BIOLOGIA PLANTARUM 49 (1): 111-116, 2005

Leaf construction cost in *Avicennia germinans* as affected by salinity under field conditions

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Abstract

This study assessed the effects of salinity and drought upon leaf construction (CC) and maintenance (MC) costs in *Avicennia germinans* growing under contrasting soil salinities in the field. Additionally, an analysis of leaf chemical composition was carried out to determine the underlying reasons for possible variation in CC. With the increase of external salinity, leaf osmotic potential (ψ_s) declined and a significant negative correlation was found between both variables. Changes in average CC were about 15 % when ψ_s changed by 85 %. Despite this, leaf ash-free heat of combustion per unit of leaf dry mass remained relatively constant at 21.3 ± 0.9 kJ g^{-1} in all conditions. The estimated maximum and minimum CC for the synthesis of protein present in leaves increased significantly with total leaf CC. Conversely, the estimated CC for lipid synthesis shows a tendency to decrease with total leaf CC. Under conditions of high salinity, ash content increased by 78 % compared to that of leaves collected at low salinity. It is concluded that undergoing changes in proteins and lipids (expensive) and ash (cheap) explain the changes in CC under contrasting salinities. Additionally, the average MC remained almost constant at moderate salinity and declined by 6 % under hyper-saline conditions. Thus, differences in CC and MC with salinity are explained in part by changes in the chemical composition of different compound fractions.

Additional key words: ash, crude fiber, heat of combustion, leaf maintenance cost, lipids, mangroves, osmotic potential.

Introduction

Mangrove species are subjected to spatial and temporal changes in soil salinity as the result of variations in rainfall patterns and terrain topography (Lugo and Snedaker 1974, Tomlinson 1986). *Avicennia germinans* is one of the most tolerant mangroves to both high and fluctuating salinity. Under natural conditions, it tolerates salinities ranging from close to freshwater up to three times that of seawater (Tomlinson 1986, Mallery and Teas 1984). The intensity and duration of exposure to salinity stress may affect the balance between the costs and benefits associated with the construction and maintenance of leaves. Leaf life span of mangroves has been related to soil water salinity (Suárez 2003). Additionally, leaf life span is positively correlated with the ratio between leaf construction cost and maximum photosynthetic rate in a number of species, which shows the existence of a balance between leaf costs and benefits (Merino *et al*. 1984, Chapin 1989, Poorter 1994).

Costs of leaf construction (CC) and maintenance (MC) vary with the chemical composition of the leaf tissue and are often related to the habitat and microhabitat of a particular species (Merino *et al*. 1984, Poorter 1994). Comparisons of CC in leaves within the same species resulted in variations of 5 - 7 % (nitrogen; Lafitte and Loomis 1988, Griffin *et al*. 1993), ≈11 % (light; Sims and Pearcy 1991), and 3 - 4 % (CO_2; Griffin *et al*. 1993).

Received 11 September 2003, *accepted* 27 April 2004.

Abbreviations: CC - construction cost; Chl - chlorophyll; d.m. - dry mass; Hc - heat of combustion; HS - high salinity; K - oxidation state of nitrogen; LMA - leaf dry mass to leaf area ratio; LS - low salinity; MC - maintenance cost; N - nitrogen; ψ_s - leaf osmotic potential.

Acknowledgements: Financial support was provided by the Decanato de Investigaciones-USB-S1-CB-008. INTECMAR-USB provided the field facilities. I thank Prof. Ernesto Medina for his support and for allowing me the use of his laboratory and field equipments. Lyz Avendaño and Carlos Grau for their patient assistance in the field. I am grateful to editor, anonymous referees, and M.A. Sobrado for helpful suggestions to improve this manuscript.

