

DECLINE OF PHOTOSYNTHETIC CAPACITY WITH LEAF AGE AND POSITION IN TWO TROPICAL PIONEER TREE SPECIES¹

KAORU KITAJIMA,^{2,3,4} STEPHEN S. MULKEY,² MIRNA SAMANIEGO,³ AND S. JOSEPH WRIGHT³

²Department of Botany, University of Florida, Gainesville, Florida 32611 USA; and

³Smithsonian Tropical Research Institute, Box 2072, Balboa, Ancon, Panama

The effect of leaf age on photosynthetic capacity, a critical parameter in the theory of optimal leaf longevity, was studied for two tropical pioneer tree species, *Cecropia longipes* and *Urera caracasana*, in a seasonally dry forest in Panama. These species continuously produce short-lived leaves (74 and 93 d, respectively) during the rainy season (May–December) on orthotropic branches. However, they differ in leaf production rate, maximum number of leaves per branch, light environment experienced by the leaves, leaf mass per unit area, and nitrogen content. Light-saturated photosynthetic rates for marked leaves of known ages (± 1 wk) were measured with two contrasting schemes (repeated measurements vs. chronosequence within branch), which overall produced similar results. In both species, photosynthetic rates and nitrogen use efficiency were negatively correlated with leaf age and positively correlated with light availability. Photosynthetic rates declined faster with leaf age in *Cecropia* than in *Urera* as predicted by the theory. The rate of decline was faster for leaves on branches with faster leaf turnover rates. Nitrogen per unit leaf area decreased with leaf age only for *Urera*. Leaf mass per unit area increased with leaf age, either partly (in *Cecropia*) or entirely (in *Urera*) due to ash accumulation.

Key words: leaf age; leaf longevity; leaf nitrogen content; leaf position; photosynthetic capacity; self-shading; tropical trees.

Photosynthetic capacity of a leaf generally exhibits a monotonic, often linear, decline after full expansion (Koike, 1988; Zotz and Winter, 1994; Ackerly and Bazzaz, 1995; Kitajima, Mulkey, and Wright, 1997a). This decline is not an uncontrolled physiological deterioration, but is caused by a redistribution of resources, especially nitrogen, to younger leaves for optimization of whole-shoot photosynthetic income (Field and Mooney, 1983; Hikosaka, Terashima, and Katoh, 1994; Ackerly, 1996). Consideration of the effect of leaf age on photosynthetic capacity (A) is necessary to estimate the long-term carbon budget of a leaf and of the whole crown. The effect of leaf age on photosynthetic capacity is also critical in cost-benefit theories of optimal leaf longevity (Kikuzawa, 1991) in which total daily photosynthetic income, not photosynthetic capacity, is modeled. However, the former can be estimated as a linear function of the latter (Zotz and Winter, 1994). Thus, if we can approximate the functional form of the decrease in photosynthetic capacity with leaf age, it will be possible to model long-term carbon budgets for whole crowns.

A linear decrease of photosynthetic capacity (A_t) with time after full leaf expansion (t) may be expressed in a following equation (Kikuzawa, 1991):

$$A_t = a(1 - t/b)$$

where a and b correspond to the y and x intercepts of the linear regression between photosynthetic rates and leaf age, respectively. The parameter a (y intercept of the regression) may be directly measured as the initial photosynthetic capacity at the

time of leaf full expansion. In contrast, the parameter b (x intercept of the regression) is a statistical extrapolation determined as a function of the initial photosynthetic rate (a) and the rate of its decline (a/b). The parameter b approximates the actual leaf longevity when the same leaf is repeatedly measured for plants with very short life spans (< 50 d; Evans, 1983; Makino, Mae, and Ohira, 1984; Ackerly and Bazzaz, 1995). However, in tropical tree species with greater leaf lifetimes of 174–315 d, b was significantly greater than actual leaf longevity (Kitajima, Mulkey, and Wright, 1997a).

A cost-benefit analysis incorporating this function suggests that leaf longevity is expected to be short when the initial net photosynthetic rate of the leaf (a) is high and/or the decline rate (a/b) is fast (Kikuzawa, 1991). A negative correlation between mean leaf longevity and initial photosynthetic capacity (model parameter a , or y intercept of regression) has been demonstrated across a variety of plant species both locally and globally (Koike, 1988; Reich et al., 1991; Reich, Walters, and Ellsworth, 1992; Mulkey, Kitajima, and Wright, 1995). In contrast, data for the interspecific relationship between leaf longevity and the slope of the A –leaf age relationship (parameter a/b) are scarce, but our earlier study (Kitajima, Mulkey, and Wright, 1997a) supported Kikuzawa's prediction.

Two sampling schemes are commonly employed for determining the A –leaf age relationship: repeated measurements of the same individual leaves (Scheme 1) and measurements of leaves with contrasting ages and positions within a branch on a given sampling day (Scheme 2, possible only for species with successive leaf production). These two methods, however, may yield different results because leaves produced in different seasons may differ in initial photosynthetic capacity and rates of decline (Field and Mooney, 1983; Kitajima, Mulkey, and Wright, 1997a, b). Even if all sampled leaves are produced within the same season, Scheme 2 may underestimate the decline rate, because leaf lifetime varies within branches, trees, and populations, and rapidly aging leaves that die young are

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⁴ Author for correspondence (e-mail: kitajima@botany.ufl.edu; FAX: 352-392-3993).

TABLE 1. Leaf characteristics (phenology, expansion time, size, lifetime, production rate) of the two pioneer tree species in the study. Both species become deciduous during the dry season (January–March).

