparent quantum yield (mol CO_2 mol^{-1} photon^{-1}) and measures of the light environment at the leaf. Sample sizes are the same as for Table 2. Different letters indicate that slopes are significantly different between species at $P < 0.05$

	Piper arieianum		*Piper sancti-felicis*	
Independent variable	Slope	r^2	Slope	r^2
Mean daily PFD (mol m^{-2} day^{-1})	0.001[a]	0.111	0.003[a]	0.798***
Weighted canopy openness (%)	0.001[a]	0.165	0.003[b]	0.821***
Estimated daily PFD (mol m^{-2} day^{-1})	0.001[a]	0.253	0.003[a]	0.848***
Minutes of direct radiation	0.000[a]	0.214	0.000[b]	0.775***
10-day mean estimated PFD (mol m^{-2} day^{-1})	0.001[a]	0.249	0.003[a]	0.864***
10-day mean minutes of direct radiation/100	0.006[a]	0.237	0.027[a]	0.805***
Yearly mean estimated PFD (mol m^{-2} day^{-1})	0.001[a]	0.020	0.004[b]	0.857***
Yearly mean minutes of direct radiation	0.009[a]	0.199	0.040[b]	0.892***

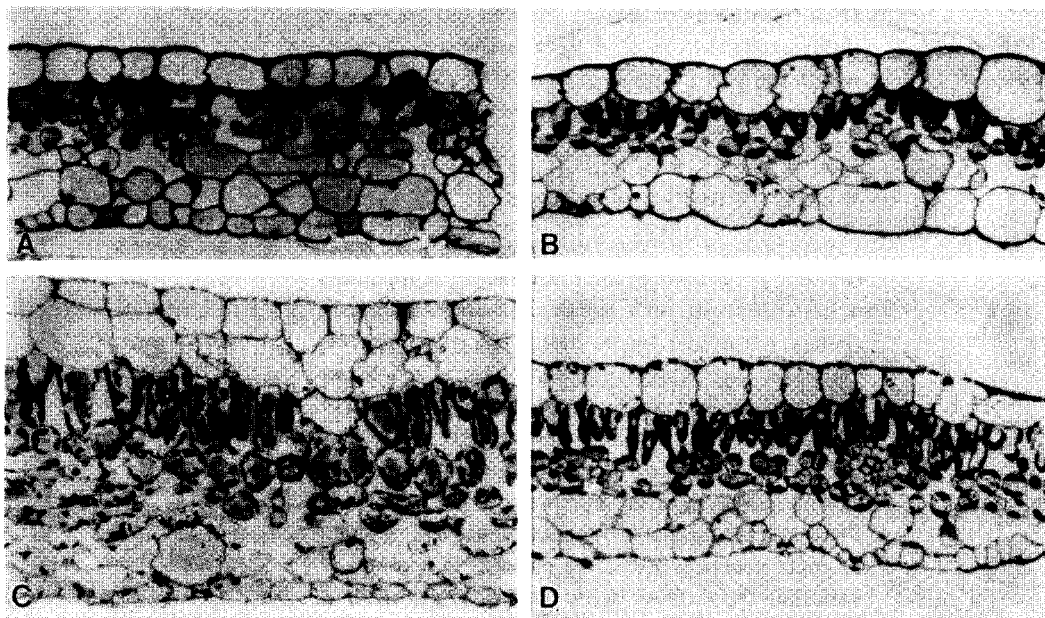* $P < 0.05;$ ** $P < 0.01;$ *** $P < 0.001.$

Fig. 3. Photomicrographs of representative leaf cross-sections of *Piper arieianum* (A, C) and *Piper sancti-felicis* (B, D) from gap microsites characterized by low-light (A, B) and high-light (C, D) availability. For all photomicrographs, magnification ×207.

