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Leaf longevity, construction, and maintenance costs of three mangrove species under field conditions

N. SUÁREZ

Laboratorio de Fotosíntesis y Metabolismo Vegetal, Departamento de Biología de Organismos,
Universidad Simón Bolívar, Apartado 89.000, Caracas 1080A, Venezuela

Abstract

This study assessed the effect of leaf age on construction cost (CC) in the mangrove species *Avicennia germinans, Laguncularia racemosa,* and *Rhizophora mangle* growing in their natural habitat. Leaf osmolality values were species-specific, the highest in *A. germinans* (1 693 mmol kg^{-1}) and the lowest in *L. racemosa* (1 270 mmol kg^{-1}). In the three species, contents of chlorophyll (*a*+*b*) (Chl$_{a+b}$) and nitrogen (N) per unit of leaf area were maximal in adult leaves and tended to decline with age. Leaf mass to leaf area ratio (LMA) and ash content increased during leaf ageing. Similarly, as leaves aged, a significant increase in leaf construction cost per leaf area (CC$_a$) was observed, while per leaf mass (CC$_m$) it remained almost constant, suggesting a sustained production of leaf compounds as leaves became older. CC was positively correlated with LMA and heat of combustion (Hc) per leaf area, suggesting differences among species in the quantity and composition of expensive compounds. Leaf half lifetime (t$_{0.5}$) showed contrasting values in the three mangrove species (60, 111, and 160 d in *L. racemosa, R. mangle,* and *A. germinans*, respectively). Overall, *L. racemosa* was the species with less expensive leaves to construct while leaves of *A. germinans* and *R. mangle* had the highest CC$_m$ and CC$_a$, respectively. Leaf longevity was positively correlated with the ratio between CC and maximum photosynthetic rate (P_{max}), clearly showing the existence of a balance between leaf costs and benefits.

Additional key words: ash; *Avicennia germinans*; heat of combustion; *Laguncularia racemosa*; leaf age; leaf half lifetime; *Rhizophora mangle*; salinity.

Introduction

In mangrove environments, the life span of leaves would depend on the relation between the salt discharge rate in the leaf and its assimilation capacity, which in turn might be modulated by the salt concentration in the substrate and by the time of exposure to salt (Clough *et al.* 1982). The leaf life span is related to the balance between costs and benefits associated with leaf construction and maintenance (Chabot and Hicks 1982, Harper 1989, Kikuzawa 1991, Reich *et al.* 1991). Thus, leaf longevity may be considered as a balance between lifetime carbon gain of a leaf and its construction and maintenance costs (Chabot and Hicks 1982, Givnish 1983, Reich *et al.* 1991, Kitajima *et al.* 1997). Cost-benefit analyses of leaf longevity in a wide range of species suggest that longevity increases when the ratio of construction cost (CC) to maximum photosynthetic rate (P_{max}) increases (Williams *et al.* 1989, Reich *et al.* 1991, Sobrado 1991, Griffin 1994) or when the initial photosynthetic rate decreases slowly as leaf ages (Kikuzawa 1991).

Leaf cost varies with the chemical composition and ageing of the leaf tissue and is often related to the habitat and microhabitat of a particular species (Merino *et al.* 1984). Leaf benefits, in terms of photosynthetic capacity, depend on the biochemical limitations due to nitrogen (N) investment in the photosynthetic machinery that finally are reflected in the N use efficiency (Field and Mooney 1983, Evans 1989). In addition, limitations on the photosynthetic processes are reflected in compromises between carbon gain and water loss (Farquhar and Sharkey 1982).

Under saline stress, the intensity and duration of exposure may affect the balance between the costs and benefits associated with the construction and maintenance of a leaf. Additionally, costs and benefits may be related to salt tolerance mechanisms that differ among species.

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Fax: 58-212-906 3064, e-mail: natsuare@usb.ve

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Thus, this study was designed to test the hypothesis that salt tolerance mechanisms may influence leaf costs while soil salinity may affect the payback time in the mangrove species *Avicennia germinans, Laguncularia racemosa,* and *Rhizophora mangle*. We investigated the effect of leaf age on construction cost for each of these species, and the relationships of leaf longevity to construction cost (CC) and gas exchange were compared among species. The three species under study are commonly found in fringe mangrove areas along coastal lines of Tropical and Subtropical America (Tomlinson 1986). These fringe environments are characterised by short and erratic rainfall periods and by the presence of areas with contrasting salinity and water availability as a result of variations in rainfall patterns and topographic characteristics (Tomlinson 1986, Lin and Sternberg 1993). The three mangrove species differ in their salt tolerance and in their physiological mechanisms to avoid salt accumulation. *R. mangle* is considered very efficient in excluding salt by roots and is found near shore areas where water movement is continuous and salinity fluctuations are low, whereas the salt secreting species *L. racemosa* and *A. germinans* grow in temporally dry areas with wider fluctuations in soil salinity.