Species	Season of new leaf production	Leaf expansion (d) ^a	Leaf size (cm ²)		Leaf lifetime (d) (weekly census) ^b		Leaf lifetime (d) (monthly census) ^c			Mean leaf no. per branch		Leaf production rate ^d (no. · shoot ⁻¹ · wk ⁻¹)
			N	mean ± SD	N	mean ± SD	N	mean	90%	13 May	19 Aug	mean ± SD
<i>Cecropia</i>	April–December	21	15	1705 ± 539	72	73.7 ± 2.5	279	76	121	4.0	6.2	0.63 ± 0.06
<i>Urera</i>	April–November	28	37	801 ± 456	129	93.4 ± 2.7	397	93	151	6.0	13.4	1.15 ± 0.21

^a Mean time from leaf emergence to leaf full expansion.

^b Estimated for all fully expanded leaves between 6 May and 3 August as time from full expansion to abscission with the Kaplan-Meier method that accounts for right-censored leaves (alive at the end of monitoring on 3 September).

^c Mean and 90th percentile leaf lifetime from monthly census in 1993, for all leaves produced in April–July for which all leaves were monitored until their death (S. J. Wright, unpublished data).

^d Mean ± SD of leaf production per week between 6 May and 5 August 1996 ($N = 9$ branches per species).

underrepresented in Scheme 2. This bias has not been evaluated in most studies that have examined A–leaf age relationships.

In a species with successive leaf production, the slope of the A–leaf age relationship should be a function of rates of leaf production and of the development of within-branch self-shading (Kikuzawa, 1995; Ackerly, 1996), which in turn is a function of light and nitrogen availabilities experienced by the leaves (Hikosaka, Terashima, and Katoh, 1994; Ackerly and Bazzaz, 1995). Within a single architectural type (e.g., orthotropic branch with successive leaf production), functional leaf traits that affect self-shading, such as leaf size, leaf mass per area, and leaf production rates, vary greatly among tropical tree species. In addition, the consequences of heterogeneity in light and nitrogen availability among branches and individuals on the A–leaf age relationship and leaf longevity have not been adequately addressed for adult canopy trees.

Here, we report the rate of decline of photosynthetic capacity with leaf age for two tropical canopy tree species whose leaf longevity (74–94 d) is much shorter than the five tree species studied earlier at the same site (Kitajima, Mulkey, and Wright, 1997a) but longer than *Heliocarpus appendiculatus* studied by Ackerly and Bazzaz (1995). Leaf age from the time of leaf full expansion was determined more precisely with weekly censuses than in our earlier study with monthly censuses. The objectives of our current study are: (1) to fill a gap in empirical data of a/b parameter in the A–leaf age relationship for tropical species with intermediate leaf longevity; (2) to evaluate the difference between the two sampling schemes; (3) to examine whether among-leaf variation in the A–leaf age relationship is related to leaf production rates among branches; (4) to compare two species that are similar in leaf longevity and overall architecture (orthotropic branches and successive leaf production) but differ in the maximum leaf number per shoot and range of light environments experienced by the leaves; (5) to explore the functional basis for the A–leaf age relationship by examining relationships among leaf age, leaf position, light microenvironment, leaf mass per area, nitrogen contents, and photosynthetic capacity in greater detail than in our earlier study.

MATERIALS AND METHODS

Site and canopy approach—The study was conducted in a seasonally dry forest in the Parque Natural Metropolitano (8°59' N, 79°31' W) near Panama City, Panama. Annual rainfall averages 1798 mm at the site, most of which occurs during the wet season from May through December. The study was conducted during May–August 1996, a year of somewhat higher than average rainfall (2016 mm). The forest is 75–150 yr old second growth with tree

heights up to 40 m. We used a 42 m tall tower crane with a 51-m jib to reach the upper canopy (Parker, Smith, and Hogan, 1992).

Species, leaf census, and sampling—*Cecropia longipes* Pitt. (Cecropiaceae, mature height 10–15 m) and *Urera caracasana* (Jacq) Griseb (Urticaceae, mature height 5–10 m) are common pioneer trees at the study site (nomenclature following D'Arcy, 1987). They are deciduous during the dry season and start producing leaves in mid-April. Both species successively produce leaves throughout the rainy season on orthotropic branches in whorls. None of the marked terminal branches developed a secondary branch during the study. The total number of leaves per branch increased in both species between the first census (13 May 1996) and the last census in which we marked new leaves (19 August 1996) (Table 1). From the monthly census data of all leaves produced during 1993, 56% and 68% of annual leaf production takes place during this period in *Cecropia* and *Urera*, respectively. The total crown leaf area of these two tree species reach their maximum values in mid- to late rainy season (S. J. Wright, unpublished data).

We studied leaves fully expanded in May–July on three marked branches in each of three trees per species. All expanding leaves were marked and monitored for weekly changes of leaf length to determine the timing of full leaf expansion within ±7 d. Leaves of *Cecropia* expanded faster to reach their larger final size than *Urera* (Table 1). Mean (±SD) leaf lifetime was estimated for each species as the time from full leaf expansion to abscission with the Kaplan-Meier method, which accounts for censored leaves (leaves alive at the final survival census on 3 September).

In each marked branch, 1–2 leaves that became fully expanded between 6 May and 3 June were selected for repeated in situ measurements of light saturated photosynthetic rates (Scheme 1). In the second sampling scheme (Scheme 2), leaves of known ages within a branch, encompassing the full range of leaf age and position gradient, were measured on a given sampling date between late June and early August. A total of three and four branches (one branch per tree plus a second branch in one of the trees) were sampled with Scheme 2 for *Cecropia* and *Urera*, respectively.