In both species, leaf mass per area was positively correlated with all measures of light availability ($P < 0.05$; data not shown). Moreover, leaf mass per unit area was also closely correlated with total leaf thickness ($r = 0.658$ for *Piper arieianum*; $r = 0.650$ for *Piper sancti-felicis*). Among all the features of leaf anatomy examined, leaf mass per area was most strongly correlated with the thickness of the combined palisade and spongy mesophyll layers. Both species showed a significant regression of leaf mass per area on mesophyll thickness (Fig. 4), although the slope of the regression lines was signifi-

cantly greater for *Piper sancti-felicis* ($P < 0.001$). Chloroplast density decreased significantly with canopy openness in leaves of *Piper arieianum* ($P = 0.009$), whereas no significant regression was observed in *Piper sancti-felicis* ($P = 0.126$).

RELATIONSHIPS BETWEEN PHOTOSYNTHETIC RESPONSES AND LEAF ANATOMY

Photosynthetic capacity of *Piper arieianum* was generally poorly correlated with leaf anatomical measurements, although photosynthetic capacity per

Table 5. Coefficient of determination (r^2) for regression analyses of leaf anatomy on measures of the light environment at the leaf for *Piper arieianum*. For all regression, $n = 10$. For significant regressions, all slopes were positive

	Dependent variable					
Independent variable	Total leaf thickness (μm)	Upper epidermis (μm)	Palisade mesophyll thickness (μm)	Spongy mesophyll thickness (μm)	Hypodermis (μm)	Lower epidermis (μm)
Measured daily PFD (mol m^{-2} day^{-1})	0·538*	0·371	0·532*	0·604**	0·002	0·018
Weighted canopy openness (%)	0·502*	0·232	0·469*	0·654**	0·003	0·023
Estimated daily PFD (mol m^{-2} day^{-1})	0·778***	0·730**	0·775***	0·824**	0·000	0·001
Minutes of direct radiation	0·704**	0·846***	0·724**	0·716**	0·000	0·000
10-day mean estimated PFD (mol m^{-2} day^{-1})	0·723**	0·581**	0·722**	0·778***	0·007	0·000
10-day mean minutes of direct radiation	0·701**	0·740***	0·719**	0·716**	0·008	0·005
Yearly mean estimated PFD (mol m^{-2} day^{-1})	0·461*	0·208	0·439*	0·597**	0·007	0·013
Yearly mean minutes of direct radiation	0·416*	0·349	0·457*	0·549*	0·008	0·015

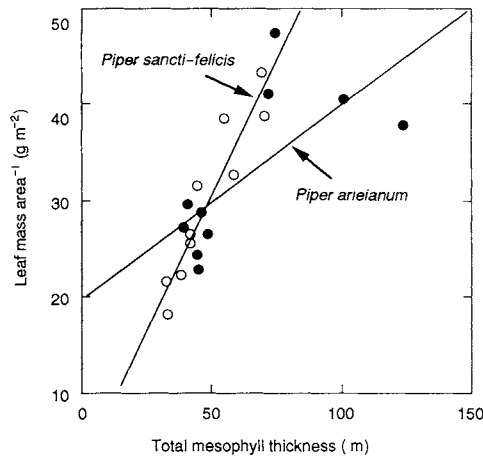* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 4. Leaf mass per area as a function of total mesophyll thickness, including both palisade and spongy mesophyll layers. Regression statistics are provided in the text. Symbols for species are as in Figs. 1 and 2.

unit area was positively correlated with thickness of the upper epidermis, and negatively correlated with the per cent airspace in leaves (Table 7). Photosynthetic capacity per unit dry mass was not correlated with any anatomical measures. Photosynthetic capacity per mol chlorophyll was negatively correlated with the measure of chloroplast area within mesophyll tissues. In fact, chloroplast density decreased as mesophyll thickness increased in this species (Fig. 5; $P = 0.056$).

In contrast, leaves of *Piper sancti-felicis* showed significant positive correlations between photosynthetic capacity (area, mass, chlorophyll and nitrogen bases) and mesophyll tissue thickness

(Table 8). Chloroplast density increased significantly with mesophyll thickness in this species (Fig. 5; $P = 0.020$).

In both species, rates of daytime dark respiration were correlated with increased thickness of palisade and spongy mesophyll layers, as well as with total leaf thickness. Apparent quantum yield was positively correlated with mesophyll tissue thickness in *Piper sancti-felicis* only (Tables 7 and 8).

