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Dynamics of herbivory in Belizean mangal

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ABSTRACT. We report long-term observations on leaf herbivory in the mangroves *Rhizophora mangle* and *Avicennia germinans*, on the Placencia Peninsula of Belize. We labelled and traced leaves and recorded the amount of damage appearing over a period of 310 days. Herbivores had damaged 1–4% of total mean leaf area within the first 10 days, but damage rates levelled off thereafter, to a final total of 4–10% damage. Herbivores did not concentrate on leaves of younger age classes. *Rhizophora mangle* growing in the high tidal zone (HHW) exhibited the highest initial and total attack rates at all but the final sampling dates. We also documented the dynamics of injury expansion and necrosis following artificial injury to seedling and tree leaves. Artificial holes in leaves were associated with higher subsequent rates of herbivory that varied among leaf age classes, and particular damage types were more prevalent in previously injured leaves. Artificial holes expanded significantly more in young leaves of both species. Holes in young leaves of both species expanded by up to 45.1% in area over 50 days, consistent with a similar study in Australia. Substantial necrotic areas developed around the injury site; a secondary effect of damage not often considered in herbivory studies. We discuss the implications of hole expansion and necrosis in estimating error in herbivory measurements. Temporally dynamic studies of herbivory are especially needed in tropical systems that show non-deciduous phenologies and long-lived leaves.

KEY WORDS: *Avicennia germinans*, Belize, biomass, herbivory, leaf growth, mangroves, *Rhizophora mangle*.

INTRODUCTION

Mangroves form extensive forests along protected coastlines throughout the tropics. Despite centuries of research on these common and unusual trees (Bowman 1917, MacNae 1967, Rollet 1981, Tomlinson 1986), relatively few investigations have concentrated on the relationships between mangroves and their direct consumers, and the relative importance of non-detritivore trophic links in mangrove ecosystems has not been elucidated. Herbivores that feed directly on mangrove leaves (Beever *et al.* 1979, Camilleri 1989, Farnsworth & Ellison 1991, Johnstone 1981, Newbery 1980, Onuf *et al.* 1977, Robertson & Duke 1987, Schoener 1988), twigs (Feller & Mathis, in manuscript), flowers

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(Farnsworth, unpublished data), roots (Ellison & Farnsworth 1990, Perry 1988, Rehm & Humm 1973, Simberloff *et al.* 1978), and propagules (Lacerda *et al.* 1988, Smith 1987, Smith *et al.* 1989) influence mangrove establishment, reproduction, growth, and architecture. The foregoing studies demonstrate that herbivores remove variable, and frequently substantial, proportions of primary production from the well-documented detritivore pathway in mangrove ecosystems (Lugo & Snedaker 1974, Odum 1971, Odum & Heald 1972). Herbivore damage varies widely among mangrove species, tidal and flooding regimes, and islands and mainland sites (Farnsworth & Ellison 1991, Schoener 1988).

The majority of mangrove herbivory studies have examined static samples; leaves have been assessed for damage at a single sampling date (Beever *et al.* 1979, Farnsworth & Ellison 1991, Johnstone 1981, Newbery 1980, Schoener 1988). However, herbivore-inflicted injuries, such as bites and holes, may themselves change in size as the leaf grows. One-time estimates of herbivore damage cannot distinguish the damage directly inflicted by herbivores from the secondary expansion that ensues as a function of normal leaf development. Thus, the actual amount of leaf tissue removed by herbivores may be overestimated if this expansion is not taken into account. Likewise, leaves may exhibit scarring and necrosis associated with certain types of damage, which reflect physiological responses, secondary infection, and healing processes that are only an indirect by-product of the original activities of herbivores.

Studies in terrestrial habitats have emphasized the need for long-term monitoring of herbivory to capture the temporal dynamics of leaf consumption and injury expansion (e.g. Coley 1983, Lowman 1984, Nielson 1978, Reichle *et al.* 1973). Recent investigations of mangrove herbivory have incorporated methods that trace the evolution of injury, growth and healing over time (Onuf *et al.* 1977, Robertson & Duke 1987). Robertson & Duke (1987) tracked the expansion of artificial wounds in young leaves of three mangrove species, and found that holes more than doubled in size over 3 months, enlarging on average 208–237%. They applied the resulting correction factor to their estimates of herbivory from static samples across all leaves surveyed, reducing their estimates by a factor of 2.08 to 2.37. We performed an artificial damage experiment in a Belizean mangrove swamp to determine: (1) if the assumptions and applications of the Robertson & Duke model appertain to Neotropical mangal; (2) if the magnitude of expansion error is comparable to their findings; (3) whether secondary necrosis regularly appears around foliar injury; and (4) whether this secondary necrosis is sizable enough to warrant attention in mangrove herbivory studies.

The vast literature on plant–herbivore interactions also suggests that prior injury can influence subsequent feeding by herbivores. The stimulus of herbivore damage may induce increased production of secondary defensive compounds that deter subsequent foraging (e.g. Bhaskaran & Kandaswamy 1978, Bryant 1981, Coley 1983, Green & Ryan 1972, Haukioja & Niemela 1979, Hillis & Inoue 1968, Ryan 1978, Schultz & Baldwin 1982). The ability of plants to allocate resources to the production of secondary compounds is dependent on nutritional status and resource availability (Bazzaz *et al.* 1987, Coley *et al.* 1985) and the intensity of herbivory. Mangroves possess a variety of secondary compounds including tannins and phenolics that may discourage herbivory (Camilleri 1989, Ghosh *et al.* 1985, Lacerda *et al.* 1986, 1988, Neilson *et al.* 1986). We sought to determine if artificial injury would affect later herbivore activity on mangrove leaves. We also documented the dynamics of folivory on two mangrove species, *Rhizophora mangle* L. and *Avicennia germinans* (L.) Stearn, over 9 months, to observe whether herbivory rates fluctuate temporally. Herbivory rates on leaves may decline over time as herbivores induce the production of plant secondary defenses, as leaves age and toughen, or as herbivore populations oscillate. Long-term field herbivory studies such as the one we report here provide evidence that herbivory in mangal is more prevalent than previously acknowledged, that plant–herbivore interactions are variable over time, and that phenomena such as induction of defensive compounds and secondary infection that are being noted in other tropical systems, may occur in mangal as well.