Materials and methods

Study site and plants: The mangrove communities studied are located at the Bocaína Lagoon (10º28'N, 67º55'W) in the San Esteban National Park, Puerto Cabello (Estado Carabobo, Venezuela), where rainfall averages 489 mm and exhibits a bimodal distribution with peaks in June-July and November-December, the latter being generally higher. Annual temperature averages 26.7 ºC and tank A evaporation is 1 826 mm. Mangrove species are represented around the Bocaína lagoon by *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* Gaertn. (Combretaceae), and *Avicennia germinans* (L.) Stearn (Avicenniaceae). *R. mangle* is found at the lagoon fringe, where salinity is similar to average seawater; *L. racemosa* is found a few meters behind the fringe, where soil saturates with rain water during the wet season; *A. germinans* occurs at the outermost inland ring, with hyper-saline soils that are flooded with rain water during the wet season. Additional details on the site characteristics for each species in the study area can be found in Suárez *et al.* (1998) and Sobrado (1999a,b).

The experiments were conducted during the wet (November 2001) and dry (February 2002) seasons. A relative scale based on leaf colour, size, and texture, and position in the branch was used to classify the leaves according to age. Quantitative differences among these classes were determined by leaf mass to leaf area ratio (LMA) and the amount of ash in leaf tissue. During the wet season, leaves were classified as: Young leaves (age 1), soft, still expanding, first pair of leaves in the branch; adult leaves (age 2) of healthy appearance, second leaf pair in *A. germinans* and *R. mangle*, and second to third leaf pair in *L. racemosa*; and old leaves (age 3) with yellowish or brownish spots representing the third leaf pair in *R. mangle* and the third to fourth leaf pair in *A. germinans* and *L. racemosa*. During the dry season no expanding leaves were found and only adult leaves were collected and classified as mature leaves (age 4). In *A. germinans* leaves of age 4 showed yellowish spots that indicated beginning of senescence. However, the leaves were not comparable during the dry season because of their different longevity.

Measurements: Two hundred leaves of 4–8 plants per species were tagged with coloured plastic rings during the wet season, when leaf production was active. At the time of tagging, leaves had attained 40 (*L. racemosa*), 50 (*R. mangle*), and 34 (*A. germinans*) % of their full size as determined *a posteriori*, when expansion was completed.

Leaves were counted two and four months after tagging, recording the number of remaining leaves. These records were used to estimate the leaf half lifetime ($t_{0.5}$) and mortality rate (λ) for each species according to Lieberman *et al.* (1985). The half lifetime ($t_{0.5}$) is defined as the time that would be taken for a leaf population to lose 50 % of its leaves:

$$t_{0.5} = (\log_e{}^2)/\lambda.$$

The mortality rate (λ) was estimated for each species through the non-linear adjustment of the survival probability as a function of time (Lieberman *et al.* 1985):

$$\lambda = (\log_e No - \log_e Nf)/t$$

where λ is considered constant through time, and No and Nf are the leaf counts at the beginning and at the end of the measurement interval t (Lieberman *et al.* 1985).

Leaves from four different individuals per species and per age were analysed to characterise the different ages during the wet season (ages 1, 2, and 3) and mature leaves in the dry season (age 4). LMA, contents of chlorophyll (Chl_{a+b}), nitrogen (N), and ash, and ash-free heat of combustion (Hc) were determined. For Chl_{a+b} determinations, five to six leaves of each age per tree were frozen after collection. Chl was extracted with 80 % acetone and the amount calculated according to Lichtenthaler and Wellburn (1983).

For each species, fresh leaves from three shoots from four trees were cleaned and their area determined after removing the middle vein. The sampled leaves were dried in a ventilated oven (60 ºC, 48 h), their dry mass measured, and thereafter ground and homogenised for subsequent analyses. Nitrogen content was measured by the micro-Kjeldahl method (Williams 1984). Ash content was determined gravimetrically in a muffle oven for 4 h at 550 °C. Hc was determined with a Parr Adiabatic Calorimeter model *1241* (*Parr Instrument Co.*, Moline, IL, USA) with correction for nitric acid formation and ignition wire melting. Crude lipid content was measured only in adult leaves collected in the wet season. The lipids, extracted with diethyl ether, were quantified gravimetrically (Williams 1984).