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Moreover, a comparison among species with different growth forms and leaf types or classes from different habitats resulted in differences between 3 to 12 % (Merino *et al.* 1984, Williams *et al.* 1987, Chapin 1989, Sobrado 1991, Eamus *et al.* 1999). In a previous study, we found that CC and MC differ among mangrove species grown at different salinities in the field (Suárez 2003). However, a full account of the effects of salinity on leaf chemical composition, and consequently on CC and MC, is lacking. Therefore, the present study with *A. germinans* represents a contribution towards the understanding of changes in CC and MC within species as a response to environmental stress. The occurrence of changes in leaf construction and maintenance with increasing salinity may be due to quantitative and qualitative variations in the chemical composition of plant tissues. The enhanced synthesis of expensive compounds and membrane material as consequence of intracellular salt accumulation and compartmentation is well documented (Yeo 1983, Venkatesan and Chellappan 1998, Del Zoppo *et al.* 1999, Mansour *et al.* 2002), and consequently salinity may influence leaf costs. Thus, this study aimed to assess the effects of salinity and drought upon leaf construction and maintenance costs in *A. germinans* growing under contrasting soil salinities in the field. Additionally, an analysis of leaf chemical composition was carried out to determine the underlying reasons for possible variation in CC.

Materials and methods

Study site and plants: Mangroves were studied in the Bocaína Lagoon (10º 28' N, 67º 55' W) within the San Esteban National Park, Puerto Cabello, Venezuela. The average annual precipitation is 489 mm, with peak rainfall occurring during June - July and November - December. The annual average air temperature is 26.7 ºC and tank A evaporation is 1826 mm. Further details about this site could be found elsewhere (Suárez *et al.* 1998, Sobrado 1999). High and low salinity sites were selected within this area. The high salinity site (HS) was near the lagoon, in an area that is flooded during the rainy season and severely drought during the dry season. Here the salinity was 17 ± 3 ‰ during the wet season and increased to 68 ± 7 ‰ during the dry season. The low salinity site (LS) was located at 100 m behind the fringe; it was characterised by a saturated, though not flooded, soil during the wet season. In this zone, the salinity was 10 ± 6 ‰ during the wet season and changed to 56 ± 2 ‰ during the dry season.

Plant measurements: The experiments were performed on four to six selected *Avicennia germinans* L. trees from each of the sites during the wet (November 2001) and the dry (February 2002) seasons. Leaf material for all the analyses was sampled in the early morning and cleaned carefully with tissue paper. The sampled leaves were mature and had a healthy appearance. In spite of that, leaf age was not necessarily comparable between the wet and dry seasons. The leaves of mangrove continue to build-up salt throughout their life span, and thus leaf sampling in these species is critical (Cram *et al.* 2002). However, the leaf age effect was considered minor since, in a previous study with mangrove species, including *A. germinans*, CC remained relatively constant until leaf senescence (Suárez 2003).

For leaf osmotic potential (ψ_s) measurements, eight leaf samples per salinity and season were collected. After removing their middle veins, they were stored in plastic syringes, and frozen in liquid nitrogen. Once in the laboratory the leaf samples were thawed for a half hour before measuring ψ_s using a vapour pressure osmometer model *5500* (*Wescor*, Logan, UT, USA). Additionally, the relationship of Na^+ and Cl^- as a function of ψ_s for this species was estimated from data obtained by Medina and Francisco (1997). The Na^+ and Cl^- contribution to ψ_s were 75 and 69 %, respectively in *A. germinans*. Afterwards, it was possible to calculate the leaf concentration of Na^+ and Cl^- for each salinity condition in this study. For chlorophyll (Chl) determinations, 12 - 30 fresh leaves per site per season were stored frozen in plastic bags after removing their middle veins. Chl was extracted with 80 % acetone and the amount thus extracted was calculated according to Lichtenthaler and Wellburn (1983). A total of 200 leaves from four branches per tree per season were collected for determination of leaf dry mass to leaf area ratio (LMA), content of nitrogen (N), lipids and ash, and ash-free heat of combustion (Hc). Leaf area was measured excluding the middle vein. The samples were then dried in a ventilated oven (60 ºC, 48 h) and weighed for calculation of LMA. Thereafter, leaf samples were ground and homogenised for subsequent analyses. N was measured by the micro-Kjeldahl method (Williams 1984). Ash content was determined gravimetrically in a muffle oven for 3.5 h at 500 ºC. 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, ash-free Hc being calculated afterward. Leaf crude fiber was determined after digestion in both acid, and basic media, and total lipids were extracted with diethyleter and quantified gravimetrically (Williams 1984).