Discussion

These data confirm the differences in acclimation potential previously observed for the same set of plants after only 6 months of growth along the gap transects (Chazdon 1992). For *Piper arieianum*, the lack of significant variation in A_{max} with light availability after 1 year of growth could well be a consequence of the smaller number of plants measured, since the earlier statistical relationship, based on larger sample sizes, was weak. Moreover, these results are consistent with previous studies of light acclimation and dark respiration in *Piper* species that differ in ecological distribution within rain forest habitats (Chazdon & Field 1987a; Walters & Field 1987; Fredeen & Field 1991). *Piper sancti-felicis*, an early-successional pioneer and gap species, demonstrates a high capacity to adjust photosynthetic capacity to changes in light availability, whereas acclimation potential is more restricted in *Piper arieianum*, the shade species (Fig. 1).

The constraints on light acclimation in *Piper arieianum* do not appear to be anatomical in nature. This species shows significant variation in thickness of both palisade and spongy mesophyll layers across

Table 6. Coefficient of determination (r^2) for regression analyses of leaf anatomy on measures of the light environment at the leaf for *Piper sancti-felicis*. For all regressions, $n = 10$, except for measured daily PFD, where $n = 8$. For significant regressions, all slopes were positive, except when the dependent variable was lower epidermis

Independent variable	Dependent variable					
	Total leaf thickness (μm)	Upper epidermis thickness (μm)	Palisade mesophyll thickness (μm)	Spongy mesophyll thickness (μm)	Hypodermis (μm)	Lower epidermis (μm)
Measured daily PFD (mol m^{-2} day^{-1})	0.147	0.001	0.379	0.509*	0.108	0.638*
Weighted canopy openness (%)	0.197	0.018	0.746***	0.626**	0.132	0.541*
Estimated daily PFD (mol m^{-2} day^{-1})	0.169	0.000	0.461*	0.552*	0.080	0.482*
Minutes of direct radiation	0.222	0.002	0.461*	0.575*	0.105	0.412*
10-day mean estimated PFD (mol m^{-2} day^{-1})	0.155	0.001	0.466*	0.552*	0.076	0.493*
10-day mean minutes of direct radiation	0.178	0.000	0.513*	0.588**	0.076	0.432*
Yearly mean estimated PFD (mol m^{-2} day^{-1})	0.259	0.006	0.718**	0.693**	0.193	0.589**
Yearly mean minutes of direct radiation	0.296	0.004	0.588**	0.654**	0.192	0.465*

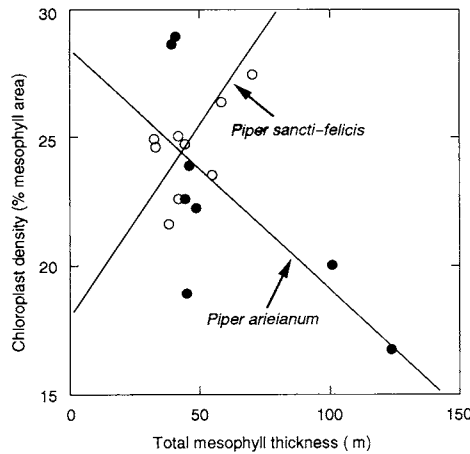* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 5. Chloroplast density (per cent of mesophyll area in cross-section) as a function of total mesophyll thickness. Regression statistics are provided in the text. Symbols for species are as in Figs 1 and 2.

the gap transects, and greater variation in total leaf thickness than *Piper sancti-felicis* (Table 5, Fig. 3). Leaf structural characteristics vary closely with changes in daily PFD across gap microsites, as demonstrated in growth chamber studies for other species (Nobel 1976; Chabot, Jurik & Chabot 1979). Yet, for *Piper arieianum*, these anatomical adjust-

ments do not lead to increased photosynthetic capacity per unit area under high-light conditions as observed for other forest understorey species (Chabot *et al.* 1979; Sims & Pearcy 1992). The increase in mesophyll tissue volume with light availability in this and other forest understorey species has the effect of diluting rather than enhancing photosynthetic activity on a dry mass basis (Chabot *et al.* 1979; Sims & Pearcy 1992). This effect is clearly seen in the decrease in chloroplast density as mesophyll thickness increased (Fig. 5). Chloroplast density also showed significant decreases with light availability in this species.