Leaf construction costs per unit leaf mass (CC_m) [kg(glucose) kg^{-1}] were estimated from N [kg kg^{-1}], ash [kg kg^{-1}], and Hc [MJ kg^{-1}] following Williams *et al.* (1987):

$$CC_m = \{[(0.06968\ Hc - 0.065)\ (1 - ash)] + \\ + [(K\ N/14.0067)\ (180.15/24)]\}/0.89$$

where the oxidation state of nitrogen as substrate (K) was –3 and +5 for ammonium and nitrate, respectively. The maintenance cost per unit dry matter per day (MC_m) was estimated in adult leaves from the wet season (age 2) and was determined from contents of lipids, protein, and ash in leaf tissue. The protein fraction was estimated by multiplying N content by 6.25 (Williams 1984). The conversion efficiency of all fractions is expressed in grams of glucose required for synthesising one gram of the fraction per day. Accordingly, the maintenance coefficients in leaf tissue were 0.0425 for lipids, 0.028–0.053 for proteins, and 0.006–0.010 for ash (Merino *et al.* 1984). Maximum and minimum construction and main-tenance costs were estimated and expressed per unit of leaf area (CC_a and MC_a) and of dry mass (CC_m and MC_m).

Leaves of each age, similar to those collected to estimate leaf composition, were used to determine osmolality. Leaves were rinsed with distilled water and dried with soft tissue to eliminate salt from the surface. After eliminating the middle vein, the blade was placed in a syringe and frozen in liquid nitrogen. The frozen samples were thawed during 0.5 h at room temperature before extracting the sap to determine osmolality with a vapour pressure osmometer model *5500* (*Wescor*, Logan, UT, USA). A total of 10–25 leaves from each species were analysed.

Gas exchange parameters were measured on sunny mornings at the end of the wet season, on 20–28 intact fully sun exposed adult leaves (age 2) from five different plants from each species using an open system (*LCA-3*, *ADC*, Hoddesdon, UK). Photosynthesis measurements were made at irradiances over 1.0 mmol m^{-2} s^{-1}, which are generally saturating; thus, photosynthesis rates were close to maximal (P_{max}). Leaf temperature was 29–39 °C and airflow was 300 mmol s^{-1}. Relative humidity and ambient partial pressure of CO_2 were kept at ambient levels (44 % and 35 Pa, respectively). Additionally, the potential instantaneous nitrogen use efficiency (NUE) was estimated from the ratio between P_{max} and leaf N concentration.

Statistical analyses: Leaf osmolality, LMA, Chl_{a+b}, N, and ash contents, Hc, CC_m, and CC_a at different ages were compared separately for each species using one-way ANOVA (Sokal and Rohlf 1969). Comparisons between species were restricted to adult healthy leaves during the wet season; the three species were compared in their Hc, lipid, MC, and CC by using a one-way ANOVA test. LSD and Dunnet's T3 were performed as *a posteriori* tests, when homogeneous and non-homogeneous variance was found in the data, respectively. A significance value of $p \leq 0.05$ was used throughout.

Results and discussion

Leaf characteristics and composition: During the wet season, soil salinity was close to seawater values at the site occupied by *R. mangle*, whereas salinity at the sites thrived by *A. germinans* and *L. racemosa* was about 50 and 20 % that of seawater, respectively (Table 1). On drought onset, soil salinity increased slightly at the *R. mangle* site (55 %) in comparison to the severe increase in salinity observed at sites where *L. racemosa* (450 %) and *A. germinans* (300 %) grow.

Leaf osmolality values were species-specific, with the highest values measured in *A. germinans* and the lowest in *L. racemosa* (Table 1). During the dry season, the largest change in leaf osmolality was detected in *R. mangle* (~33 %), intermediate in *A. germinans*

Table 1. Soil salinity [‰] and leaf osmolality [mmol kg^{-1}] in leaves classified as young (1), adult (2), and old (3) during the wet season (WS) and in mature leaves (4) during the dry season (DS), from the three mangrove species studied. The number of samples was 6 for soil salinity and 10-25 for leaf osmolality. Means ± SD for each variable. In the same row, means followed by different letters were statistically different at $p \leq 0.05$.