METHODS

All experiments were conducted between January and December 1988, in the Placencia Lagoon, Point Placencia, Stann Creek District, Belize (16° 31′ N, 88° 21.5′ W). Farnsworth & Ellison (1991) present a full site description. Mangroves in Placencia Lagoon exhibit a distinct zonation of species and morphology correlated with tidal height, that is typical of zonation patterns noted elsewhere (Lugo & Snedaker 1974, MacNae 1967, Rützler & MacIntyre 1982). *Rhizophora mangle* predominates in a 20–100 metre-wide band from low water (LW) to mean water (MW). We examined only large trees of fringe morphology (*sensu* Lugo & Snedaker 1974). From MW to highest high water (HHW) *Avicennia germinans* occurs in mixture with *Rhizophora mangle*, *Conocarpus erectus* L., and *Laguncularia racemosa* (L.) Gaertn.

Leaf tracing experiment

Leaf tracings were performed on *Rhizophora mangle* trees in each of three tidal zones (LW, MW and HHW) and *Avicennia germinans* trees in the HHW zone of Placencia Lagoon. Fifty leaves per population were haphazardly selected for tracing, with the following requirements: (1) all leaves were intact, exhibiting no visible herbivore damage; and (2) all leaves occurred <2 m above the ground, to permit careful tracing and easy re-sampling. Up to five leaves per tree were marked. Leaves were labelled with a plastic numbered parakeet leg band (National Band and Tag Company, Newport, Kentucky), which we affixed loosely around the petiole. As both leaf growth rate and herbivory rate may correlate with age, it was necessary to account for leaf age at the outset. Terminal branches of *Rhizophora mangle* and *Avicennia germinans* possess opposite, paired leaves, with successively older leaf pairs arrayed from the apex back along the twig. The pair number of each labelled leaf was noted, as a relative index of initial leaf age.

Leaf damage was monitored at 13 sampling dates over a period of 310 days between 27 January and 1 December 1988. We carefully flattened each leaf on a clipboard, then traced the leaf margin onto a clear plastic overlay using a colour marker. At each successive tracing date, we recorded new leaf damage on the original overlay using a pen of a different colour. We also recorded the (rare) shrinkage or disappearance of damage seen on previous dates. We retraced all leaf margins on 19 March and 7 November 1988 to account for increases in total leaf area through the year. All percentage damage figures calculated during January to March, March to November, and November to December, were adjusted for these updated leaf area measures.

At the conclusion of the experiment, all leaf tracings were xeroxed onto opaque paper. Whole leaf-tracings were cut out of the paper, and run through a desk-top Licor leaf area meter to determine actual leaf area in cm^2. Areas marked as damaged on the tracings were then excised from the leaf stencils, and the decrease in total leaf area measured as before with the leaf area meter. Leaf areas were measured for each of the 13 sampling dates in this manner, equalized for updated leaf expansion measures in March and November.

Artificial damage experiment

In November 1988, we used a hand-held metal hole punch to create a 6.2 mm diameter hole in each of 60 previously undamaged leaves of *Rhizophora mangle* in the LW zone. These leaves fell into three categories: (1) seedling leaves (N = 30 leaves, one per seedling) occurring on plants <1 m tall with no secondary cortification of the hypocotyl; (2) young leaves occupying the first or second leaf pair on a tree branch (N = 15 leaves, 3 per tree); and (3) older tree leaves from the 3rd through 7th leaf pairs (N = 15 leaves, 3 per tree). Sixty similar leaves on other plants (30 seedling leaves, and 15 of each tree age class) were marked and left intact as controls. All leaves were tagged with small numbered bird bands as above. We repeated the protocol for both *Rhizophora mangle* leaves (N = 60) and *Avicennia germinans* (N = 60) in the HHW zone of the lagoon, for a grand total of 180 hole-punched leaves and 180 intact controls.

Leaf areas were determined *in situ* for all leaves at the outset of the experiment by laying a clear plastic grid of 5 × 5 mm squares over each leaf and counting the number of squares occupied by the leaf (Coley 1983, Ernest 1989, Farnsworth & Ellison 1991, Schoener 1988). We later compared field grid estimates of leaf area with values for a subset of the same leaves given by a Li-Cor Model 3100 leaf area meter. Gridded leaf areas deviated from true leaf area by an average 1.7% ±5.4%. There was no systematic relationship between the percentage deviation from true leaf area based on grid estimate, and the actual size of the leaf ($r = 0.069$; $P = 0.668$; $N = 41$).

The diameter of the artificial hole was measured using calipers (±0.1 mm); this diameter was used to calculate hole area (mm^2). The diameter of any necrosis appearing directly around the hole was similarly measured. Necrosis normally appeared as a concentric ring of dead tissue directly surrounding the hole. Therefore, the area calculated from the diameter of the necrotic ring minus the hole area gave the area of necrotic tissue.

All leaves remaining (grand total = 348) were harvested after 50 days and scored for size, change in hole area on treated leaves, appearance and size of necrotic areas associated with the artificial injury, and other herbivore damage. Seven artificially damaged leaves and five control leaves had disappeared by the harvest; there appeared to be no difference in leaf longevity as a function of treatment. Damage types were identified on the basis of qualitative visual distinctions; several damage types were found to be characteristic of certain herbivore species (Farnsworth & Ellison 1991). The prevalent damage categories were bites (incursions along the leaf margin) and holes (punctures within the leaf) by Lepidoptera and Coleoptera larvae; trails (excavations of the epidermis by leaf mining Lepidoptera larvae, genus *Marmara*); and necrotic spots caused by Homoptera, certain Lepidoptera, fungi and other pathogens. Bagworm moths (probably *Oiketicus* sp.) and scale insects also caused distinctive damage to the leaf epidermis. Crescent-shaped brown scars on *Rhizophora mangle* were left by gastropods (*Littoraria angulifera*) feeding on epiphyllous fungi. We counted the number of grid squares occupied by the various damage types to quantify the percentage of total leaf area lost to herbivores. Wet masses of whole leaves were measured using a 5 g Pesola field scale (to ±0.1 g) to develop an estimate of leaf biomass lost to herbivores (facilities to dry leaves to constant weight were unavailable).

