Effects of Nutrient Enrichment on Leaf Anatomy of Dwarf Rhizophora mangle L. (Red Mangrove)

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ABSTRACT

I fertilized dwarf Rhizophora mangle trees for two yr to determine how the morphology and internal anatomy of leaves are affected by increased nutrient availability. The sclerophyllous structure of R. mangle leaves decreased on P- and NPK-fertilized trees, but not on N-fertilized and control trees. Leaves from trees treated with phosphorus-containing fertilizers were 40 percent to 80 percent as thick as leaves on control trees. Differences in the hypodermis accounted for most differences in leaf thicknesses. In P- and NPK-fertilized trees, the thickness of the hypodermis in basal leaves was 225 (±13) μm and 207 (±11) μm, respectively, compared to 572 (±13) μm in N-fertilized and 594 (±13) μm in Control trees. Area per leaf and leaf length:width ratio did not change in response to increased nutrient availability. Nutrient deficiency rather than salinity and physiological drought appears to be the proximate cause of well-developed xeromorphic traits in leaves of dwarf red mangrove. This study provides experimental evidence of the oligotrophic-xeromorphic hypothesis that states that sclerophyll is an adaptation to P-deficient soils rather than to drought or physiological drought.

Key words: leaf anatomy; hypodermis; nutrient enrichment; phosphorus deficiency; Belize; Rhizophora mangle; xeromorphy; sclerophyll.


The stature of mangrove forests varies across forest types that range from dwarf trees in the flat coastal fringe to towering trees along rivers (Lugo & Snedaker 1974). Primary productivity, forest stature, and growth rates in mangrove forests have been correlated with numerous environmental variables, including latitude, salinity, nutrient availability, flooding frequency, oxidation-reduction status of soil, sulfide concentrations, surface hydrology, and tidal force (MacNae 1968, Lugo & Snedaker 1974, Onuf et al. 1977, Pool et al. 1977, Boto & Wellington 1984, CINTRÓN et al. 1985, Lugo 1989, Jiménez & Sauter 1991, McKee 1993). Based on a recent experimental fertilization study in Belize, phosphorus deficiency is a major factor limiting growth in an island stand of dwarf R. mangle trees and, sclerophyll, measured as leaf mass per unit area (g/cm²) and as leaf toughness, decreased significantly in response to increased P availability (Feller 1995).

The attributes that produce the characteristic physiognomy of dwarf R. mangle trees, i.e., low stature, slow growth, and long-lived scleromorphic leaves, are similar to many other tree species that occupy infertile ecosystems (Grubb 1977, Miller & Stoner 1979, Medina et al. 1990). This suite of traits was originally described for vegetation associated with xeric Mediterranean climate (Schimper 1903). However, in the tropics they are also characteristic of wet lowland forests associated with the blackwater rivers of the Río Negro region in the northern Amazon basin (Bates 1863, Janzen 1974, Sobrado & Medina 1980, Medina et al. 1990), as well as montane forests (Camerik & Werger 1981, Grubb 1989). Experimental addition of fertilizers has shown that tropical montane trees in Jamaica and Venezuela with these traits are nitrogen-limited (Tanner et al. 1990, 1992). Based on nutrient inventories of soils and vegetation, Vitousek (1984) predicted that most wet lowland tropical forests are phosphorus limited.