		Age	*L. racemosa*	*R. mangle*	*A. germinans*
WS	Soil salinity		7.3 ± 4.6	31.7 ± 3.5	17.0 ± 2.5
	Leaf osmolality	1	1234 ± 79 a	1411 ± 124 a	1675 ± 306 a
		2	1291 ± 65 a	1481 ± 96 b	1703 ± 303 a
		3	1286 ± 47 a	1568 ± 118 c	1701 ± 344 a
DS	Soil salinity		40.3 ± 9.1	49.0 ± 4.4	67.9 ± 6.6
	Leaf osmolality	4	1308 ± 119 a	1982 ± 140 d	2053 ± 89 b

(~21 %), and slight in *L. racemosa* (~3 %). *R. mangle* is a non-secreting mangrove, so its leaves exhibit a higher accumulation of salt as they mature during the wet season, and this effect was enhanced during drought. The basic mechanism of salt exclusion in this species occurs at root level, which may not be very efficient in environments with large fluctuations in salinity (Scholander *et al.* 1962, Atkinson *et al.* 1967). Lin and Sternberg (1993) reported that fluctuating salinity has significant negative effects on the photosynthesis and growth of *R. mangle*. In contrast, *A. germinans* and *L. racemosa* are salt secreting mangroves, which in fact contributes to salt balance with leaf age, and excessive salt accumulation was not detected during the dry season. Consistent with this, leaf Na^+ and Cl^- contents in secreting mangrove species do not change significantly with ageing (Atkinson *et al.* 1967, Popp 1984). Near constant salt content of mature leaves suggests that ion compartmentalisation within the vacuole is limited once cells reach expansion (Waisel *et al.* 1986). Once the leaf reaches maturity, salt must be removed, both by leaf gland secretion and by retranslocating ions out of the mature leaf (Scholander *et al.* 1962, Drennan and Pammenter 1982, Waisel *et al.* 1986). Thus, ion exclusion by roots may be a less efficient mechanism in environments where soil salinity fluctuates than in those where soil salinity is relatively constant. Nevertheless, leaf osmolality correlated better with soil salinity averaged over wet and dry seasons than with soil salinity at each season separately. Thus, *A. germinans* had higher leaf osmolality than *L. racemosa* and *R. mangle*, possibly reflecting the higher permeability to salts of the roots (Scholander *et al.* 1962; Table 1). Overall, the changes that are observed in leaf osmolality during drought are similar to those found in previous studies with these species (Medina and Francisco 1997, Suárez *et al.* 1998). In the three mangrove species, Na^+ and Cl^- concentrations explained the changes in osmolality as a consequence of soil salinity enhancement by drought (Medina and Francisco 1997).

In all three species, Chl_{a+b} and N per unit leaf area showed maximal contents at age 2 with a tendency to decline with age or drought (Table 2). This is a common feature in leaf ageing; it has been observed that in mangrove N declines by 23 to 53 % depending on the species (Popp *et al.* 1984). Overall, leaf N and Chl_{a+b} values were similar to those previously reported in these mangrove species (Medina and Francisco 1997, Sobrado 1999a,b). As expected, LMA was lower in young leaves and tended to increase with the accumulation of structural compounds during leaf ageing. Ash contents per mass unit increased slightly with leaf ageing and were similar among species: 9.9–16.6, 9.5–17.3, and 11.4–19.4 g kg^{-1} in *L. racemosa, R. mangle,* and *A. germinans*, respectively. These values are relatively high when compared with those found in a wide range of non-mangrove species (Merino *et al.* 1984, Pipp and Larcher 1987, Sobrado 1991). Thus, ash content in mangrove may reflect the large accumulation of inorganic ions generally occurring in halophytic species.

Table 2. Chlorophyll (Chl_{a+b}), nitrogen (N), and ash contents in leaves classified as young (1), adult (2), and old (3) during the wet season (WS) and in mature leaves (4) during the dry season (DS), from the three mangrove species studied. Number of samples analysed was 18 for Chl_{a+b}, 12 for N, and 4-6 for ash. Means ± SD for each variable. In the same row, for each variable means followed by different letters were statistically different at $p \leq 0.05$.