Measurement of photosynthesis and light in the field—We measured the light-saturated net CO₂ assimilation rate per unit leaf area (= photosynthetic capacity, A) and stomatal conductance to water (G_s) with a portable infra-red gas analyzer (LI-6400, Li-COR, Lincoln, Nebraska, USA). Light with the photon flux density (PPFD) at 1500 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ was supplied with red light emitting diodes (LI-6400-02). The CO₂ concentration of the reference air entering the leaf chamber was adjusted with a CO₂ mixer control unit such that the “sample” air exiting the chamber contained 350 ppm of CO₂. This resulted in CO₂ concentration of the reference air to be in the range of 360–388 ppm and most typically close to 370 ppm. All gas exchange data were collected in the mornings between 0800 and 1200. The chamber temperature was controlled by maintaining the Peltier block temperature at 28°C. The relative humidity of the reference air was kept as close to ambient (usually 70–85%) as possible. The air flow rate was 500 mL/min. We also measured A for a smaller number of leaves during their expansion; the increase of A was sharp during expansion and continued for an additional 1–2 wk after leaves reached their full sizes. These gas exchange data during leaf expansion,

including estimated intercellular CO_2 concentration, have been reported elsewhere (Terwilliger et al., 2001). In this paper, we report only the results of photosynthesis after leaves reached their full sizes.

Light availability (daily total PFD) at the leaf surface was measured continuously for 5 d for a subset of leaves sampled with Scheme 2, immediately before or after the field gas exchange measurements (5–6 leaves per branch). A calibrated GaAsP sensor (Hamamatsu, Japan) was attached to the adaxial surface of each sampled leaf and hourly means for PFD sampled every 5 s were recorded with LI-1000 data loggers (LI-COR). The results were expressed as %PFD (the mean percentage of the total daily PFD above the canopy) for each leaf.

Laboratory measurements—Photosynthetic light response curves were determined for incident PFD between 0 and $1900 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a leaf-disk oxygen electrode and data acquisition software (Hansatech, Norfolk, UK) for one arbitrarily selected branch of each species (Scheme 2). The leaf disks (10 cm^2 each) were sampled just after dawn on the day of measurement and kept in dark aerated plastic containers lined with moist filter paper until measured. Light provided by a Björkman-type lamp (Hansatech) was increased in steps by combinations of neutral density glass filters after oxygen evolution rate reached quasi-steady state at each light level. The electrode chamber was supplied with humidified air with 10% CO_2 and cooled by circulating 28°C water. Quantum yield (initial slope) and dark respiration rate were calculated from regression analysis of the linear region of the photosynthetic light response curve of each leaf. The age effects on photosynthetic light-response curves were summarized by fitting non-rectangular hyperbola (Lieth and Reynolds, 1987) for mean values for 2–3 leaves of each age group.

We determined leaf mass per area (LMA, in grams per square meter) from 3.43 cm^2 leaf disks for leaves sampled for Scheme 2 field gas exchange and all disks used for oxygen electrode measurements after drying them at 60°C for ≥ 5 d. Nitrogen contents per unit mass (N_m) and per unit area (N_a) were determined for the same leaf disks from leaves used in the Scheme 2 field gas exchange with a Perkin-Elmer CHNO/S Model II elemental analyzer (Perkin-Elmer, Shelton, Connecticut, USA). Leaf disks used for the oxygen electrode measurements were used for determination of mineral ash contents (as a percentage of dry mass) after ashing at 500°C in a muffle furnace for 6 h.

Statistical analyses, including leaf survival analysis and regression and correlation analyses among leaf traits, were done with JMP V.3.0 (SAS, 1994). Species difference in initial photosynthetic capacity was tested with a t test for estimated y intercepts for individual leaves sampled with Scheme 1 ($N = 10$ leaves per species). Species difference in slopes of the A -leaf age relationships was tested as a significant species-by-age interaction in ANCOVA with pooled data with species as the main factor and leaf age as covariate.

RESULTS

Leaf demography—Although both are pioneer species with relatively similar leaf longevity, *Cecropia* and *Urera* differed in many aspects of leaf production and loss rates. *Cecropia* produced fewer, larger leaves that expanded more rapidly than *Urera* (Table 1). Leaf production rates were constant within each study branch, as shown by the lower examples in Fig. 1; the number of distal (and younger) leaves above each focal leaf increased linearly with time. During April–July, leaf loss rates were lower than leaf production rates, resulting in a net increase in leaf number per branch between May and August (Table 1). By August, however, leaf production rates were in equilibrium with leaf loss rates (data not shown). Although both species bore large leaves with long petioles in whorls on orthotropic branches, *Urera* had a greater equilibrium number of leaves per branch than *Cecropia*. There was large variation in leaf lifetime within each species; the proportion of leaves surviving declined linearly with leaf age. *Cecropia* had a significantly shorter mean leaf lifetime (74 d) than *Urera* (93 d). These values were similar to the mean leaf lifetime for leaves