Xeromorphic traits that prevail in wet environments were originally thought to be adaptations to drought where water is abundant but physiologically unavailable to plants (Schimper 1903, Eames
Physiological drought may occur in cold climates where water is unavailable when it is frozen, in tropical rain forests where water is quickly lost due to high evaporation rates, or in salt marshes where water is unavailable because of high salt concentration (reviewed in Grubb 1986). More recently, xeromorphy in Mediterranean regions and in wet areas with infertile soils was considered the result of parallel or convergent evolution in response to different selective forces (Givnish 1978, More recently, xeromorphy in Mediterranean regions and in wet areas with infertile soils was considered the result of parallel or convergent evolution in response to different selective forces (Givnish 1978, Loveless 1981, Beadle 1967, Medina et al. 1990). Because hard leaves tend to be long lived with physical and chemical properties that reduce decomposition rates and slow nutrient release, sclerophyll may help retain nutrients in the plant and reduce leaching losses from the system; consequently, several authors have suggested that this leaf type may be an adaptation for nutrient conservation in nutrient-poor habitats (Small 1972, Janzen 1974, Chapin 1980, Chabot and Hicks 1982, Horner et al. 1988). Experimental studies in raised sphagnum peat bogs in Canada (Small 1972), montane forests in Jamaica (Buckley et al. 1980, Kapos & Tanner 1985), heath forests in Sarawak, Malaysia (Peace & Macdonald 1981), and tropical rain forests of the Upper Rio Negro region in South America (Medina et al. 1990) have shown that scleromorphic leaves in these wet climates are not more drought resistant than mesomorphic leaves. Although these studies have rejected the part of the drought-resistance hypothesis that xeromorphic traits are adaptations to physiological drought, differences in sclerophyll in mangroves are typically attributed to a salinity gradient (Clough et al. 1982, Saenger 1982, Camilleri & Ribi 1983, Lugo 1989).

The goal of this investigation was to determine the responses by R. mangle leaves to nutrient enrichment and to test the oligotrophic-xeromorphism hypothesis (Loveless 1961, Beadle 1967, Medina et al. 1990). Leaves of fertilized R. mangle were examined to determine how their morphology and internal anatomy change in response to increased nutrient availability.

**MATERIALS AND METHODS**

**Study site.**—Field work was conducted on Twin Cays, a 92-ha range of mangrove islands, located in the outer lagoon just inside the barrier reef of central Belize, approximately 22 km SE of the coastal town of Dangriga (16°50'N, 88°06'W) (Fig. 1). Radiocarbon dating of peat cores, 7-m deep, shows that Twin Cays developed as a mangrove system approximately 7000 yr ago atop a Pleistocene high (I. A. Macintyre, unpublished data). McKee (1993) provides an extensive description of the hydroedaphic and the physicochemical properties of peat substrate of Twin Cays.

These islands are intertidal with a 21-cm tidal range. Tidal exchange with interior areas occurs through deep, narrow creeks and across broad, shallow ebb-flood channels (Wright et al. 1991). The tide in this region of the Caribbean is microtidal and mixed semidiurnal (Kjerfve et al. 1982).

The mangrove vegetation at Twin Cays is physiognomically varied and interrupted by numerous tidal creeks, open flats, and shallow interior ponds. Like many mangrove systems in the Caribbean, it is dominated by R. mangle, Avicennia germinans (L.) Stearn (black mangrove), and Laguncularia racemosa (L.) Gaertn.f. (white mangrove). In 1980, this island range became the primary study site and experimental field laboratory for the Smithsonian Institution’s Marine Field Station on nearby Carrie Bow Cay (Rüttler & Feller 1988 and in press).

**Experimental design.**—During July 1989, trees to be fertilized were chosen from a 1.2-ha stand of approximately 5300 dwarf R. mangle trees, ≤1.5-m tall, growing in a shallow ponded area in the interior of Twin Cays. The bottom topography of this pond creates a north-south water-depth gradient. The tides ebb and flood this pond across the water-depth gradient, creating an east-west tidal-elevation gradient. Water depth at each experimental tree, measured at mid-flood tide during the new moon, ranged from 11 to 22 cm on the shallow side and from 30 to 54 cm on the deep side. The entire site is permanently flooded except for the margin of the shallow side which drains during extreme low tides. The pore-water salinity at 15-cm depth at the study site ranges from near seawater (33‰) to slightly hypersaline with some seasonal variation across the site: January 1990, (mean ± 1 SE) 36.9 ± 3‰; July 1990, 38.8 ± 3‰; January 1991, 34.9 ± 2‰; July 1991, 37.3 ± 3‰ (Feller, 1995). Pore-water salinity is slightly higher during the summers than during the winters, and trees in the low tidal elevation portions of the site have pore-water salinities that are slightly higher than trees in the high tidal elevation portion. Based on 6 yr of monitoring, pore-water salinity in the low tidal elevation portion of the site is generally 1–3 ppt higher than in the...
FIGURE 1. Map of Twin Cays, in relation to the mainland of Belize, the barrier reef, and the Smithsonian Institution Marine Field Station on Carrie Bow Cay. The study site is indicated by an arrow on the southeastern coastline of Twin Cays.
Nutrient Treatment Levels:
NPK