			L. racemosa	*R. mangle*	*A. germinans*
Longevity, $t_{0.5}$[d]			59.7	100.6	159.7
		Age			
Chl_{a+b} [μmol m^{-2}]	WS	1	362.3 ± 54.0 a	242.8 ± 40.1 a	329.7 ± 41.5 a
		2	411.4 ± 58.1 b	284.7 ± 33.1 b	341.9 ± 41.0 a
		3	271.2 ± 41.8 c	178.6 ± 39.9 c	321.6 ± 30.3 a
	DS	4	444.7 ± 56.7 b	244.5 ± 33.8 a	225.4 ± 53.7 b
Nitrogen [mmol m^{-2}]	WS	1	125.8 ± 17.6 a	152.6 ± 11.5 ab	230.1 ± 14.8 a
		2	131.8 ± 15.1 a	164.8 ± 14.5 a	253.3 ± 25.6 b
		3	107.7 ± 9.9 b	148.2 ± 24.2 b	221.2 ± 24.8 ac
	DS	4	131.5 ± 13.1 a	146.5 ± 12.6 b	209.3 ± 28.2 c
Ash [g m^{-2}]	WS	1	11.37 ± 1.09 a	16.01 ± 1.16 a	16.83 ± 2.56 a
		2	16.65 ± 1.74 b	19.84 ± 3.02 a	19.37 ± 3.15 ab
		3	24.02 ± 1.46 c	35.50 ± 5.04 b	21.30 ± 2.30 b
	DS	4	18.56 ± 2.82 b	27.16 ± 4.42 b	33.68 ± 2.33 c

Leaf half-life ($t_{0.5}$) was markedly different among the three mangrove species, and longer survival periods in *R. mangle* and *A. germinans* than in *L. racemosa* were accompanied by larger values of N and ash (Table 2), and LMA. Therefore, the differences in leaf longevity were strongly related to variations in leaf physiological and structural traits, as shown with other species (Williams *et al.* 1989, Reich *et al.* 1991, Sobrado 1991). On the other hand, leaf longevity in the three species was negatively correlated with soil salinity during the dry season ($r^2 = 0.99$, $p < 0.05$), implying that leaf longevity is modulated by the salinity intensity experienced by this species when water availability is low and growth is limited. Leaves of *L. racemosa* collected during the dry season (age 4) had similar values of Chl_{a+b}, N, and ash as age 2 leaves collected during the wet season (Table 2), which is consistent with the lowest leaf longevity exhibited by this species. In contrast, during the dry season (age 4) values of Chl_{a+b}, N, and ash in *A. germinans* and *R. mangle* leaves suggest that these were older, or of similar age, than leaves collected during the wet season (Table 2). An alternative explanation is that salinity, in combination with drought, leads to reductions of leaf N and Chl_{a+b} and increment of ash, specially in *A. germinans*. Thus, we do not discard that leaves collected during the dry season were produced during that season. Similarly, Medina and Francisco (1997) reported that the N content in mangrove species decreases in the dry season. In addition, LMA in *L. racemosa* and *A. germinans* increased during the dry season. In *A. germinans* LMA is also higher during the dry season than during the wet season (Sobrado 1999).

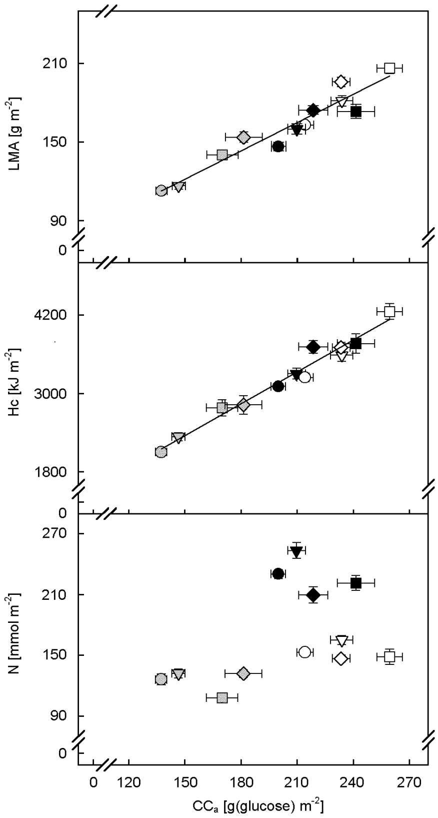

Fig. 1. Leaf mass to leaf area ratio (LMA), heat of combustion (Hc), and nitrogen content (N) as a function of minimum leaf construction cost on area basis ($\mathrm{CC_a}$) in *L. racemosa* (*grey symbols*), *R. mangle* (*white symbols*), and *A. germinans* (*black symbols*). Measurements were taken in leaves classified according to age as young (age 1, *circles*), adult (age 2, *triangles*) and old (age 3, *squares*) during the wet season and mature leaves (age 4, *diamonds*) during the dry season. Means ± SE. $n = 12$ for LMA and N, $n = 4$–6 for Hc and $\mathrm{CC_a}$. Lines fitted by linear regression were statistically significant at $p \leq 0.05$.