CC per unit leaf dry mass [g(glucose) g^{-1}(d.m.)] was estimated from Hc [kJ g^{-1}(d.m.)], N [g g^{-1}(d.m.)], and ash [g g^{-1}(d.m.)] following Williams *et al.* (1987):

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

where the oxidation state of nitrogen as substrate (K) was -3 and +5 for ammonium and nitrate, respectively. Nitrate and ammonium were considered as the only source of nitrogen for calculation of maximum and minimum CC, respectively. MC per unit dry mass per day [mg(glucose) g^{-1}(d.m.) d^{-1}] was determined from the content of lipid, 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 mg of glucose required for synthesising 1 g of the respective fraction per day. Thus, the following maintenance coefficients were used: lipids: 4.25; protein: 2.8 (minimum), 5.3 (maximum); ash 0.6 (minimum), 1.0 (maximum) according to Merino *et al.* (1984). Costs for lipids and protein synthesis were estimated by multiplying leaf lipids and protein contents by the construction costs, derived from Penning de Vries *et al.* (1974). Costs and concentrations are expressed per unit dry mass to avoid the problem of variation in leaf dry mass to leaf area ratio (Poorter 1994).

Statistical analyses: The experimental results for the different variables were compared using a one-way *ANOVA* (Sokal and Rohlf 1969). Afterwards, Least Significant Difference and Dunnet's T3 tests were performed as *a posteriori* tests, when homogeneous and non-homogeneous variances, respectively, were found in the data. A significance value of $P < 0.05$ was used throughout.

Results and discussion

The leaf dry mass to leaf area ratios (LMA) showed a tendency to increase with salinity and drought (Table 1). The maximum increase, between the lowest salinity (wet season) and the highest salinity (dry season), was 30 %. This corroborates previous findings with mangroves regarding the increase of LMA as salinity becomes more severe (Ball and Pidsley 1995, Medina and Francisco 1997, Sobrado 1999). Conversely, Chl content decreased by 65 % at high salinity during the dry season compared with values measured at low salinity during the wet season (Table 1). Nitrogen concentration (N) was on average 19 % lower during the dry season as compared to the wet season (Table 1). Overall, leaf osmotic potential, N, and Chl values were in the ranges reported for mangrove species (Downton 1982, Popp *et al.* 1984, Medina and Francisco 1997, Sobrado 1999, Suárez 2003). Consequently, Chl/N ratio decreased as the result of both salinity and drought, suggesting that both factors lead to enhancement of N investment in cell walls, specialised cells, and other compounds not associated with photosynthesis (Lambers and Poorter 1992). With the increase of external salinity, leaf osmotic potential declined and a significant negative correlation was found between both variables ($r^2 = 0.93$, $P < 0.05$). This may be the result of Na^+ and Cl^- leaf accumulation, necessary for osmotic adjustment in order to maintain a water potential gradient for water uptake (Scholander *et al.* 1966, Clough *et al.* 1982, Medina and Francisco 1997, Suárez *et al.* 1998, Del Zoppo *et al.* 1999, An *et al.* 2002).

Table 1. Soil salinity, leaf dry mass to leaf area ratio (LMA), chlorophyll (Chl), nitrogen (N) contents, Chl/N ratio, ash free heat of combustion (Hc), content of protein, total lipids, ash and crude fiber, and crude fiber/protein and $Na^+ + Cl^-$/ash ratios on a leaf mass basis in adult leaves of *A. germinans* grown at low (LS) and high (HS) salinity and collected during the wet and dry seasons. Number of samples analysed was 12 - 30 for Chl and 12 - 14 for the other variables. Means ± SD for each variable. In the same row, means followed by different letters were statistically different at $P < 0.05$.