Table 1. Nutrient treatment and water-depth gradients to accommodate the four levels of the Nutrient treatment in three replicates (4 Nutrients × 2 Tidal Elevation × 2 Water Depth × 3 replicates = 48 trees). As a precaution against lateral migration of nutrients, 10-m buffer zones were left between transects and trees.

The four levels of the Nutrient treatment, in 300-g doses, were: NPK (10:15:15) as NH₄PO₄; K₂O; P (0:45:0) as P₂O₅; N (45:0:0) as NH₄; and control (no enrichment). The Nutrient treatment level for each tree within each replicate was randomly determined. The NPK level was applied in two 150-g Jobe® tree stakes per tree. Granular triple superphosphate and ammonium were applied in two 150-g doses per tree encased in dialysis tubing (Spectrapor Membrane Tubing®, 40-mm diameter, 6000–8000 molecular weight cut off). To apply the fertilizers at the start of the experiment, I cored two holes, 7-cm diameter by 30-cm deep, into the peat substrate on opposite sides of each tree, directly beneath the outermost margin of the canopy. After tree stakes or filled sections of dialysis tubing were inserted into cored holes, each hole was sealed with a peat plug taken from a nearby unvegetated patch and was covered with a concrete weight. In controls, holes were cored and sealed but no nutrient was added. To minimize damage to roots and surrounding peat, the same holes were used for each fertilizer application. Fertilizer treatments were applied to each of the 48 trees twice a year, in July and January, starting from July 1989 through July 1991. This method has been extremely effective in restricting the lateral spread of the added nutrients. Growth responses clearly demonstrated that only plants within 1 m of the point of application were affected by the treatments.

For July 1990 and 1991, area per leaf (cm²) and leaf length-to-width ratios were measured with a Li-Cor 3000 Portable Leaf Area Meter. Values for July 1989 were calculated using a regression equation obtained from 300 leaf lengths and area-meter values for similar length and length-to-width ratios. Initially, leaf measurements were made on five randomly selected first-order branches. Repeated measurements also included leaves on lateral shoots that these branches subsequently produced. In addition, maximum area per leaf (cm²) for each of the 48 trees was based on leaves from the basal positions on these five branches after 1 yr and 2 yr of fertilization.

To evaluate specific treatment-induced changes
in leaf internal anatomy, I measured thickness of leaves and leaf-tissue layers using transections of fresh leaves and compound light microscopy equipped with a calibrated ocular micrometer. Measurements were made on leaf material harvested after 2 yr of treatment in July 1991. By that time, all the leaves on each of the 48 trees had been produced since July 1989, when the trees had received their first fertilizer treatments. Leaf position rather than leaf age was used as a standard for comparing leaves among the treatments because the 6-mo sampling and refertilization schedule precluded knowing exactly when leaves first emerged from the bud. Young, fully-expanded sun leaves from a penapical stem position and old, green leaves from a basal stem position on each of the trees were collected, stored in a cooler, and hand sectioned within 5 hr of collecting. Measurements were made on sections taken from the lamina midway between the leaf tip and base and between the right leaf margin and the midvein. Sections were photographed.