Leaf construction cost: During the wet season, in the three species, a significant increase in leaf construction cost per leaf area ($\mathrm{CC_a}$) was observed as leaves aged (Fig. 1). However, construction cost per leaf mass ($\mathrm{CC_m}$) remained almost constant until leaf senescence. Increases in $\mathrm{CC_a}$ and relatively constant $\mathrm{CC_m}$ suggest a sustained production of expensive and inexpensive leaf compounds as leaves became older. These results are in contrast with the view that during leaf lifetime, CCs decrease as a result of the synthesis of less expensive fractions, translocation, molecular inter-conversion, and leaching, as well as N dilution by non-structural saccharides (Merino *et al.* 1984, Diamantoglou *et al.* 1989). Indeed, the heat of combustion (Hc) per leaf mass unit did not decrease as leaves aged (18.3–19.2, 19.5–20.3, and 21.1–21.6 MJ kg^{-1} in *L. racemosa, R. mangle,* and *A. germinans*, respectively). Overall, Hc per leaf mass unit values were similar to those in other halophytic species (Pipp and Larcher 1987). Leaf caloric content is a reliable estimate of the synthetic cost of leaves (McDermitt and Loomis 1981). We found that N content decreased in senescing leaves (age 3) suggesting translocation of this highly expensive and mobile element. However, contents of other high cost compounds may increase with age.

The meaning and usefulness of mass-based and area-based estimates of leaf CC are not clear when experimental treatments of environmental variables influence LMA (Griffin 1994). $\mathrm{CC_a}$ results from multiplying LMA by the energetic cost of constructing leaf tissues. Griffin (1994) reported that CC and Hc per mass of leaf are relatively constant and therefore variations in $\mathrm{CC_a}$ are the consequence of changes in LMA. According to this view, when data from the three species were pooled, LMA values were positively correlated with $\mathrm{CC_a}$ ($r^2 = 0.92$, $p < 0.05$). Increases in LMA might be caused either by an increase in the number of cell layers per unit area or by the accumulation of non-structural saccharides. If added cell layers were of similar biochemical composition, CC would increase on an area basis, but remain constant on a mass basis (Griffin 1994). In fact, leaves are smaller and tightly arranged in response to elevated soil salinity (Sobrado 1999). However, Hc per leaf area was also correlated with $\mathrm{CC_a}$ ($r^2 = 0.96$, $p < 0.05$) suggesting differences in the quantity and composition of expensive compounds among species.

Overall, leaves from *L. racemosa* were the least expensive to construct, both per leaf area and mass (Fig. 1, Table 3). In contrast, leaves of any age of *A. germinans* were the most expensive to construct per leaf mass while *R. mangle* had the highest $\mathrm{CC_a}$. High $\mathrm{CC_m}$ in *A. germinans* may be related with the high N content,

Table 3. Heat of combustion (Hc), content of total lipids, minimum and maximum construction (CC), and maintenance (MC) costs in adult leaves of *L. racemosa*, *R. mangle,* and *A. germinans* during the wet season (WS). Means ± SD for each variable. For each variable and different species means followed by different letters were statistically different at $p \leq 0.05$; n = 4–6.

		L. racemosa	*R. mangle*	*A. germinans*
Hc [kJ m^{-2}]		2336 ± 137 a	3582 ± 236 b	3299 ± 186 c
Hc [MJ kg^{-1}]		19.13 ± 0.41 a	19.80 ± 0.27 b	21.53 ± 0.37 c
Lipids [g m^{-2}]		4.69 ± 0.90 a	6.92 ± 0.45 b	4.59 ± 0.45 a
CC_m [kg(glucose) kg^{-1}]	Min	1.20 ± 0.03	1.29 ± 0.03	1.37 ± 0.05
	Max	1.28 ± 0.02 a	1.35 ± 0.03 b	1.48 ± 0.05 c
MC_a [g(glucose) m^{-2} d^{-1}]	Min	0.62 ± 0.04	0.81 ± 0.04	0.94 ± 0.04
	Max	0.97 ± 0.06 a	1.25 ± 0.06 b	1.57 ± 0.06 c
MC_m [g(glucose) kg^{-1} d^{-1}]	Min	5.3 ± 0.3	4.5 ± 0.1	5.9 ± 0.2
	Max	8.3 ± 0.3 a	6.9 ± 0.2 b	9.9 ± 0.4 c