Changes in photosynthetic capacity in *Piper sancti-felicis* across the gap transects reflect both anatomical adjustments as well as biochemical adjustments to changes in light availability. The significant increase in photosynthetic capacity per unit leaf mass with increased light availability (Fig. 1B) suggests that light acclimation occurs at the cellular level as well as the whole-leaf level in this species. The increase in chloroplast density as mesophyll thickness increases is further evidence of adjustments to light availability at the cellular or subcellular level. Moreover, chlorophyll-use efficiency and nitrogen-use efficiency of photosynthesis both increase significantly with mesophyll tissue thickness in *Piper sancti-felicis* (Table 8),

Table 7. Pearson correlation coefficients between photosynthetic characteristics and leaf anatomical characteristics for *Piper arieianum*. $n = 10$ for all correlations

Independent variable	Dependent variable					
	A_{max} area^{-1}	Dark respiration	Apparent quantum yield	A_{max} mass^{-1}	A_{max} Chl^{-1}	A_{max} N^{-1}
Total leaf thickness	0·554	−0·841**	0·509	−0·082	0·456	−0·036
Upper epidermis	0·662*	−0·665*	0·405	0·196	0·156	−0·018
Palisade mesophyll thickness	0·587	−0·813**	0·538	0·064	0·450	−0·078
Spongy mesophyll thickness	0·610	−0·882**	0·500	−0·069	0·601	0·100
Hypodermis	−0·342	0·343	0·318	−0·481	−0·136	−0·347
Lower epidermis	−0·004	0·154	0·157	0·064	−0·049	−0·307
Chloroplast density	−0·007	0·286	−0·178	0·490	−0·797**	−0·134
Per cent airspace	−0·749*	0·560	−0·180	−0·243	−0·439	−0·346

$* P < 0.05; ** P < 0.01.$

Table 8. Pearson correlation coefficients between photosynthetic characteristics and leaf anatomical characteristics for *Piper sancti-felicis*. $n = 10$ for all correlations. For lower epidermis, all correlations were negative

Independent variable	Dependent variable					
	A_{max} area^{-1}	Dark respiration	Apparent quantum yield	A_{max} mass^{-1}	A_{max} Chl^{-1}	A_{max} N^{-1}
Total leaf thickness	0·331	−0·648*	0·494	0·125	0·496	0·351
Upper epidermis	−0·195	−0·069	0·108	−0·182	−0·051	−0·110
Palisade mesophyll thickness	0·804**	−0·886***	0·720*	0·647†	0·872**	0·762**
Spongy mesophyll thickness	0·714*	−0·852**	0·752*	0·524	0·816**	0·718*
Hypodermis	0·218	−0·606	0·378	−0·044	0·388	0·211
Lower epidermis	−0·697*	0·724*	−0·692*	−0·536	−0·751*	−0·701*
Chloroplast density	0·462	−0·619	0·402	0·252	0·548	0·415
Per cent airspace	−0·301	−0·011	−0·370	−0·448	−0·207	−0·353

indicating that changes in photosynthetic capacity are due to cell-based differences in biochemistry and photochemistry as well as anatomical adjustments (Table 6). These biochemical changes lead to increased efficiency of carboxylation under high-light conditions.

Although leaf mass per area may be a good predictor of variation in leaf photosynthetic capacity within a species (Pearce *et al.* 1969; Jurik 1986), our results show that this trait is not always a good predictor of leaf photosynthetic capacity (Björkman 1981). For *Piper sancti-felicis*, A_{max} area^{-1} is highly dependent on leaf mass per area ($r^2 = 0.783$, $P < 0.001$), whereas for *Piper arieianum*, there is no significant regression ($r^2 = 0.118$, $P = 0.229$). Changes in leaf structure and leaf anatomy are not necessarily coincident with changes in photosynthetic responses.