Variables based on material harvested in July 1991 at the end of this experiment were analyzed as an independent measures three-factor ANOVA using the Systat® 1991 software package for inferential statistics. For sequential measurements of leaf area, I used a repeated-measures ANOVA to look for possible differences over the 2-yr period. To analyze for heteroscedasticity, probability plots of all variables and ANOVA residual were examined. Data were transformed as needed to reduce variance. When an ANOVA found a significant Nutrient effect, I used a pairwise *a priori* orthogonal contrast matrix to locate differences among the four Nutrient treatment levels. Null hypotheses were rejected at $P < .05$.

**RESULTS**

Addition of P and NPK, but not N, fertilizers had a dramatic impact on the internal anatomy and texture of *R. mangle* leaves (Fig. 3). The leaf-tissue layers of this species are dorsoventrally arranged (Tomlinson 1986). Beginning with its adaxial surface, a leaf has a waxy cuticle, followed by a three cell-layer epidermis, a three cell-layer hypodermis, a single-layer palisade mesophyll, a loose spongy mesophyll, and a two or three cell-layer lower epidermis with slightly sunken stomata. There was a significant difference in total leaf thickness among Nutrient levels (Fig. 4; $F = 14.688, P < .001$). Penapical and basal leaf thicknesses were similar among P- and NPK-fertilized trees and among N-fertilized and control trees. On N-fertilized and control trees, penapical leaves were approximately 15 percent thicker and basal leaves were 60–70 percent thicker than on P- and NPK-fertilized trees. The cuticular, epidermal, and palisade layers were not significantly different among Nutrient levels and leaf positions (Table 1). Differences in hypodermis thickness account for most of the variation in total leaf thickness that distinguishes penapical and basal leaves in P- and NPK-fertilized trees from N-fertilized and control trees. In basal leaves on N-fertilized and control trees, dorsoventral elongation of the three cell layers that comprise the hypodermis accounts for approximately 55 percent of the total leaf thickness. Thickness of the hypodermis more than doubles as these leaves age from penapical to basal stem positions. The hypodermis in penapical and basal leaves is similar among P- and NPK-fertilized trees (ANOVA, $P < .05$) and comprises about 30 percent of leaf-lamina thickness. In basal leaves, the spongy mesophyll thickness also differed significantly among Nutrient levels ($F = 36.330, P < .001$). It was approximately 20 percent thicker in N-fertilized and control tree leaves than in either P- or NPK-fertilized tree leaves. Although the 6-mo sampling interval forced comparisons of leaf anatomy based on stem position rather than age, there were no differences in leaf longevity among the four Nutrient treatment levels. The number of leaves...
that survived through successive sampling intervals was not different among Nutrient levels (ANOVA, P > .05). Approximately half of the leaves monitored during this study survived through three 6-mo sampling intervals. These data are consistent with Twilley (unpublished data) who determined that 18 mo is the average longevity for _R. mangle_ leaves at Twin Cays. Despite slight difference in pore-water salinity along the tidal-elevation gradient, neither Tidal Elevation nor Water Depth had a significant effect on leaf anatomy (ANOVA, P > .05).

Fertilization with NPK and P resulted in dramatic increases in growth in both leaf area and woody tissue per shoot for both years of this study, and there was no indication that the effect of the fertilizer was wearing off after two yr (Feller 1995). However, area per leaf varied somewhat by Nutrient treatment level during the course of the experiment.