	Wet season		Dry season	
	LS	HS	LS	HS
Soil salinity [‰]	10.0 ± 6.27a	17.0 ± 2.5a	56.0 ± 1.41b	67.9 ± 6.6c
LMA [g m^{-2}]	131.9 ± 11.5a	159.6 ± 13.1b	155.1 ± 9.8b	173.0 ± 10.8c
Chl [μmol g^{-1}(d.m.)]	3.90 ± 0.31a	2.34 ± 0.59b	2.57 ± 0.52b	1.35 ± 0.44c
N [mmol g^{-1}(d.m.)]	1.57 ± 0.10a	1.56 ± 0.11a	1.35 ± 0.18b	1.19 ± 0.14c
Chl/N [mmol(Chl) mol^{-1}(N)]	2.48	1.48	1.90	1.13
Ash-free Hc [kJ g^{-1}]	21.6 ± 0.5ns	21.4 ± 0.8ns	20.9 ± 0.6ns	21.1 ± 1.3ns
Protein [mg g^{-1}(d.m.)]	137.7 ± 8.5a	136.4 ± 9.3a	118.5 ± 16.0b	104.0 ± 12.1c
Lipids [mg g^{-1}(d.m.)]	26.6 ± 6.3a	34.3 ± 3.2b	37.4 ± 3.6b	37.8 ± 6.1b
Ash [mg g^{-1}(d.m.)]	111.5 ± 7.80a	121.0 ± 12.6a	151.6 ± 13.3b	198.4 ± 30.4b
Crude fiber [mg g^{-1}]	230.9 ± 6.3a	236.5 ± 19.0a	186.7 ± 23.7b	199.3 ± 20.0b
Fiber/Protein	1.71 ± 0.14ns	1.84 ± 0.15ns	1.58 ± 0.25ns	1.98 ± 0.39ns
$Na^+ + Cl^-$/ash	0.322	0.372	0.398	0.396

As external salinity increased, CC tended to decrease (Fig. 1*A*). Thus, the difference between the average values of CC estimated at low and compared to that at high salinity was about 15 % [from 1.31 to 1.51 g(glucose) g^{-1}(d.m.)]. This CC variation in *A. germinans,* when salinity increased by 680 %, was much higher than previous reports of variation for any given species (Lafitte and Loomis 1988, Griffin *et al.* 1993, Sims and Pearcy 1991). Leaf CC varied from 1.28 to 1.48 g(glucose) g^{-1} across mangrove species in the field (Suárez 2003). Leaf maintenance costs (MC) remained relatively constant at moderate salinity and declined under hyper-saline conditions (Fig. 1*B*). The total MC is a result of the integration of protein and lipid maintenance costs while the cost of maintaining ionic concentrations is considered as a constant (Merino *et al.* 1984). Thus, at moderate salinity, the relative constancy observed in the MC was the result of a compensating protein concentration decrease and lipid concentration increase (Table 1, Fig. 1*B*). At high salinity during the dry season the MC of protein fraction decreased from 5.48 to 4.19 mg(glucose) g^{-1}(d.m.) d^{-1} whereas that of the lipid fraction only increased from 1.13 to 1.61 mg(glucose) g^{-1}(d.m.) d^{-1}, compared with low salinity during the wet season, consequently total MC declined. In fact, high salinity might negatively influence many processes in the leaf such as the synthesis of lipids, nucleic acids, and protein (Mansour 1995, Venkatesan and Chellappan 1998, Arshi *et al.* 2002).