Statistical analyses for both experiments were conducted using SPSS-PC version 3.1. Percentage damage figures were arcsin square root transformed to normalize and equalize variances (Sokal & Rohlf 1981). Specific statistical tests are indicated where appropriate, below.

RESULTS

Leaf tracing experiment

The proportion of *Rhizophora mangle* leaves impacted by herbivores increased over the course of the monitoring period, with much of the damage appearing within the first week of sampling. Within 4 days, 46% of all labelled *Rhizophora* mangle leaves in the LW zone, 62% in the MW zone, and 77% in the HHW zone showed some evidence of attack (Table 1). In contrast, *Avicennia germinans* showed lower initial and overall rates of attack. By day 4 only 26% of *Avicennia germinans* leaves exhibited damage. By 7 November 1988, all remaining *Rhizophora mangle* leaves and 89% of the remaining *Avicennia germinans* leaves showed damage (Table 1).

The fraction of leaf area damaged by herbivores rapidly increased within the first few days, then tailed off over the course of the year. Figure 1 depicts the accumulation of leaf damage as a percentage of total leaf area for *Rhizophora*

Table 1. Percentage of labelled leaves of two mangrove species attacked by herbivores over an 11 month period, from the leaf tracing experiment. Numbers in parentheses are absolute number of leaves with damage/total no. of remaining leaves. Time is expressed as elapsed days, with day 0 showing all leaves undamaged at time of first tracing. The tidal zones are LW = low water; MW = mean water; and HHW = highest high water.

Species		*Rhizophora*			*Avicennia*
Time (days)	Zone	LW	MW	HHW	HHW
0		0	0	0	0
2		18.0 [50]	44.0 [50]	56.0 [50]	16.0 [50]
3		26.0 [50]	46.0 [50]	71.4 [49]	24.0 [50]
4		46.0 [50]	62.0 [50]	76.6 [47]	26.0 [50]
11		74.5 [47]	92.0 [50]	93.5 [46]	48.0 [50]
57		93.0 [43]	95.7 [47]	90.9 [44]	79.2 [48]
285		100.0 [7]	100.0 [15]	100.0 [7]	89.3 [28]
310		100.0 [7]	100.0 [11]	NR	NR

NR = none remaining.

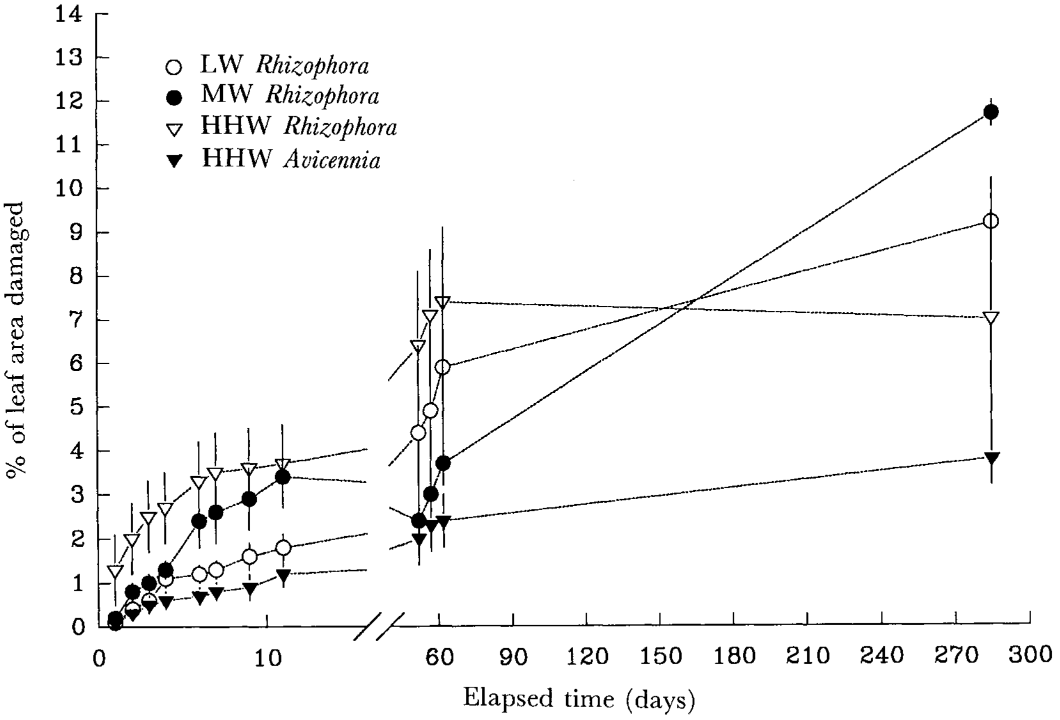

Figure 1. Herbivore damage on traced leaves of *Rhizophora mangle* and *Avicennia germinans* in three tidal zones (abbreviations as in Table 1) over an 11 month period ($\bar{X}$ ±SE at each sampling date). Sample sizes as in Table 1.

mangle (LW, MW and HHW zones) and *Avicennia germinans* (HHW zone), pooled across all leaf age classes and damage types. By day 11, the mean percentage of leaf area lost to herbivores ranged from 1.2% among *Avicennia germinans* trees, to >3.7% among *Rhizophora mangle* trees in the HHW zone. Leaves that were re-censused in March exhibited new leaf growth and new damage. Mean percentage leaf damage hovered between 1% and 6.3% for all leaves sampled at this time (Figure 1). By 7 November, mean percentage leaf damage approached 12% on *Rhizophora mangle* leaves from the MW zone, 9.2% in LW *Rhizophora mangle*, 7.0% in HHW *Rhizophora mangle* leaves, and only 3.8% among HHW *Avicennia germinans* leaves. High variance in damage among leaves as well as small remaining sample populations contributed to the high standard errors (Figure 1).

Mean relative herbivory rates (% damage accruing per day) were calculated at each sampling date as:

$$(\%\mathrm{dam}_{t2} - \%\mathrm{dam}_{t1})/t_2 - t_1,$$

where %dam is the percentage of total leaf area damaged, t_1 and t_2 are consecutive sampling dates (the denominator gives elapsed days). Figure 2 shows that relative herbivory rates accelerated briefly during the first two days of observation. Thereafter, herbivory rates declined among all species in all zones over 310 days, most by a factor of 5 (Figure 2).