<table>
<thead>
<tr>
<th>Leaf-tissue layers</th>
<th>NPK</th>
<th>P</th>
<th>N</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penapical leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle</td>
<td>$8^\pm1$</td>
<td>$8^\pm1$</td>
<td>$7^\pm1$</td>
<td>$8^\pm1$</td>
</tr>
<tr>
<td>Upper epidermis</td>
<td>$52^\pm1$</td>
<td>$53^\pm2$</td>
<td>$52^\pm1$</td>
<td>$50^\pm1$</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>$183^\pm8$</td>
<td>$194^\pm11$</td>
<td>$229^\pm13$</td>
<td>$231^b^\pm16$</td>
</tr>
<tr>
<td>Palisade mesophyll</td>
<td>$191^\pm4$</td>
<td>$194^\pm12$</td>
<td>$189^\pm9$</td>
<td>$210^\pm9$</td>
</tr>
<tr>
<td>Spongy mesophyll</td>
<td>$188^\pm6$</td>
<td>$187^\pm5$</td>
<td>$210^\pm10$</td>
<td>$190^\pm12$</td>
</tr>
<tr>
<td>Basal leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle</td>
<td>$9^\pm1$</td>
<td>$8^\pm1$</td>
<td>$9^\pm1$</td>
<td>$9^\pm1$</td>
</tr>
<tr>
<td>Upper epidermis</td>
<td>$54^\pm1$</td>
<td>$55^\pm1$</td>
<td>$56^\pm2$</td>
<td>$53^\pm1$</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>$225^\pm13$</td>
<td>$208^\pm16$</td>
<td>$572^\pm70$</td>
<td>$594^b^\pm65$</td>
</tr>
<tr>
<td>Palisade mesophyll</td>
<td>$193^\pm3$</td>
<td>$196^\pm4$</td>
<td>$204^\pm11$</td>
<td>$201^\pm5$</td>
</tr>
<tr>
<td>Spongy mesophyll</td>
<td>$193^\pm7$</td>
<td>$200^\pm6$</td>
<td>$237^\pm6$</td>
<td>$236^\pm5$</td>
</tr>
</tbody>
</table>
In July 1990, at the end of the first year of fertilization, the maximum area per leaf for basal leaves on both P- and NPK-fertilized trees was significantly larger than similarly positioned leaves on either N-fertilized or control trees (Fig. 5). This effect disappeared by the end of the second year, and there were no significant differences in maximum area per leaf among all levels of Nutrient, Tidal Elevation, or Water Depth after 2 yr of treatment. Similarly, there were no significant differences in leaf length: width ratios among treatments (ANOVA, \( P > .05 \)).

**DISCUSSION**

In a controlled nutrient-enrichment experiment, leaf thickness decreased significantly in P- and NPK-fertilized dwarf *R. mangle* trees following 2 yr of treatment. Similar to previous findings (Tomlinson 1986), variation in thickness of the hypodermis in *R. mangle* leaves accounts for most differences in leaf thickness among Nutrient levels. Relative to control trees, P and NPK fertilizers, but not N fertilizer, caused reductions in thickness of the leaf lamina primarily by reduction of the hypodermis. The hypodermis in unfertilized controls is approximatly 20 percent thicker in penapical leaves and over 60 percent thicker in basal leaves than in leaves at similar positions on P- and NPK-fertilized trees. These data, along with the growth responses and changes in nutrient balance in trees supplied with P and NPK fertilizers (Feller 1995), provide experimental evidence in support of the oligotrophic-xeromorphism hypothesis (Loveless 1961, Beadle 1967, Medina et al. 1990) by demonstrating that sclerophylly in dwarf *R. mangle* trees is related to phosphorus deficiency. Although these results are consistent with findings in other ecosystems (Loveless 1961, Beadle 1967, Small 1972, Specht & Moll 1983, Vitousek et al. 1988, Medina et al. 1990), they deviate from current hypotheses regarding xeromorphy in mangroves (Saenger 1982, Hutchings & Saenger 1987, Lugo et al. 1989). It has been proposed that extreme leaf thickness in some mangrove species is succulence, or water storage in fleshy tissue, and is an adaptation to physiological drought caused by high salinity (Mullan 1931, Shields 1950, Wylie 1954, Saenger 1982, Hutchings & Saenger 1987). However, in my experiment the slight differences in pore-water salinity had no effect on leaf thickness. The expanded hypodermis in *R. mangle* leaves has been described as aqueous tissue that may function for water storage, salt accumulation or osmoregulation (Saenger 1982, Camilleri & Ribi 1983). Data presented here do not support this. Similar water content and sodium concentration in leaf tissue among the four Nutrient levels (Feller 1995) despite large differences in leaf thickness indicate that leaf thickness was not a function of succulence or salt accumulation. These results are consistent with interpretations by Clough et al. (1982) for Australian mangroves. The presence of a thickened waxy cuticle has also been interpreted as an adaptive response to physiological drought in mangrove environments (Stace 1966, Sidhu 1975). In my experiment, similar thickness of the cuticle among Nutrient levels in both young and old leaves in penapical and basal positions shows that variation in sclerophylly is not caused by differential thickening of this layer.