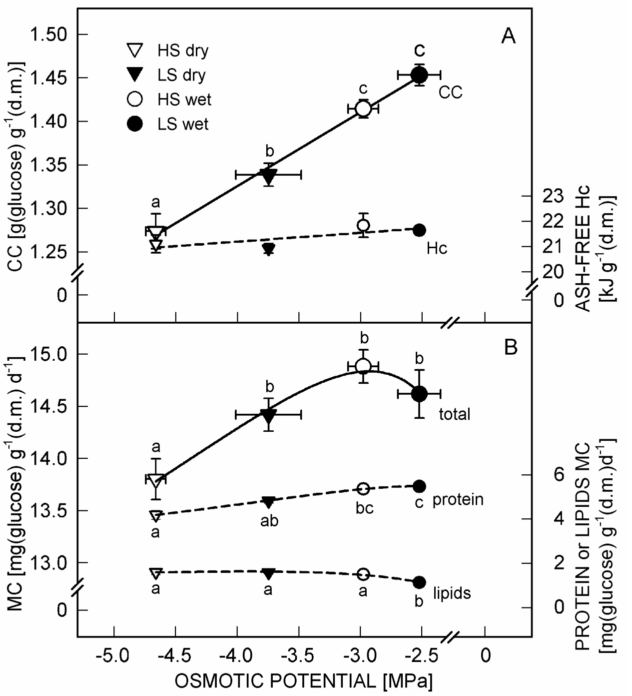

Fig. 1. Average construction cost (CC), ash-free heat of combustion (Hc) (*A*), average total maintenance cost (MC) and maintenance cost of protein and lipids (*B*), all on dry mass basis as a function of osmotic potential in leaves of *A. germinans* grown at low (LS) and at high salinity (HS), during the wet and dry seasons. Measurements were taken in adult leaves. Means ± SE. $n = 8$ for osmotic potential, $n = 12 - 14$ for CC, and $n = 6 - 7$ for MC. *Points* followed by different letters were statistically different at $P < 0.05$. *Lines* were fitted by linear regression (*A*) and by quadratic regression (*B*), both being statistically significant at $P < 0.05$.

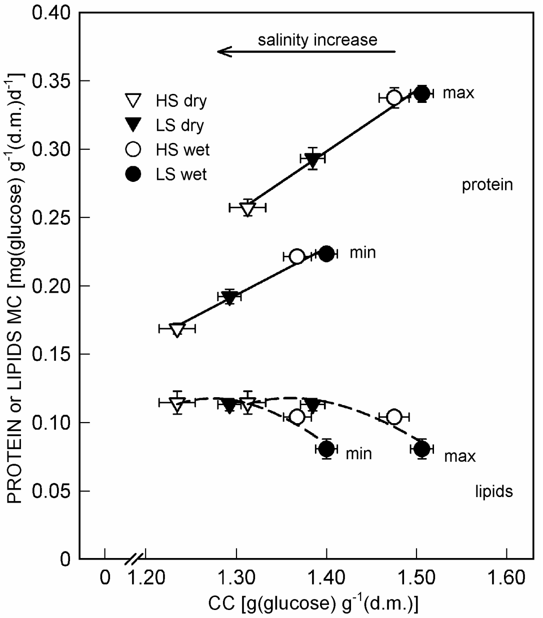

Fig. 2. Estimated construction costs for protein and lipid synthesis versus total leaf construction cost (CC), both on mass basis in *A. germinans*. Maximum and minimum CC are presented separately. The arrow indicates the increase of salinity in correspondence with Fig. 1. Measurements were taken in adult leaves. Means ± SE. $n = 12 - 14$ for protein CC and $n = 6 - 7$ for lipid CC. *Lines* were fitted by linear regression for protein CC and by quadratic regression for lipid CC, both being statistically significant at $P < 0.05$.