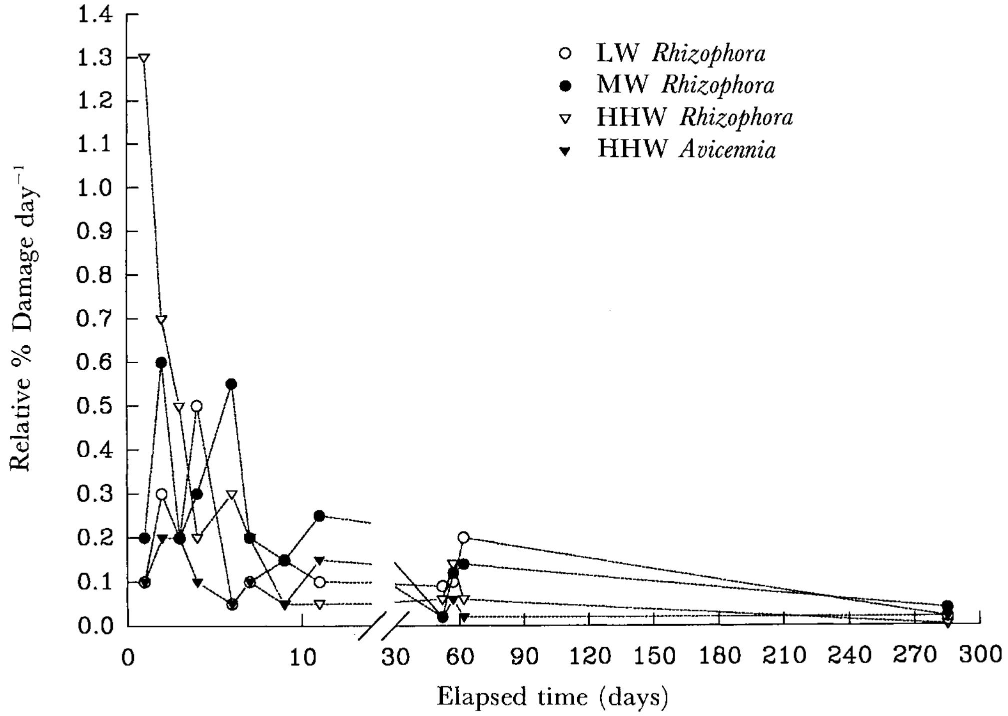

Figure 2. Relative rates of herbivory (mean percentage damage per leaf per day) on leaves of *Rhizophora mangle* and *Avicennia germinans* in three tidal zones (abbreviations as in Table 1) over an 11 month period, from leaf tracing data.

Herbivory on *Rhizophora mangle* trees varied with tidal height. At all but the final sampling date, *Rhizophora mangle* growing in the HHW zone experienced significantly higher rates of herbivory than *Rhizophora mangle* from either the LW or MW zones (Scheffé *a posteriori* test for multiple comparisons among means, $0.0001 < P < 0.029$ for all tests; $0.45 < P < 0.595$ at final date).

Within the HHW zone, *Rhizophora mangle* leaves exhibited significantly higher percentage damage than neighbouring HHW *Avicennia germinans* leaves at all but the final sampling date, when only seven *Rhizophora mangle* leaves were left for comparison ($P < 0.0001$, ANOVA on species nested within HHW zone, with transformed values of percentage damage as dependent variable; $P = 0.225$ at final sampling date). Although damage types appeared superficially similar, careful inspection of the insects responsible showed that *Rhizophora mangle* and *Avicennia germinans* trees did not share overlapping suites of herbivores, even when growing in close proximity.

Herbivores did not appear to concentrate initially on leaves of a particular age class. Transformed percentage damage values were regressed on leaf pair separately for each species and tidal zone. Whilst percentage damage tended to be higher on older leaves, there were no significant ($P < 0.05$) relationships between herbivory and leaf age for either 30 January, 7 February, or 24 March. Thereafter, with new leaves emerging, many of the original cohort were moving into new age classes; further age distinctions became problematic and additional analyses of age effects were not possible.

Leaves of *Rhizophora mangle* grew slowly throughout the year (Table 2), and growth rates differed between species, tidal zone, and leaf age classes. In the HHW and MW zones, young *Rhizophora mangle* leaves occurring in the first leaf pair grew significantly faster than older leaves during the first three months of monitoring, and second-pair leaves grew consistently (but not significantly) faster than third-pair or older leaves. Differences in growth rate by leaf age class were not pronounced in LW *Rhizophora mangle* leaves, however (Table 2). *Rhizophora mangle* leaves of all age classes grew significantly faster in the HHW zone than in the MW or LW zones (January to March: $P < 0.009$, $F_{2,133} = 4.9$; ANOVA with *a posteriori* Tukey HSD test to distinguish between three zones; March to November: $P < 0.0001$, $F_{2,26} = 16.08$).

Young *Avicennia germinans* leaves exhibited slightly, but not significantly higher growth rates than older leaves (Table 2). Pooled over all age classes, *Avicennia germinans* leaves grew very little over the year; the normalized change in leaf size between January and March, March and November did not differ significantly from 1.00 (by overlap of 95% confidence intervals). By contrast, young *Rhizophora mangle* leaves of the same zone grew by a factor of 1.8 (Table 2). Older leaves of both species did not differ in overall growth rates.

Artificial damage experiment

Artificial holes punched into mangrove leaves tended to expand over 50 days (Table 3), although the magnitude of this expansion was variable. Holes

Table 2. Growth of *Rhizophora mangle* (in 3 tidal zones) and *Avicennia germinans* leaves, expressed as normalized change in leaf area, for all leaves from 27 January to 7 November, and for each of the first three pairs for both species from 27 January to 19 March. Normalized values are ratios of leaf area relative to updated initial leaf areas. Leaf pair number was used as an index of initial leaf age. Significant changes in leaf size between 27 January and 19 March, and between 19 March and 7 November (by non-overlap of 95% confidence intervals) are denoted by asterisk. Values within tidal zones with different superscripts are significantly different by non-overlap of 95% confidence intervals. Abbreviations for zones as in Table 1.