Leaf toughness and leaf mass per unit area (g/cm²) decreased significantly in response to enrichment with P and NPK, but not N, fertilizers (Feller 1995). These measures indicate that leaves on phosphorus-enriched trees were less sclerophyllous than leaves on N-fertilized or control trees. However, after 2 yr of fertilization, area per leaf in dwarf *R. mangle* at Twin Cays was unaffected by increased nutrient availability. This result is inconsistent with Geeske et al. (1994) suggestion that decreasing leaf size within a species along an elevational gradient also may be correlated with nutrient availability.

Studies that contrast xeric species with species from nutrient-poor habitats suggest that the anatomical and physiological attributes associated with xeromorphy have arisen via convergent or parallel evolution in response to a complex of environmental stresses (Givnish 1978, 1979; Connor & Doley 1981; Lamont & Kelly 1988; Stock 1988; Uttowski & Lamont 1991). Other authors have suggested that sclerophylly is an adaptive defense mechanism against herbivory (Feeny 1970, Janzen 1974, Chabot & Hicks 1982, Coley 1983, Grubb 1986). However, despite large differences in the degree of sclerophyll exhibited by *R. mangle* leaves, this study at Twin Cays showed no difference in levels or rates of damage by herbivores that fed on expanded leaves (Feller 1995). Alternatively, increased growth along with decreased nutrient-use efficiency and sclerophyll in response to fertilization suggest that xeromorphy in this species may encompass a suite of adaptations related to nutrient conservation.

Although this study determined that phosphorus limitation is the proximate cause of increased sclerophylly in dwarf *R. mangle* trees at Twin Cays, nutrient limitation in mangrove swamps cannot be interpreted from plant responses at a given soil-nutrient concentration. Because of the complexity
of soil chemistry in wetlands, nutrient availability may be altered by high salinity and other edaphic factors related to anaerobic conditions in waterlogged mangrove soils, such as low redox potentials and high sulfide content, that interfere with nutrient uptake (Clough et al. 1982, Ball et al. 1982, Howes et al. 1986, Mendelssohn & McKee 1988, Burdick et al. 1989, Lugo et al. 1989, McKee 1993).

Mangrove ecosystems are affected by multiple stresses acting simultaneously and perhaps interactively to determine the forest structure (McKee, in press). Spatial variability of soil factors such as nutrient availability, salinity, and oxidation status have variable impact on the fertility of mangrove soil, and these impacts depend largely on tolerances of individual mangrove species. These results demonstrate that *R. mangle* is phenotypically plastic and not restricted in its growth potential in the manner which Grime (1977) and Chapin et al. (1986) have suggested is typical of plant species adapted to low-resource availability.

This study provides evidence that phosphorus deficiency affects the internal anatomy of *R. mangle* leaves. However, it does not provide any answers regarding the mechanisms or adaptive significance of a variably thickened hypodermis, which is primarily responsible for the differences in leaf thickness. The differences in leaf thickness in response to phosphorus availability are similar to thickness differences between sun and shade leaves on *R. mangle* (pers. obs.). This parallel occurrence of thick leaves induced by different environmental variables, *i.e.*, phosphorus deficiency and light, in the same plant species may involve similar mechanisms. A thick hypodermis may protect photosynthetic tissue in the leaf when *R. mangle*’s metabolic rate is limited by either nutrient deficiency or excess light.

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**LITERATURE CITED**