The ash-free heat of combustion (Hc) was comparable to that of other halophytic and glycophytic species and tended to remain relatively constant [21.3 ± 0.9 kJ g^{-1}(d.m.)] under all conditions (Table 1; Pipp and Larcher 1987, Williams *et al.* 1987, Larcher and Thomaser-Thin 1988, Sobrado 1991, Suárez 2003). Previous studies have also shown that Hc per unit leaf dry mass is relatively constant among and within species (Griffin 1994). Additionally, Hc has been suggested to be a reliable estimate of the synthetic cost of leaves (McDermitt and Loomis 1981, Williams 1984). However, in this study, CC values in *A. germinans* leaves were weakly correlated with those of ash-free Hc ($r^2 = 0.55$). The estimated maximum and minimum construction costs for the synthesis of protein present in leaves increased significantly with total leaf CC ($r^2 = 0.98$, $P < 0.05$; Fig. 2). Conversely, the estimated cost of construction for lipid synthesis shows a tendency to decrease with leaf CC (Fig. 2). However, lipids showed a tendency to increase with salinity up to a certain level, above which further increases in salinity do not result in a proportional increase in lipid content (Table 1, Fig. 2).

Proteins and lipids are considered expensive compounds (Penning de Vries *et al.* 1974, Williams *et al.* 1987, Lafitte and Loomis 1988). Therefore, if the proportion of nitrogen invested in protein build-up did not change with salinity, the negative effect of salinity on leaf CC is a result of a reduction in protein content, while other expensive compounds, in this case lipids, changed in an opposite direction. It is worth noting that, at any salinity, protein construction cost represents about 20 % of the total leaf CC, while that for lipids represents only about 5 - 10 %. Consequently, higher lipid content at high salinity was not enough to compensate for the reduction in protein, and leaf CC diminished with salinity. Variations in CC are determined by the net balance of changes in costly fractions (protein, lipids, and lignin) and cheap fractions (organic acids and minerals) (Chapin 1989, Poorter 1994). Corresponding with this view, ash contents per dry mass increased by 78 % at high salinity during drought as compared with those of leaves collected at low salinity during the wet season (Table 1). Furthermore, ash values were about twice those found over a wide range of glycophyte species, but compared well with values reported for other mangrove species (Merino *et al.* 1984, Pipp and Larcher 1987, Larcher and Thomaser-Thin 1988, Sobrado 1991, Poorter 1994, Larcher 1995, Suárez 2003). Plants growing on saline soils commonly have high ash content, and the ash contains high amounts of Na^+, Mg^{2+}, and Cl^- (Larcher 1995). Thus, ash content in *A. germinans* may reflect the large accumulation of Na^+ and Cl^- ions occurring in this halophytic species. In fact, estimates of $Na^+ + Cl^-$ were 36, 45, 60, and 79 mg g^{-1} at a ψ_s of -2.52, -2.98, -3.75, and -4.66 MPa, respectively. In consequence, the relationship between leaf ash and estimated Na^+ plus Cl^- contents was highly significant ($r^2 = 0.98$, $P < 0.05$) and suggested that Na^+ and Cl^- represent 60 ± 8 % of the total leaf ash content (Table 1). The values of ash free of Na^+ and Cl^- were estimated by subtracting the Na^+ and Cl^- from total ash values. Values were 76 mg g^{-1} (low salinity during the wet season) and 120 mg g^{-1} (high salinity and drought), which compared well with those typical for glycophyte species (30 - 120 mg g^{-1}; Poorter 1994). This confirmed that the high ash values obtained in the present study reflected the increased salt accumulation with salinity in this species.

The crude fiber content was higher during the wet season and lower during the drought (Table 1). Of the crude fiber content, 97 % is represented by cellulose and lignin (Pomeranz and Meloan 1994). Lignin has a high specific construction cost and it has been suggested that its accumulation in the leaf increases CC (Larcher 1995).

In conclusion, this study clearly shows that salinity involves changes in leaf construction and maintenance costs. An increase in protein and a decrease in lipids fractions with increasing salinity were responsible for this variation. It was also shown that ash content increased under high salinity, reflecting the relative increase in Na^+ and Cl^- in leaf tissue. However, further studies should be addressed for a more detailed understanding of how salinity affects leaf chemical composition (*i.e.* osmotically compatible solutes) and its effect on CC and cost/benefit analysis.