			Normalized change in leaf size ($\bar{X} \pm 1$ SE[N])		
		Dates	27 Jan to 19 Mar		19 Mar to 7 Nov
Species	Zone	Leaves			
Rhizophora mangle	LW	All	1.00±0.02 [43]	NS	1.07±0.07 [7]
		Pair 1	0.96±0.05 [11]^{a}		—
		Pair 2	1.02±0.04 [22]^{a}		—
		Pair 3	1.01±0.01[8]^{a}		
	MW	All	1.02±0.02 [49]	NS	1.05±0.03 [15]
		Pair 1	1.13±0.08 [9]^{a}		—
		Pair 2	1.02±0.03 [24]^{a}		—
		Pair 3	0.98±0.03[12]^{a}		—
	HHW	All	1.15±0.05 [44]	*	1.60±0.12 [7]
		Pair 1	1.80±0.08 [5]^{a}		—
		Pair 2	1.33±0.09 [13]^{b}		—
		Pair 3	0.94±0.02[15]^{c}		—
Avicennia germinans	HHW	All	0.96±0.02 [49]	NS	0.96±0.01[29]
		Pair 1	1.01±0.04 [18]^{a}		—
		Pair 2	0.96±0.02 [15]^{a}		—
		Pair 3	0.96±0.05[8]^{a}		—

Table 3. Change in hole area and area of necrotic tissue surrounding holes on leaves of *Rhizophora mangle* and *Avicennia germinans* recorded for a 50 day period ($\bar{X}$ ±1 SE). For each species in a given tidal zone, values with different superscripted letters are significantly different ($P \leq 0.05$, Scheffé test for multiple comparisons between means). Necrotic areas were not significantly different by Scheffé test across populations. 'Age' refers to leaf age class. Abbreviations for zones as in Table 1.

Zone	Species	Age	N	Change in hole area (mm^2)	% change in hole area	Change in area of necrosis (mm^2)	% leaves with necrosis
LW	*R. mangle*	young	15	12.1±4.5^{a}	40.1	14.4±2.2	100.0
		old	15	−0.1±0.4^{b}	−0.3	17.8±3.2	100.0
		seedling	30	2.2±1.1^{b}	7.3	11.9±0.8	100.0
HHW	*R. mangle*	young	13	9.5±1.7^{a}	31.5	14.7±3.0	100.0
		old	14	0.9±0.4^{b}	2.9	11.1±1.1	100.0
		seedling	28	0.5±0.4^{b}	1.7	12.2±1.3	100.0
HHW	*A. germ.*	young	14	7.2±3.1^{ab}	23.9	10.9±1.6	92.9
		old	15	1.2±0.5^{a}	3.9	11.6±1.9	100.0
		seedling	29	13.6±3.2^{b}	45.1	12.7±1.8	89.7

expanded regularly in all directions, and the reported expansion was not a product of herbivore feeding around the hole. The two mangrove species exhibited comparable, broadly variable rates of artificial hole expansion, from −0.33% to 45.07% changes in hole area.

The majority of treated leaves developed areas of necrotic tissue directly associated with the artificial hole within a few days of the treatment. At harvest, 100% (N = 115) of *Rhizophora mangle* leaves and 93.1% (54 of 58) of *Avicennia germinans* leaves showed some form of necrosis around the hole. Necrotic areas were consistent in size across leaf age classes, species, and tidal zones, ranging from 11.1 mm^2 to 17.8 mm^2 (Table 3). Normally green leaf tissue became dark brown and brittle in a ring around the artificial hole. Such areas did not develop in control leaves, except around certain naturally damaged areas, especially holes left by Lepidoptera. Traced leaves also exhibited secondary necrosis around bites and holes.

Holes in adult young leaves expanded significantly faster than holes in seedling leaves among *Rhizophora mangle* trees of both zones (by Scheffé test, Table 3). However, opposite results were found for *Avicennia germinans*. Holes expanded 45% on average among these seedlings, and 24% among young tree leaves, but high variance swamped apparent differences between these means ($P > 0.05$, Scheffé test). The mean rates of hole expansion in both species were consistently higher among young tree leaves than among older tree leaves, although differences in hole growth rate were significant only in *Rhizophora mangle* (by Scheffé test, Table 3). To determine the extent to which leaf age could explain differences in hole expansion, values of percentage change in hole area were regressed on leaf age, separated by tree and seedling samples, for each species. Prior inspection of residuals indicated a roughly normal distribution of values, so variables were not transformed. Significant regressions of hole expansion on leaf age were found only for tree leaves of *Rhizophora mangle* at LW and HHW ($P < 0.01$, Model I regressions; $r^2 = 21-23\%$).

We also tested the premise that changes in hole area were a function of leaf growth over the experimental period, with the hypothesis that rapidly-expanding holes would occur in faster growing leaves. Model II regressions of hole expansion rates on percentage changes in leaf area were performed. The relationship between leaf growth and hole growth was only significant for *Rhizophora mangle* tree leaves in the LW zone ($P < 0.0001$, $r^2 = 42\%$).

Leaves with artificial holes grew on average 50% to 90% slower than control leaves over the 50 days. However, high variability in growth rates obscured any significance in these trends (unpaired t-test; *Rhizophora* $F_{1,118} = 0.218$, NS; *Avicennia* $F_{1,58} = 0.396$, NS). Uninjured *Rhizophora mangle* leaves grew 19.9% (SE ±0.333), while hole-punched leaves grew 9.8% (SE ±0.319). *Avicennia germinans* leaves grew less than *Rhizophora mangle* leaves, and exhibited the same treatment effect (controls, $\bar{X} = 16.6\% \pm 0.305$; with holes, $\bar{X} = 1.6\% \pm 0.225$).

Artificial injury to leaves appeared to influence the amounts of damage subsequently appearing on leaves of *Rhizophora mangle* and *Avicennia germinans*. Previ-

Table 4. Percentages of leaf area attacked ($\bar{X} \pm 1$ SE; sample size N), and percentage of leaves attacked by herbivores for *Rhizophora mangle* and *Avicennia germinans*. For each species in a given tidal zone, within each treatment (leaves with artificial holes or leaves without artificial holes), mean % damage values with different superscripted letters are significantly different ($P \leq 0.05$, Scheffé test for multiple comparisons between means). For comparisons of mean leaf area damaged between treatments, * indicates significant ($P \leq 0.05$, t-test). All statistics were performed on arcsin square root transformed data.