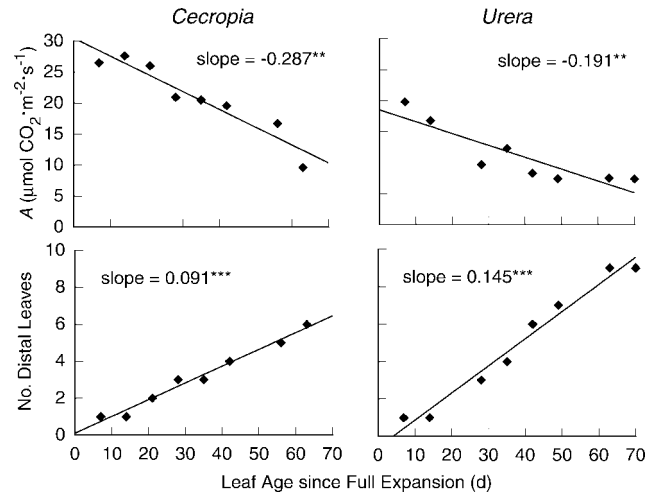


Fig. 1. Examples of repeated measurements of a single leaf (Scheme 1) for light-saturated assimilation rates (A) and number of distal leaves as a function of leaf age (days since full expansion) for *Cecropia* and *Urera*. Solid lines represent significant regression slopes (*** $P < 0.0005$, ** $P < 0.005$).

produced during the same season from monthly censuses in which all leaves were monitored until they abscised during 1993 (Table 1).

A -leaf age relationship: comparison of species and sampling schemes—Figure 1 shows an example of the decrease of photosynthetic capacity with leaf age for each species from repeated sampling of a single leaf (Scheme 1 sampling). For individual leaves, the physiological maximum of leaf photosynthetic capacity was often not observed until 7–14 d after the date of full leaf expansion. The data points for young leaves yet to reach their physiological maxima were not included in the linear regression analyses of the A -leaf age relationship below.

Within each species, there was a substantial variation in the slopes and intercepts of the A -leaf age relationships among leaves sampled with Scheme 1, such that the range of values overlapped considerably between the two species (Table 2). This variation was largely explained by leaf production rates; the slope was steeper for leaves on branches that had higher leaf production rates (Fig. 2; $P = 0.05$ for *Cecropia*). On average, *Cecropia* had a higher initial photosynthetic capacity (y intercept, t test $P < 0.003$) and steeper negative slope (significant species-by-leaf age interaction in ANCOVA, $P < 0.02$), which was expected from its shorter leaf longevity. However, estimated x intercepts (= leaf age at which A would reach zero in a linear extrapolation) did not differ significantly between the two species. In both species, x intercepts were greater than the mean leaf lifetime, but less than the 90th percentile leaf lifetime.

Overall, the pattern of decline of A with leaf age was similar between Schemes 1 and 2. Examples of the A -leaf age relationship from Scheme 2 sampling are shown in Fig. 3. There was a considerable variation among the 3–4 branches sampled with this method (Table 2). The mean slope was more negative with Scheme 2 than with Scheme 1 in *Urera*, as predicted, but the opposite was observed for *Cecropia* (Table 2). The intercepts and slopes did not differ significantly between the two species in Scheme 2.

Photosynthetic light response curves measured with an ox-

TABLE 2. Regression statistics (y intercepts, x intercepts, and slopes) for light-saturated photosynthetic net assimilation rate (A , in micromoles of CO_2 per square meter per second) against leaf age (days since full expansion) according to the two sampling schemes. Means (and ranges) of parameters estimated for individual leaves and branches are given. A total of nine (one leaf for each of three marked branches per tree) plus one extra leaf were sampled in Scheme 1, while a total of three (one per tree) or four (one per tree and one extra) branches were sampled with Scheme 2 for *Cecropia* and *Urera*, respectively.

Scheme	<i>Cecropia longipes</i>				<i>Urera caracasana</i>			
	N	y intercept	Slope	x intercept	N	y intercept	Slope	x intercept
1	10 leaves	25.2 (17.6–32.5)	-0.257 (-0.510–-0.112)	109 (57–185)	10 leaves	19.0 (16.0–21.3)	-0.203 (-0.337–-0.08)	111 (62–200)
2	3 branches	23.7 (21.0–25.8)	-0.179 (-0.206–-0.160)	133 (122–152)	4 branches	21.2 (16.7–24.6)	-0.255 (-0.348–-0.107)	98 (61–156)

xygen electrode under CO_2 -saturated conditions are summarized in Fig. 4, by plotting means of contrasting age groups. Light- and CO_2 -saturated photosynthetic rates clearly decreased with leaf age in both species. Dark respiration (expressed as positive values) and quantum yields had slightly negative correlations with leaf age in *Urera*, while *Cecropia* showed no change in dark respiration and quantum yields with leaf age (Table 3).

Leaf age effects on light, LMA, and nitrogen—Within each branch sampled with Scheme 2, photosynthetic capacity was significantly correlated with the light microenvironment of the leaves (Fig. 3B); it was greater for younger leaves at the distal position within each shoot that received higher %PFD. To evaluate possible functional reasons for changing photosynthetic capacity with leaf age, we examined correlations among variables that were expected to vary with leaf age for pooled data from the 3–4 branches of each species (Figs. 5 and 6, Table 3). Because of the tight linear relationship between leaf position and leaf age ($r = 0.93$ – 0.97), the correlation of a given trait with leaf position was very similar to its correlation with leaf age (Table 3).

Light availability at the leaf surface declined with leaf age (Fig. 5A, B) as older leaves had more distal leaves that cast shade upon them. Light availability was almost 100% for the youngest leaves of *Cecropia*, but decreased steeply with leaf age (with great variation among leaves at a given age). Leaves of *Urera*, a smaller-statured pioneer growing in gaps in the canopy of dominant trees, experienced lower light availability than *Cecropia* leaves through their lifetime. *Urera* leaves received less than 40% of full sun even when they had just

expanded and experienced increasingly shaded environment as they aged.