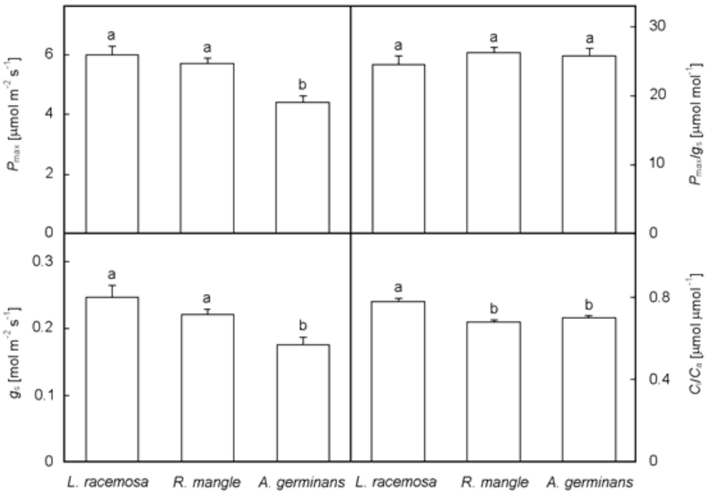

Fig. 2. Maximum photosynthetic rate (P_{max}), stomatal conductance (g_s), P_{max}/g_s ratio, and internal to ambient CO_2 concentration ratio (C_i/C_a) measured at midday in adult leaves of *L. racemosa*, *R. mangle,* and *A. germinans* at the end of the wet season. *Columns* represent the mean of 20–28 leaves and SE is indicated. *Columns* marked by different letters were statistically different at $p \leq 0.05$.

whereas the high CC_a of *R. mangle* may be a consequence of a larger fraction of lipids per leaf area (6.92 g m^{-2}) as compared with the other two species (4.7 and 4.6 g m^{-2} in *L. racemosa* and *A. germinans*, respectively) and of the high LMA. Adult leaves of *R. mangle* are the richest in lipid and ash fraction per leaf area unit, but they have the lowest protein content in comparison with the other two species. *R. mangle* and *A. germinans* leaves are more expensive to maintain per leaf area unit than the short-lived leaves of *L. racemosa*. A positive relationship between construction and maintenance costs has been found in a tropical forest species (Sobrado 1991); however, such relationship was not observed in this study of mangrove species.

CC of leaves is defined as the quantity of glucose required to provide the carbon skeletons, reductants, and ATP for the synthesis of organic compounds by standard biochemical routes (Williams *et al.* 1987). Construction and maintenance costs may increase as a consequence of salt accumulation avoidance mechanisms, because these involve the synthesis of expensive compounds, reduction and high energy investment, transport processes, and nutrient uptake (Yeo 1983). Under high salinity, ion intracellular compartmentation becomes very important as cytoplasmic enzyme activity is affected by NaCl (Flowers *et al.* 1977, Yeo 1981). Compartmentation by vesiculation of plasmalemma and tonoplast formation implies an increase of membrane material and enhanced lipid synthesis (Prud'homme *et al.* 1990). Additionally, the lipid composition of membranes changes in the presence of salt affecting the permeability, fluidity, and selectivity of the membrane (Erdei *et al.* 1980). Transport of inorganic ions and synthesis of compatible organic solutes are always higher in mangroves under hyper-salinity conditions (Flowers *et al.* 1977, Yeo 1983, Popp 1984, Popp *et al.* 1984, Ashihara *et al.* 1997) and both processes are regarded as costly in terms of energy (Yeo 1983). Organic acids have a cost, in terms of glucose, of 0.379–0.869 kg(glucose) kg^{-1} (Williams *et al.* 1987). Finally, both secretion and re-translocation by the phloem are processes that would imply expensive transport across the membrane and metabolic interactions.