	Rhizophora						*Avicennia*		
Zone:	LW			HHW			HHW		
Age:	Young	Old	Seedling	Young	Old	Seedling	Young	Old	Seedling
Leaves with artificial holes									
N	15	15	30	13	14	28	14	15	29
Mean % area damaged	10 ± 1.9^{a}	47 ± 11.8^{a}	14 ± 3.3^{a}	3 ± 0.6^{a}	9 ± 2.4^{a}	13 ± 4.0^{a}	5 ± 1.1^{a}	9 ± 1.7^{a}	7 ± 1.6^{a}
% attacked	100.0	100.0	100.0	100.0	100.0	92.6	71.4	93.3	58.6
Leaves without artificial holes									
N	16	13	29	13	15	29	15	15	30
Mean % area damaged	2 ± 0.6^{a}	2 ± 0.6^{a}	7 ± 2.5^{a}	9 ± 2.9^{a}	3 ± 0.8^{a}	6 ± 2.4^{a}	4 ± 2.8^{ab}	0.3 ± 0.2^{a}	9 ± 3.6^{b}
% attacked	66.7	78.6	89.3	76.9	93.3	50.0	42.9	20.0	60.0
Significance	*	NS	NS	NS	*	*	NS	*	NS

ously injured leaves of all treatment populations except HHW young *Rhizophora mangle* leaves and seedling leaves of *Avicennia germinans* showed higher herbivore damage (expressed as a percentage of leaf area attacked) than their corresponding control populations (Table 4). These differences in percentage damage were significant only for certain populations, however (t-tests, Table 4). A higher percentage of hole-punched leaves were attacked by herbivores than control leaves in all populations except *Avicennia germinans* seedlings (Table 4). In general, as in the leaf tracing study, more *Rhizophora mangle* leaves than *Avicennia germinans* leaves were attacked. Herbivory rates on untreated leaves over 50 days were comparable to the damage shown on leaves followed in the tracing experiment at 52 days (compare Figure 1 representing old and young leaves pooled, and Table 4).

Herbivory levels did not show a consistent relationship with leaf age class as defined by young tree, old tree or seedling groups (Table 4). Likewise, Kolmogorov-Smirnov goodness-of-fit tests relating probability of attack with leaf pair number were not significant among LW *Rhizophora mangle* trees or seedlings (treatments pooled; $z = 0.997$, two-tailed $P = 0.273$), HHW *Rhizophora mangle* leaves ($z = 0.714$, two-tailed $P = 0.687$), or *Avicennia germinans* leaves ($z = 1.134$, two-tailed $P = 0.153$).

Figure 3 depicts the distribution of particular damage types occurring on treated and control leaves of both species. The damage type spots contributed most to the disparity between total percentage damage values reported above, more than doubling in artificially injured leaves. These 'spots' were visually distinguishable from the necrotic areas associated with holes, and included white spots caused by Lepidoptera larvae (especially *Megalopyge* sp. in *Rhizophora* and an unidentified leaf-rolling larva in *Avicennia*), and apparent fungal infections. The percentage damage occurring as 'bites', however, was lower among treated tree leaves, although this difference varied by age class and was not significant overall ($P = 0.48$ by ANOVA for *Rhizophora mangle* leaves). However, treated leaves of *Avicennia germinans* seedlings exhibited lower levels of bites and spots than controls, and lower levels of damage overall (Figure 3, Table 4). The percentage damage attributable to other types of herbivores did not vary consistently between artificially damaged and control leaves.

The amount of leaf biomass lost to herbivores was calculated, incorporating a correction factor, for both hole-punched and control leaves, based on wet mass measurements undertaken at leaf harvest (Table 5). This correction factor, ranging from 1.00 to 1.45, was derived directly from hole expansion indices recorded for the different species and leaf age classes (Table 3). Biomass of leaf tissue taken by herbivores ranged from 11 to 50 g m^{-2} for *Rhizophora mangle* and 2 to 27 g m^{-2} for *Avicennia germinans* over the course of 50 days (Table 5).