References

An, P., Inanaga, S., Lux, A., Li, X.J., Ali, M.E.K., Matsui, T., Sugimoto, Y.: Effects of salinity and relative humidity on two melon cultivars differing in salt tolerance. - Biol. Plant. **45**: 409-415, 2002.

Arshi, A., Abdin, M.Z., Iqbal, M.: Growth and metabolism of senna as affected by salt stress. - Biol. Plant. **45**: 295-298, 2002.

Ball, M.C., Pidsley, S.M.: Growth responses to salinity in relation to distribution of two mangrove species, *Sonneratia alba* and *S. lanceolata*, in northern Australia. - Funct. Ecol. **9**: 77-85, 1995.

Chapin, F.S.: The cost of tundra plant structures: evaluation of concepts and currencies. - Amer. Natur. **133**: 1-19, 1989.

Clough, B.F., Andrews, T.J., Cowan, I.R.: Physiological processes in mangroves. - In: Clough, B.F. (ed.): Mangrove Ecosystems in Australia: Structure, Function and Management. Pp. 193-210. Australian National University Press, Canberra 1982.

Cram, W.J., Torr, P.G., Rose, D.A.: Salt allocation during leaf development and leaf fall in mangroves. - Trees **16**: 112-119, 2002.

Del Zoppo, M., Galleschi, L., Onnis, A., Pardossi, A., Saviozzi, F.: Effect of salinity on water relations, sodium accumulation, chlorophyll content and proteolytic enzymes in a wild wheat. - Biol. Plant. **42**: 97-104, 1999.

Downton, W.J.S.: Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. - Aust. J. Plant Physiol. **9**: 519-528, 1982.

Eamus, D., Myers, B., Duff, G., Williams, R.: A cost-benefit analysis of leaves of eight Australian savanna tree species of differing leaf life-span. - Photosynthetica **36**: 575-586, 1999.

Griffin, K.L.: Calorimetric estimates of construction cost and their use in ecological studies. - Funct. Ecol. **8**: 551-562, 1994.

Griffin, K.L., Thomas, R.B., Strain, B.R.: Effects of nitrogen supply and elevated carbon dioxide on construction cost in leaves of *Pinus taeda* (L.) seedlings. - Oecologia **95**: 575-580, 1993.

Lafitte, H.R., Loomis, R.S.: Calculation of growth yield, growth respiration, and heat content of grain sorghum from elemental and proximal analyses. - Ann. Bot. **62**: 353-361, 1988.

Lambers, H., Poorter, H.: Inherent variation in growth rate between higher plants: A search for physiological causes and ecological consequences. - Adv. ecol. Res. **23**: 187-261, 1992.

Larcher, W.: Physiological Plant Ecology. 3rd Edition. - Springer-Verlag, Berlin 1995.

Larcher, W., Thomaser-Thin, W.: Seasonal changes in energy content and storage patterns of Mediterranean sclerophylls in a northernmost habitat. - Acta œcol./Œcol. Plant. **9**: 271-283, 1988.

Lichtenthaler, H.K., Wellburn, A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. - Biochem. Soc. Trans. **11**: 591-592, 1983.

Lugo, A.E., Snedaker, S.C.: The ecology of mangroves. - Annu. Rev. Ecol. Syst. **5**: 39-64, 1974.

Mallery, C.H., Teas, H.J.: The mineral ion relations of mangroves. I. root cell compartments in a salt excluder and a salt excreter species at low salinities. - Plant Cell Physiol. **25**: 1123-1131, 1984.

Mansour, M.M.F.: NaCl alteration of plasma membrane of *Allium cepa* epidermal cells. Alleviation by calcium. - J. Plant Physiol. **145**: 726-730, 1995.