Leaf mass per area increased significantly with leaf age in both species (Fig. 5C, D). *Cecropia* leaves exhibited greater proportional increase of LMA than *Urera*. Consequently, photosynthetic capacity per mass ($A_{\text{mass}} = A$ divided by LMA) exhibited even steeper declines than A , especially in *Cecropia* (Table 3). Leaves of both species accumulated very high amounts of ash; up to 16% of leaf dry mass in *Cecropia* and 30% in *Urera*, increasing linearly with leaf age (Fig. 7). Ash-free mass increased with leaf age in *Cecropia*, but was independent of leaf age in *Urera*. Ash-free mass per leaf area was estimated for all leaves for which LMA was calculated, using the linear regression of percentage of ash on leaf age. Photosynthetic capacity per ash-free mass ($A_{\text{ash-free}}$) declined less steeply with leaf age than A_{mass} ($r = -0.70$ vs. $r = -0.82$ in both species). Nitrogen content per unit leaf area did not change with leaf age, position, and light availability in *Cecropia*, but it decreased in *Urera* (Fig. 5E, F, Table 3). However, because of the steep increase of LMA, nitrogen per unit mass declined in *Cecropia* as well as in *Urera* (Table 3).

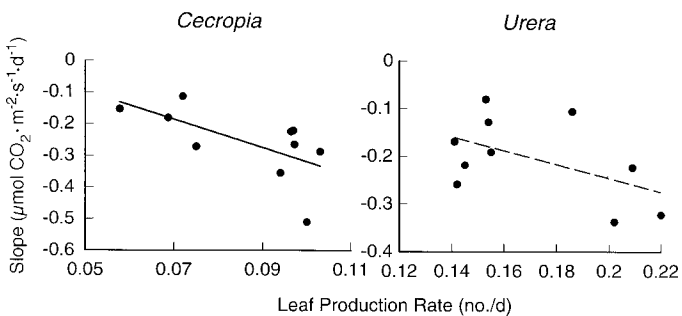


Fig. 2. Slope of A -leaf age relationship for individual leaves from repeated measurements (Scheme 1, $N = 10$ leaves) plotted against the production rate of leaves distal to the focal leaf (= regression slope of distal leaf number against time). The slope of the A -leaf age relationship was negatively correlated with the leaf production rate in *Cecropia* ($r = -0.63$, $P = 0.05$), but not in *Urera* ($r = -0.5$, $P = 0.13$).

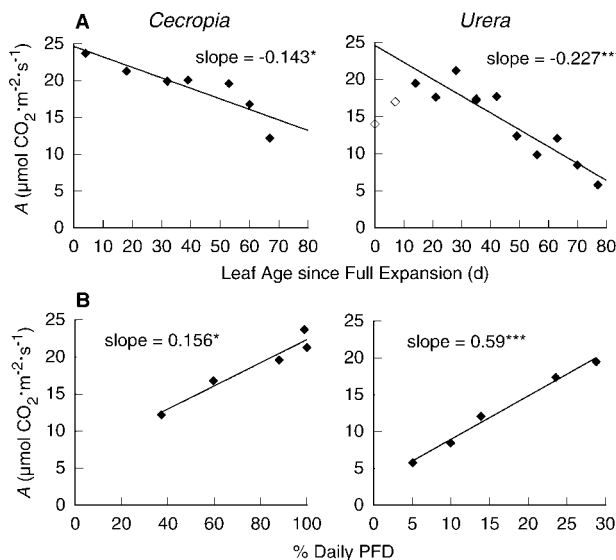


Fig. 3. (A) Examples of Scheme 2 measurements of light-saturated photosynthetic assimilation rates (A) of leaves of contrasting ages within a single branch on a single day for *Cecropia* and *Urera*. Very young leaves that had not achieved their maximum photosynthetic capacity (open symbols) are not included in the regression shown by the solid line. (B) Relationship between A and percentage of daily total photon flux density (%PFD) measured for 1 wk immediately preceding the measurements of A for a subset of the same leaves (significance of slope: *** $P < 0.0005$, * $P < 0.05$).

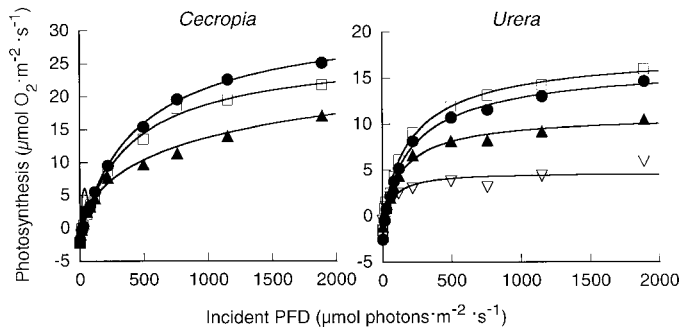


Fig. 4. Photosynthetic light response curves measured with a leaf-disk oxygen electrode. Means for contrasting age groups within a single branch are plotted against incident photon flux density. *Cecropia*: circle: 1 and 15 d; square: 29, 36, and 50 d; triangle: 57, 64, and 71 d old. *Urera*: circle: 0, 8, and 14 d; square: 35, 42, and 56 d; triangle, 63 and 78 d; inverted triangle, 84 d old.