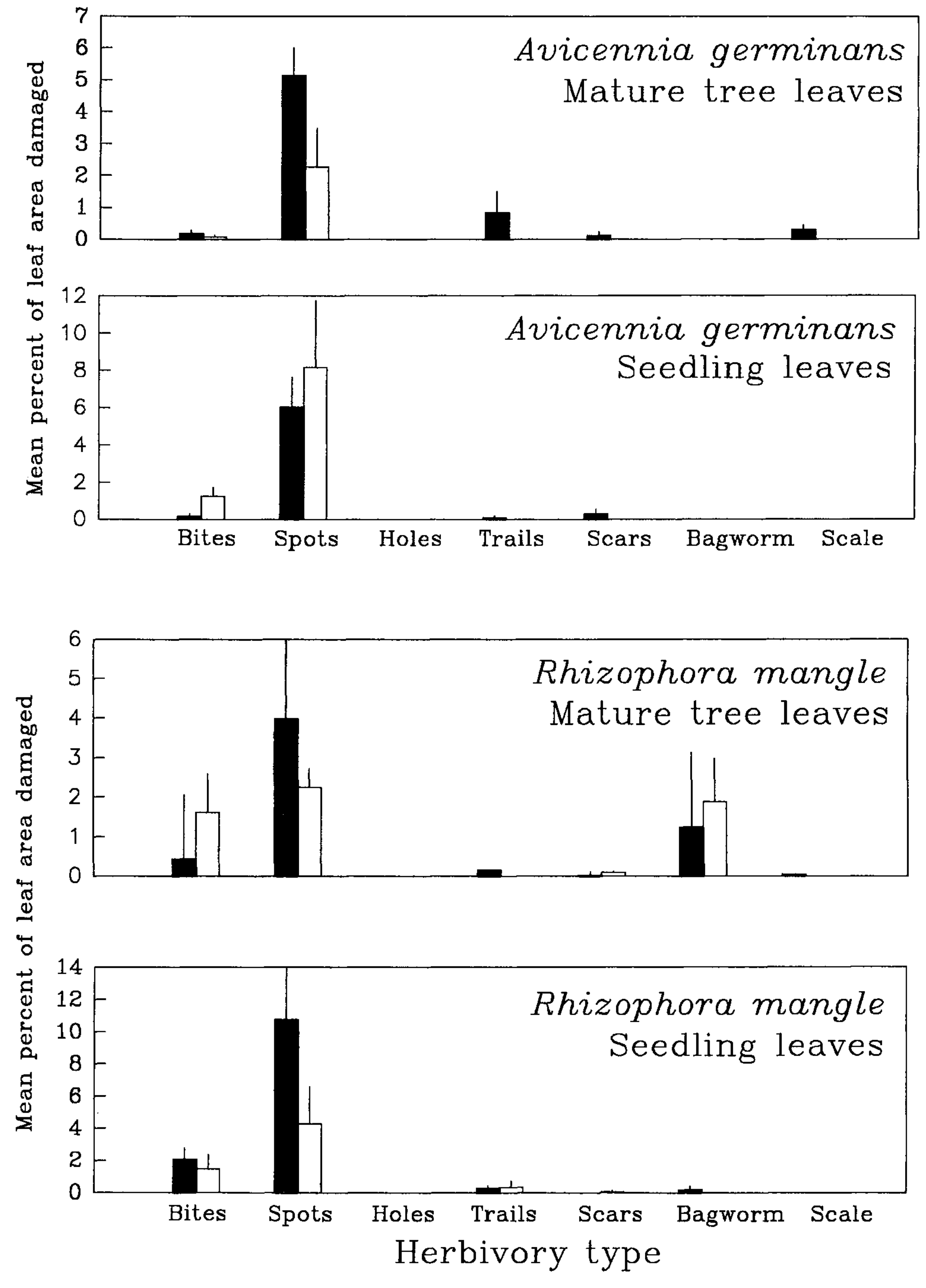

Figure 3. Types of herbivore damage occurring on mature and seedling leaves of *Rhizophora mangle* and *Avicennia germinans* with and without artificial holes recorded over a 50 day period. Bars represent mean percentage of total leaf area (±1 SD). Leaves with artificial holes ■, leaves without artificial holes □.

Table 5. Leaf tissue (g m^{-2}) lost to herbivores for leaves without artificial holes of *Rhizophora mangle* and *Avicennia germinans*, estimated with hole growth correction factor.

Species	Zone	Age	Leaf biomass (g m$^{-2)}$	% of leaf eaten	Biomass lost (g m^{-2})	Correction factor	Adjusted loss (g m$^{-2)}$
Rhizophora	LW	Young	795.8	2	15.9	1.40	11
		Old	803.7	2	16.1	1.00	16
		Seedling	765.2		53.6	1.07	50
	HHW	Young	570.5	9	51.3	1.32	39
		Old	638.4	3	19.2	1.03	19
		Seedling	568.9	6	34.1	1.02	33
Avicennia	HHW	Young	509.8	4	20.4	1.24	16
		Old	612.8	0.3	1.8	1.04	2
		Seedling	440.5	9	39.6	1.45	27

DISCUSSION

Long-term studies of herbivory elucidate sources of error in static estimates of herbivory, healing and expansion of injuries, the temporal dynamics of herbivore activity on single leaves, and the effects of herbivory on leaf growth and senescence. Both the leaf tracing observations and the artificial damage experiment allowed us to follow individual leaves over periods ranging from 50 to 310 days, and several patterns became evident from our data. Both experiments revealed highly comparable herbivory rates (Figure 1, Table 4), even though the experiments were initiated at different seasons (leaf-tracing in January, hole-punch in November). The vast majority of leaves followed in both studies were attacked by herbivores. *Rhizophora mangle* trees growing in the HHW tidal zone exhibited higher rates of herbivory than their LW counterparts in both experiments, a finding consistent with one-time samples elsewhere in Belize (Farnsworth & Ellison 1991).

Leaf injuries did expand in growing leaves. Static surveys may attribute gaps and holes seen in leaf tissue entirely to herbivores, when in fact, apparent tissue loss may simply increase with leaf growth. Robertson & Duke (1987) quantified the magnitude of this growth-related hole expansion for paleotropical mangrove species. Artificial holes in leaves of *Rhizophora stylosa* Griff. expanded by a mean factor of 2.08, and holes in *Ceriops tagal* (Perr.) C. B. Robinson by a factor of 2.37 over the course of 35 to 90 days. Our objective was to obtain error estimates for two Neotropical species, *Rhizophora mangle* and *Avicennia germinans*. The artificial holes we followed in *Rhizophora mangle* and *Avicennia germinans* also expanded, but the magnitude of this expansion was highly variable between species and leaf age classes (Table 3). Our expansion indices ranged from a factor of 1.00 to 1.45 over the course of 50 days, with a mean expansion of 11.3% for *Rhizophora mangle* and 24.3% for *Avicennia germinans*. Holes monitored for a full 90 days might have exhibited expansion indices approaching 2.00, assuming that leaf growth and hole expansion rate remained constant throughout this time. However, it was inadvisable to extrapolate these figures, as the leaf tracing data clearly indicate that leaf growth is neither constant, nor independent of leaf age (Table 2). Reichle *et al.* (1973) developed a model for estimating biomass loss to herbivores in *Liriodendron tulipifera* L., incorporating a hole expansion correction factor that was dependent on leaf age class. They applied these correction factors to deciduous leaves sampled at the end of the growing season, by which time all leaves had expanded fully. Such error estimates are more problematic in Neotropical mangal that continuously produces leaves and does not exhibit a discrete annual deciduous period. We applied correction factors obtained for each species and each leaf age class to obtain a conservative estimate of leaf biomass lost to herbivores (Table 5). We justify this correction because all leaves were initially undamaged, leaf ages were recorded throughout the experiment and all leaves were harvested and weighed simultaneously after 50 days. However, we introduce this correction with the caveat that hole expansion is a more complex phenomenon than has been assumed previously.

Should we generally apply a correction factor based on these studies to static estimates of herbivore damage? Such a blanket application would be based on three assumptions: (1) that damage will expand most rapidly in faster-growing young leaves; (2) that most herbivory occurs on young leaves that are not fully expanded; and (3) that this correction factor should be applied across herbivory estimates for leaves in all age classes. Data from the artificial damage experiment suggest that injuries do expand 7–10 times faster among leaves of younger age classes (Table 3), which themselves grew faster than older leaves (Table 2). However, hole expansion was by no means a linear function of leaf growth (Table 5). Baseline leaf growth rates noted from leaf tracings varied between mangrove species, leaf age, and tidal zone (Table 2). Moreover, leaves with artificial holes grew more slowly than undamaged leaves over 50 days. Thus, herbivory exerts its own effects on leaf growth (and possibly on leaf turnover), independent from hole expansion and leaf age.