Gas exchange: Maximum photosynthetic rate (P_{max}) and stomatal conductance (g_s) were not clearly different between *L. racemosa* and *R. mangle* but were slightly lower in *A. germinans* (Fig. 2). However, intrinsic water use efficiency (P_{max}/g_s) and ratio of intercellular and ambient CO_2 concentration (C_i/C_a) were comparable among species. A slightly higher C_i/C_a ratio was found in *L. racemosa*, indicating a greater decrease in the capacity for CO_2 assimilation as compared to g_s (Wong *et al.* 1979). C_i values were 264, 230, and 237 × 10^3 cm^3 m^{-3} in *L. racemosa, R. mangle,* and *A. germinans*, respectively. Salinity affects photosynthesis due to effects on stomata as well as to metabolic factors (Ball and Farquhar 1984, Naidoo and Willert 1995, Sobrado 1999). Overall, mangrove species under increasing salinity show that the decline in P_{max} overrides the decline in g_s resulting in improved leaf water efficiency (Ball and Farquhar 1984, Clough and Sim 1989, Naidoo and Willert 1995, Sobrado 1999a,b).

P_{max} was not related to N content, suggesting that the investment of this nutrient in the photosynthetic machinery and in protective non-photosynthetic compounds was different (Field and Mooney 1983, Evans 1989). In fact, the ratio Chl/N [mmol(Chl_{a+b}) mol^{-1}(N)] was lower in *A. germinans* (1.4) than in *L. racemosa* (3.2) and *R. mangle* (1.7). Additionally, NUE differed among species, with values of 46, 35, and 17 mmol(CO_2) mol^{-1}(N) s^{-1} in *L. racemosa, R. mangle,* and *A. germinans*, respectively. Differences in NUE may result from investment differences in protective *vs.* photosynthetic compounds (Evans 1989). Therefore, low leaf NUE in *A. germinans* may be a consequence of a large N investment in cell walls, specialised cells, and other compounds not associated with photosynthesis (Lambers and Poorter 1992). Synthesis of compatible solutes is of paramount importance in plants thriving in saline environments (Storey and Wyn Jones 1977). In fact, *A. germinans* exhibits high accumulation of quaternary ammonium compounds as principal compatible solutes (Storey and Wyn Jones 1977, Popp *et al.* 1984). Nevertheless, our results indicated that NUE and P_{max} were positively correlated ($r^2 = 0.96$, $p < 0.05$). Additionally, when leaf longevity increased and, consequently, the residence time of N also increased, NUE decreased. Accordingly, *L. racemosa*, with the lowest leaf longevity, had the highest NUE value, and the opposite occurred in *A. germinans*. This would mean that leaf longevity would be related to a balance between cost and benefits, or payback potential (Williams *et al.* 1989). In this study, leaf longevity was positively correlated with the CC_a/P_{max} ratio ($r^2 = 0.81$, $p < 0.05$). This relationship has been found in a number of species from different non-saline habitats (Williams *et al.* 1989, Reich *et al.* 1991, Sobrado 1991, Eamus *et al.* 1999). Thus, the payback interval for *A. germinans* is larger than for *R. mangle* and *L. racemosa* and thus, the high CC_a/P_{max} ratio results from the longer time taken for carbon fixation per leaf.

In conclusion, *L. racemosa*, growing in less saline soil, was the species with the lowest leaf osmolality and the shortest leaf life span. However, the costs to construct and maintain their leaves were low and consequently leaf payback would occur rapidly and leaf turnover would have a relatively low cost. Rapid replacement of older leaves by new ones with greater photosynthetic rate may be an adaptive advantage for this species (Escudero and del Arco 1987). Conversely, *A. germinans* exhibited the highest leaf osmolality and leaf longevity. Their leaves had high construction and maintenance costs per leaf mass, probably related to the greater N content. However, P_{max} of *A. germinans* was relatively low, probably because N investment in the photosynthetic machinery was reduced at the expense of investing in non-photosynthetic compounds. Therefore, high longevity in *A. germinans* may be required in order to warrant payback in nutrient-poor and hyper-saline soils. Finally, leaves of *R. mangle* presented slightly higher CC_a values than those of *A. germinans*. However, their P_{max} was comparable with that of *L. racemosa*, so this species would have an intermediate payback time as showed by its leaf longevity. Although leaf cost-benefits of the three mangrove species and their physiological responses for salt regulation did not show a clear relationship, leaf life span was related to the soil salinity of the habitat occupied by each species, indicating the existence of a balance between leaf costs and benefits.

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