The lowest photosynthetic capacity observed for old leaves right before their abscission was higher in *Cecropia* than *Urera* (Fig. 6A, B), corresponding to the higher light availability experienced by very old leaves of *Cecropia* than those of *Urera* (Fig. 5A, B). Photosynthetic capacity of *Cecropia* never dropped below 10 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, but older *Urera* leaves often had much lower A . Photosynthetic nitrogen use efficiency (A/N_a) decreased with leaf age and position in both species, slightly more steeply in *Cecropia* than *Urera* (Fig. 6C, D; Table 3). Thus, A/N_a was lower for older leaves that received lower light (Table 3). Stomatal conductance to water vapor (G_s) did not change with leaf age in *Cecropia*, but it decreased in *Urera*. In both species, G_s was positively correlated with %PFD received by the leaf.

DISCUSSION

Link between the slope of A -leaf age relationship and leaf longevity—The results of this study supported the predictions made by the cost-benefit model of Kikuzawa (1991) along with our earlier study (Kitajima, Mulkey, and Wright, 1997a). Species with shorter leaf longevity exhibit (1) a higher initial photosynthetic capacity and (2) a steeper rate of decline of photosynthetic capacity with leaf age (parameter a/b in Kikuzawa's model). The slopes for the two tropical pioneers with mean leaf lifetime of 74–93 d were between -0.2 and $-0.25 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ per day. In contrast, five other tree species at the same site with mean leaf lifetimes of 174–315 d had shallower slopes of -0.032 to -0.018 . Between the two species reported here, on average, *Cecropia*, with slightly shorter leaf longevity, had steeper slopes than *Urera* when leaves were sampled with Scheme 1. *Cecropia* also had a higher peak photosynthetic capacity than *Urera* as predicted by theoretical and empirical models (Kikuzawa, 1991; Reich, Walters, and Ellsworth, 1992).

The link between parameters a and b and optimal leaf longevity (t^*) is more explicitly predicted by the following relationship (Kikuzawa and Ackerly, 1999):

$$t^* = (2bC/a)^{1/2}$$

where C is the construction cost of the leaf. This equation predicts the observed relationship between leaf longevity and parameters a and b for various plant species, assuming a typical construction cost for leaf tissue for all species (Kikuzawa

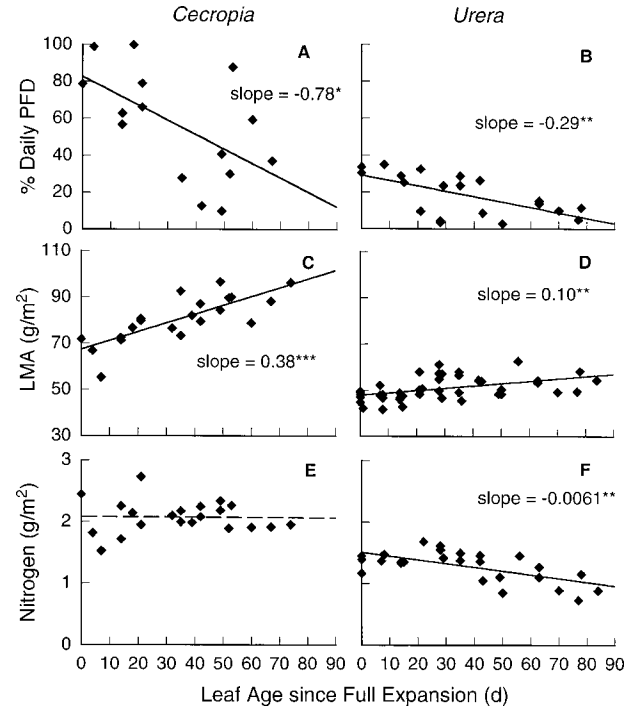


Fig. 5. Effects of leaf age on leaf traits that are known to influence photosynthetic capacity: total daily photon flux density (percentage of full sun) at leaf surface (A, B), leaf mass per area (LMA; C, D), nitrogen content per leaf area (E, F) for *Cecropia* (pooled samples of three branches) and *Urera* (pooled samples of four branches) with Scheme 2 sampling (significance of slope: *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$).

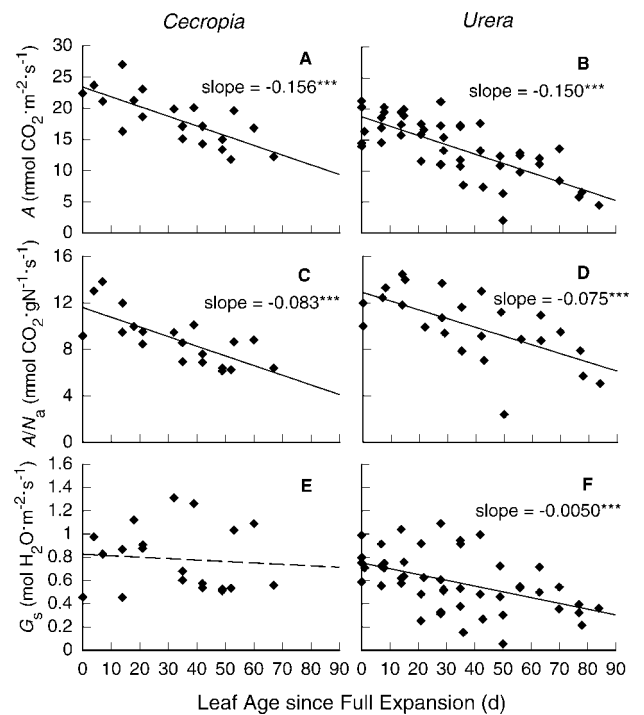


Fig. 6. Effects of leaf age on light saturated photosynthetic rates per unit leaf area (A ; A, B), photosynthetic nitrogen use efficiency (A/N_a ; C, D) and stomatal conductance for water (G_s ; E, F) for *Cecropia* (pooled samples of three branches) and *Urera* (pooled samples of four branches) with Scheme 2 sampling.