Neither the artificial damage nor the leaf tracing observations indicate that herbivores concentrate their activity on leaves of younger age classes (Table 2, Table 4). Previously undamaged older tree leaves displayed similar herbivory rates to their counterparts in the first leaf pair. In a survey of herbivory at several sites in Belize, taking leaf pair number into account, we found no correlation between herbivore damage and leaf age (Farnsworth & Ellison 1991). Our observation that Neotropical mangrove herbivores do not initially discriminate on the basis of leaf age departs from herbivory studies in other systems, but is consistent with other findings in mangrove forests. In general, nutritional quality is seen to decline in older, tougher leaves, and many herbivores preferentially attack newly emerging leaflets (e.g. Coley 1983, Crawley 1983 and references therein). However, Newbery (1980) found that coccids preferentially infested older *Avicennia marina* (Forsk.) Vierh. trees and that foliar nutrient characteristics did not differ between older and younger trees. Mattson (1982) determined that mangrove tree crabs (*Aratus pisonii* Milne Edwards) prefer older *Rhizophora mangle* leaves to younger leaves when offered a choice. Though our present study demonstrates that holes and bites occurring in younger leaves expand more rapidly than similar injuries occurring in older leaves, a correction factor is necessary if most herbivory occurs on young leaves, and if young leaves constitute the majority of the leaves sampled. The former requirement is not borne out by our data, and the latter requirement is usually not accounted for in static herbivory estimates. Most estimates are based on random samples of young and old leaves; the relative proportions of different leaf age classes are not assessed. Hence, we record relative plant age, and our error corrections incorporate separate expansion factors for young and old leaves. Our resulting estimates of biomass loss to herbivores are broadly comparable to, but somewhat higher than, those of Robertson & Duke (1987) and Onuf *et al.* (1977). Measured rates of herbivory were higher at our site, and we used wet mass rather than dry mass figures.

Artificially and naturally-damaged leaves consistently developed areas of necrosis within a few days, a phenomenon that does not appear to be an artefact of hole-punching. Whether this necrosis is due to secondary infection or scarring following leaf injury is unknown, and begs further study. While tissue death does not constitute an absolute loss of biomass to the litter pathway, such necrosis may reduce the photosynthetic area of individual leaves. Impaired photosynthetic capacity could in part explain the lower growth rates of damaged leaves. Herbivore-induced leaf necrosis may reduce primary productivity of trees and seedlings, but is largely ignored in the majority of damage surveys in mangroves.

The foregoing experiments also suggest that prior damage influences subsequent herbivore activity on mangrove leaves. The artificial damage experiment demonstrates the short-term responses of herbivores to apparent leaf damage. Chemical assays to compare levels of tannins, phenolics and other secondary metabolites among treated and control leaves were not feasible, so we focussed on a biotic assay – herbivore activity – to detect possible foliar induction responses. Leaves with holes suffered higher rates of herbivory than control leaves over 50 days (Table 4), and older leaves of HHW *Rhizophora mangle* and *Avicennia germinans* trees were significantly harder hit than their untreated counterparts. Herbivory did not significantly increase among younger leaves of these HHW trees, however, evidence that young leaves of HHW trees may respond differently to injury. Spots caused by Lepidopteran larvae and sucking insects were more prevalent on most classes of treated leaves than on controls (Figure 3), contributing most to differences in damage amounts between treatments. In contrast, treated leaves of *Avicennia germinans* seedlings exhibited lower levels of bites and spots than controls, and lower levels of damage overall (Figure 3, Table 4). Observations of leaves on adult trees yield little evidence of induction of defensive compounds that would discourage consequent herbivory. Inducible defenses may entail an integrated response over the entire tree and are only variably reflected in individual tree leaves (e.g. Edwards & Wratten 1983, Green & Ryan 1972, Haukioja & Niemela 1979). It is probably more feasible to detect induction at the seedling level, although, to our knowledge, no artificial damage experiments have explicitly addressed ontogenetic differences in integration of inducible defenses. On a smaller scale, *Avicennia germinans* seedlings may display more rapid, discernible whole-plant responses to herbivory. Alternatively, we might hypothesize that herbivore feeding rates may decline as leaf area available for consumption (or leaf apparency) decreases.

Avicennia germinans leaves exhibited significantly less herbivore damage than *Rhizophora mangle* in the HHW zone throughout the year (Figure 1), a finding consistent with other surveys in Belize (Farnsworth & Ellison 1991). It is of interest that *Avicennia germinans* leaves also displayed very low growth rates relative to *Rhizophora mangle* leaves during the same period. This finding implies a possible trade-off in resources (*sensu* Bazzaz *et al.* 1987) dedicated to growth v. herbivore deterrence in *Avicennia germinans*. Throughout the tropics, members of the Avicenniaceae display very different leaf chemistry from the Rhizophoraceae (Hogg & Gillian 1984, Ghosh *et al.* 1985, Lacerda *et al.* 1986), reflecting differences in habitat, the physiology of salt tolerance, and relative investments to secondary metabolites. *Avicennia germinans* in Belize also supports a distinct herbivore fauna, not shared with *Rhizophora mangle* (Farnsworth & Ellison 1991).

Longer-term inspections revealed a consistent decline in herbivory rates over 9 months of leaf tracings. Herbivores attacked most labelled leaves within the first week of sampling (Table 1, Figure 1). Thereafter, herbivory rates, expressed as new percentage damage accruing per day, declined for both species in all tidal zones (Figure 3). This tailing off of herbivory could result from: (1) a saturation of feeding sites on individual leaves; (2) a decrease in leaf apparancy to new herbivores after initial attack; (3) seasonality in attack rates among herbivores themselves; (4) the appearance of inducible defences in leaves, rendering them unpalatable to later herbivores. Future investigations should distinguish between these hypotheses. Together, chemical and biological assays as well as long-term monitoring reveal important patterns in herbivory and herbivore defence in mangal.

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