Mansour, M.M.F., Salama, K.H.A., Al-Mutawa, M.M., Abou Hadid, A.F.: Effect of NaCl and polyamines on plasma membrane lipids of wheat roots. - Biol. Plant. **45**: 235-239, 2002.

McDermitt, D.K., Loomis, R.S.: Elemental composition of biomass and its relation to energy content, growth efficiency and growth yield. - Ann. Bot. **48**: 275-290, 1981.

Medina, E., Francisco, M.: Osmolality and $\delta^{13}C$ of leaf tissue of mangrove species from environments of contrasting rainfall and salinity. - Estuar. coast. Shelf Sci. **45**: 337-344, 1997.

Merino, J., Field, C., Mooney, H.A.: Construction and maintenance costs of Mediterranean-climate evergreen and deciduous leaves. - Acta œcol./Œcol. Plant. **5**: 211-229, 1984.

Penning de Vries, F.W.T., Brunsting, A.H.M., Van Laar, H.H.: Products, requirements and efficiency of biosynthetic processes: a quantitative approach. - J. theor. Biol. **45**: 339-377, 1974.

Pipp, E., Larcher, W.: Energiegehalte pflanzlicher substanz. II. Ergebnisse der datenverarbeitung. - Sitzungsber. Österr. Akad. Wiss., Mathem.-naturw. Kl., Abt. I, **196**: 249-310, 1987.

Pomeranz, Y., Meloan, C.E.: Food Analysis: Theory and Practice. 3rd Edition. - Chapman & Hall, New York 1994.

Poorter, H.: Construction costs and payback time of biomass: a whole plant perspective. - In: Roy, J., Garnier, E. (ed.): A Whole Plant Perspective on Carbon-nitrogen Interactions. Pp. 111-127. SPB Academic Publishing, The Hague 1994.

Popp, M., Larher, F., Weigel, P.: Chemical composition of Australian mangroves. III. Free amino acids, total methyolated onium compounds and total nitrogen. - Z. Pflanzenphysiol. **114S**: 15-25, 1984.

Scholander, P.F., Bradstreet, E.D., Hammel, H.T., Hemmingsen, E.A.: Sap concentrations in halophytes and some other plants. - Plant Physiol. **41**: 529-532, 1966.

Sims, D.A., Pearcy, R.W.: Photosynthesis and respiration in *Alocasia macrorrhiza* following transfers to high and low light. - Oecologia **86**: 447-453, 1991.

Sobrado, M.A.: Cost-benefit relationships in deciduous and evergreen leaves of tropical dry forest species. - Funct. Ecol. **5**: 608-616, 1991.

Sobrado, M.A.: Drought effects on photosynthesis of the mangrove, *Avicennia germinans*, under contrasting salinities. - Trees **13**: 125-130, 1999.

Sokal, R.R., Rohlf, F.J.: Biometry. - W.H. Freeman and Co., San Francisco 1969.

Suárez, N.: Leaf longevity, construction, and maintenance costs of three mangrove species under field conditions. - Photosynthetica **41**: 373-381, 2003.

Suárez, N., Sobrado, M.A., Medina, E.: Salinity effects on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) L. seedlings. - Oecologia **114**: 299-304, 1998.

Tomlinson, P.B.: The Botany of Mangroves. - Cambridge University Press, London 1986.

Venkatesan, A., Chellappan, K.P.: Accumulation of praline and glycine betaine in *Ipomoea pes-caprae* induced by NaCl. - Biol. Plant. **41**: 271-276, 1998.

Williams, K., Percival, F., Merino, J., Mooney, H.A.: Estimation of tissue construction cost from heat of combustion and organic nitrogen content. - Plant Cell Environ. **10**: 725-734, 1987.

Williams, S.: Official Methods of Analysis. - AOAC, Arlington 1984.

Yeo, A.R.: Salinity resistance: physiologies and price. - Physiol. Plant. **58**: 214-222, 1983.