TABLE 3. Pearson's correlation coefficients (r) and significance levels (P) for correlation of leaf characteristics with leaf age, position from the most distal leaf (Pos), and light availability (%PFD). Samples were pooled for three branches of *Cecropia longipes* and four branches of *Urera caracasana*. N = total number of leaves.

Correlated variables	<i>Cecropia longipes</i>			<i>Urera caracasana</i>		
	N	r	P	N	r	P
Pos vs. Age	21	0.93	0.0000	51	0.97	0.0000
%PFD vs. Age	15	-0.57	0.0260	20	-0.63	0.0027
%PFD vs. Pos	15	-0.77	0.0008	20	-0.66	0.0016
LMA vs. Age	21	0.78	0.0000	43	0.48	0.0012
Ash-free LMA vs. Age	21	0.68	0.0014	41	0.06	NS
N_a vs. Age	21	-0.02	NS	27	-0.64	0.0003
N_a vs. %PFD	15	0.08	NS	15	0.90	0.0000
N_m vs. Age	21	-0.68	0.0008	20	-0.85	0.0000
A vs. Age	20	-0.74	0.0002	48	-0.74	0.0000
A vs. %PFD	15	0.68	0.0005	19	0.82	0.0000
A vs. LMA	20	-0.66	0.0016	41	-0.14	NS
A vs. N_a	20	0.23	NS	25	0.81	0.0000
A vs. G_s	20	0.52	0.0185	48	0.84	0.0000
A_{mass} vs. Age	20	-0.82	0.0000	40	-0.82	0.0000
A_{mass} vs. %PFD	20	0.64	0.0101	19	0.78	0.0001
A_{mass} vs. LMA	20	-0.87	0.0000	25	-0.43	0.0066
A/N_a vs. Age	20	-0.74	0.0000	25	-0.64	0.0006
A/N_a vs. %PFD	20	0.70	0.0038	14	0.72	0.0035
G_s vs. Age	20	-0.09	NS	48	-0.49	0.0005
G_s vs. LMA	20	-0.21	NS	48	-0.05	NS
G_s vs. %PFD	15	0.69	0.0046	19	0.68	0.0012
R_d vs. Age	8	-0.58	NS	8	-0.75	0.0295
Q_y vs. Age	8	-0.32	NS	8	-0.64	0.0201

Abbreviations: LMA = leaf mass per area (g/m^2); N_a = nitrogen per leaf area (g/m^2); N_m = nitrogen per leaf dry mass (mg/g); A = light-saturated photosynthetic net assimilation rate per leaf area ($\mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$); A_{mass} = light-saturated net photosynthetic rate per leaf mass ($\mu\text{mol CO}_2\text{-g}^{-1}\text{-s}^{-1}$); A/N_a = light-saturated net photosynthetic rate per leaf nitrogen ($\mu\text{mol CO}_2\text{-g}^{-1}\text{-s}^{-1}$); G_s = stomatal conductance to water vapor ($\text{mol H}_2\text{O}\text{-m}^{-2}\text{-s}^{-1}$); R_d = dark respiration (oxygen uptake rate) ($\mu\text{mol O}_2\text{-m}^{-2}\text{-s}^{-1}$); Q_y = apparent quantum yield (initial slope of photosynthetic light curve) (mol/mol).

and Ackerly, 1999). The data reported here fit well to this general relationship, along with five other species at the same site (K. Kitajima and K. Kikuzawa, unpublished data). The good fit of observed data to the cost-benefit model suggests that leaf longevity is primarily a function of "payback time" for the cost of construction and maintenance of leaves (Chabot and Hicks, 1982; Williams, Field, and Mooney, 1989) and leaf-support tissues (Kikuzawa and Ackerly, 1999).

The x intercept of the A -leaf age relationship is an extrapolation of the leaf age at which photosynthetic capacity would reach zero. Because total daily net photosynthetic income should reach zero before photosynthetic capacity reaches zero, the x intercept should be greater than the actual leaf lifetime for a given species. Interestingly, the discrepancy between the x intercept and the mean leaf lifetime is smaller for species

with shorter leaf lifetime. The x intercepts were approximately the same as the mean leaf lifetime of 28–37 d in *Heliocarpus appendiculatus* (Ackerly and Bazzaz, 1995). The x intercepts were similar for *Cecropia* and *Urera*; greater than the mean leaf lifetime but shorter than the 90th percentile of leaf lifetime in both species (this study). In contrast, the x intercepts for five species with longer leaf lifetime were much greater than the 90th percentile of leaf lifetime (Kitajima, Mulkey, and Wright, 1997a).

Sampling schemes and variation among individual leaves—Overall, the two contrasting sampling regimes produced similar A -leaf age relationships. In this study, Scheme 1 with a larger sample size ($N = 10$ leaves, spread among nine branches on three trees) should produce better estimates for the population than Scheme 2 ($N = 3$ –4 branches). Repeated measurements of individual leaves (Scheme 1) resulted in a steeper relationship than did measurements of a chronosequence on a single day (Scheme 2) in *Urera*. This was expected because leaves that exhibit faster physiological decline and die early would be underrepresented in Scheme 2. Yet, in *Cecropia*, the mean did not differ between the two schemes.