Analysis of the effects of vapour pressure on the light acclimation responses suggest that stomatal conductance in *Piper arieianum* may be more sensitive to vapour pressure deficits than in *Piper sancti-felicis*. Leaves of *Piper hispidum* in a Mexican rain forest have a strong stomatal response to humidity (Mooney *et al.* 1983). Although the differential sensitivity of these species to vapour pressure did not affect patterns of light acclimation, these responses could significantly influence daily carbon gain in gap and gap-edge environments.

Increases in rates of daytime dark respiration (area basis) at higher light levels are correlated with anatomical changes in both species, particularly with increased thickness of photosynthetic mesophyll tissue (Tables 7 and 8). Respiratory activity therefore appears to be strongly influenced by the metabolic demands of photosynthetic tissues. Non-structural respiratory costs can be assessed by examining the rate of dark respiration on a leaf-mass basis across the gap transects. On this basis, the two species diverge greatly — *Piper sancti-felicis* exhibits increasing respiratory rates with increases in light availability, whereas *Piper arieianum* shows no significant change (Fig. 2). The increased rates of dark respiration under high-light conditions in *Piper sancti-felicis* reflect both increased physiological activity within photosynthetic tissues as well as increased photosynthetic tissue per unit area. These data further emphasize that the limited acclimation potential in *Piper arieianum* reflects an inability to adjust metabolic processes at the cellular level (Figs 2 and 5). This species appears to have a low ceiling for both maximum photosynthetic rates as well as respiratory rates (Figs 1 and 2). Acclimation studies of seedlings of six Australian rain forest tree species also indicate that the sensitivity of leaf respiration to increases in specific leaf mass was greatest in early-successional species compared to species characteristic of mid- and late-succession (Turnbull 1991).

Leaves of *Piper sancti-felicis* showed decreases in apparent quantum yield with decreased light availability across gap transects, whereas quantum yields of *Piper arieianum* remained constant (Table 4). These results indicate that photoinhibition was not occurring in either species during this time of year, despite the appearance of yellowed leaves in *Piper arieianum* plants in the large gap. Measurements of photosynthetic capacity of 6-month-old plants suggested that plants of *Piper arieianum* growing in exposed gap microsites suffered from chronic photoinhibition, although quantum yields were not measured at this earlier sampling date (Chazdon 1992). Quantum yields often decrease in leaves grown under high-light conditions compared to low-light-grown plants, even for species characteristic of high-light environments (Björkman 1981; Sims & Pearcy 1989; Turnbull 1991). Turnbull (1991) found that for five out of six rain forest tree species studied, quantum yield decreased at the lowest light level at which each survived. Moreover, measurements of quantum yield were found to be significantly different under filtered shade compared to neutral shade (Turnbull 1991). In the absence of field data for other species, it is unclear whether the species differences observed here are characteristic of other species growing in natural light environments.

The increase in thickness of the upper epidermis in *Piper arieianum* may have a protective function under high-light conditions (Fig. 3C). Less clear is an interpretation of decreases in thickness of the lower epidermis under high light in *Piper sancti-felicis* (Table 6). Although multi-layered epidermises have been identified in a variety of plant families (Esau 1977; Roth 1984), our study is the first to demonstrate variation in epidermal thickness in response to a natural gradient in light availability. A second upper epidermal layer may increase leaf reflectance (Carter 1991), alter light-scattering processes within mesophyll tissue (Bone, Lee & Norman 1985), or protect photosynthetic tissues from excessive irradiance (Roth 1984). A role in water storage has also been suggested (Roth 1984).

Few studies have examined natural variation in leaf anatomy in relation to photosynthetic light acclimation (Jurik 1986). Further studies in this area may elucidate the ecological significance of species differences in leaf-level adaptation to heterogeneous light environments. A key distinction in light acclimation potential between shade-adapted and high-light-adapted species may reflect the extent to which photosynthetic capacity on a leaf-mass basis varies with light availability. Anatomical adjustments in response to variation in light availability may therefore not always be coincident with changes in photosynthetic capacity within leaves.

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