There was large variance among individual leaves in lifetime and rate of decline of photosynthetic rates. A part of the within-species variance appears to be explained by different leaf production rates among branches. All else equal, branches with higher leaf production rates should develop self-shading more rapidly, and consequently, leaves should exhibit more rapid declines of photosynthetic capacity (Fig. 2), as predicted by the theories of optimal nitrogen allocation from old, shaded leaves to young leaves (Field and Mooney, 1983; Hirose and

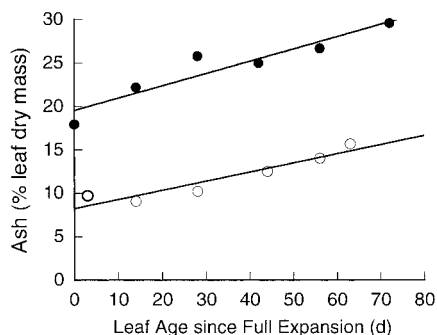


Fig. 7. Increase of ash content (percentage of dry mass) with leaf age in *Cecropia* (open symbols; $\text{Ash}[\%] = 0.106[\text{leaf age}] + 6.0$, $P = 0.004$) and *Urera* (closed symbols; $\text{Ash}[\%] = 0.142[\text{leaf age}] + 15.6$, $P = 0.005$).

Werger, 1987a; Ackerly, 1992; Hikosaka, Terashima, and Kato, 1994; Ackerly and Bazzaz, 1995).

Leaf age, nitrogen reallocation, and photosynthetic capacity—In both species, the decrease in photosynthetic nitrogen use efficiency (A/N_a) contributed to the decline of photosynthetic capacity with leaf age. Such decreases in A/N_a with leaf age have been observed commonly (Hirose and Werger, 1987b; Reich, Walters, and Ellsworth, 1991; Sobrado, 1992; Witkowski et al., 1992; Reich et al., 1994; but see Mooney et al., 1981; Field and Mooney, 1983). In *Urera*, decreasing N_a with leaf age, presumably due to nitrogen reallocation from old to new leaves, also contributed to the decline of photosynthetic capacity. However, in *Cecropia*, N_a did not change with leaf age.

While the pattern found for *Urera* was consistent with theories of optimal resource allocation, the lack of nitrogen reallocation in *Cecropia* was surprising. The equilibrium number of leaves for *Cecropia* was only six per branch. These leaves were displayed in a manner that minimized self-shading within the orthotropic branches. Although the estimated leaf area index of a mature *Cecropia* crown was only ca. 1 (S. Mulkey, unpublished data), there was a sharp decline of light availability with leaf age within each branch (Fig. 5A, B). Based on photosynthetic light-curve characteristics and ambient light measurements, *Cecropia* leaves senesced and abscised before self-shading caused midday light levels to drop below saturating PFD. In contrast, orthotropic terminal branches of *Urera* held more than twice as many leaves, which experienced a lower and narrower range of light than *Cecropia*. Although *Urera* experienced lower light availability than *Cecropia* leaves, *Urera* maintained higher A/N_a (Fig. 6C, D) through acclimation and redistribution of nitrogen. The significant decrease of dark respiration in *Urera*, but not in *Cecropia*, also suggests a greater acclimation potential of *Urera* leaves (Table 3).

The increase in LMA with leaf age contributed to the decrease of N_m and A_{mass} in both *Cecropia* (which did not change N_a) and *Urera* (which reallocated nitrogen to younger leaves). Leaf mass per area also increased with leaf age in the five other tree species we studied at the same site. These ubiquitous increases in LMA with leaf age were at least partly due to accumulation of mineral ash, especially silicon and calcium, in leaves of these species (K. Kitajima, unpublished data). In particular, the observed LMA increase was entirely due to ash accumulation in *Urera* and due to both ash and carbon accumulation in *Cecropia*. The ratio of structural and nonstructural carbon, however, did not change consistently with leaf age in either species (Terwilliger et al., 2001).

The decline in mesophyll conductance with leaf age (Loreto et al., 1994) may be another reason for lowered nitrogen use efficiency. Lower stomatal conductance (G_s) and water use efficiency (or ratio of A to G_s) are often, but not always, observed with leaf aging (Field and Mooney, 1983; Sobrado, 1992; Witkowski et al., 1992; Dawson and Bliss, 1993). In our study, only *Urera* exhibited lower G_s with leaf age (Fig. 5F), while only *Cecropia* exhibited a decline in water use efficiency (Fig. 4 of Terwilliger et al., 2001).

Summary—The effect of leaf age on photosynthetic capacity per unit leaf area was similar for the two species, but differed slightly in the direction expected by cost-benefit theory. *Urera*, with a longer leaf lifetime, exhibited a slightly shall-

lower slope in A -leaf age relationship. Repeated measurements of the same leaf demonstrated variation within species, apparently reflecting differences among branches in rates of leaf production and self-shading. Measurements of leaf age sequences in 3–4 branches produced A -leaf age slopes roughly similar to those from repeated measurements of individual leaves. We conclude that either method is acceptable for species with successive leaf production, as long as the sample size is adequate for sampling heterogeneity among branches and leaves. Interestingly, leaf age and position were more reliable predictors of photosynthetic rate than variables that are considered to be the physiological bases of the A -leaf age relationship, such as %PAR, LMA, N_a , and G_s . This suggests that the economic trade-off between leaf longevity and photosynthetic rate is the main determinant of A -leaf age relationship.

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