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Community Ecology

Fungal diversity and plant disease in mangrove forests: salt excretion as a possible defense mechanism

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Keywords. Salt excretion in leaves of some mangrove species may serve as an important defense against fungal attack, reducing the vulnerability of typically high-density, monospecific forest stands to severe disease pressure. In field surveys of a Caribbean mangrove forest in Panama, *Avicennia germinans* suffered much less damage from foliar diseases than did *Laguncularia racemosa* or

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Rhizophora mangle. Similarly, *Avicennia* leaves supported the least superficial fungal growth, endophytic colonization, and diversity, followed by *Laguncularia* and *Rhizophora*. Host specificity of leaf-colonizing fungi was greater than expected at random. We hypothesize that the different salt tolerance mechanisms in the three mangrove species may differentially regulate fungal colonization. The mangroves differ in their salt tolerance mechanisms such that *Avicennia* (which excretes salt through leaf glands) has the highest salinity of residual rain water on leaves, *Laguncularia* (which accumulates salt in the leaves) has the greatest bulk salt concentration, and *Rhizophora* (which excludes salt at the roots) has little salt associated with leaves. The high salt concentrations associated with leaves of *Avicennia* and *Laguncularia*, but not the low salinity of *Rhizophora*, were sufficient to inhibit the germination of many fungi associated with mangrove forests.

Keywords. *Avicennia germinans* - *Laguncularia racemosa* - Panama - Plant disease resistance - *Rhizophora mangle*

Introduction

When a plant species grows at high densities, the plant population is often much more susceptible to diseases and pests than the same plants would be at lower densities. Such density-dependent disease development is well documented in agricultural systems, and is also a common phenomenon in natural plant communities (Burdon and Chilvers *1982*; Augspurger and Kelly *1984*; Gilbert et al. *1994*). Moist tropical forests are widely known for their great diversity and low density of individual plant species, and many studies suggest that this is a result of density-dependent disease and herbivory dynamics (Janzen *1970*; Connell *1971*; Augspurger and Kelly *1984*; Clark and Clark *1984*; Gilbert et al. *1994*). Mangrove forests are unusual among tropical ecosystems because of their naturally low plant diversity and high population densities, a consequence of the physiological restrictions of living in the intertidal environment (Tomlinson *1986*). Unless mangrove species are particularly well defended against pathogen attack, we would expect to find high disease pressure in these systems. However,

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preliminary observations in the mangrove forests on the Caribbean coast of the Republic of Panama indicated that although saplings of *Rhizophora mangle* L. (Rhizophoraceae; red mangrove) suffered severely from leaf diseases, leaves of similarly common *Avicennia germinans* (L.) L. (Verbenaceae; black mangrove) and *Laguncularia racemosa* (L.) Gaertn. (Combretaceae; white mangrove) were nearly disease free. This paper explores the possibility that foliar salt excretion in some mangrove species may represent a previously unrecognized mechanism for resistance to fungal diseases.

While numerous studies describe fungi associated with mangrove species, most emphasize the diversity of saprophytic marine fungi growing on roots and on submerged, decomposing wood in the intertidal zone (see review in Hyde and Lee *1995*). A few studies have focused on describing the kinds and relative abundance of wood-decay basidiomycetes associated with dead and live trees (Sotão et al. *1991*; Filho et al. *1993*; Gilbert and Sousa, unpublished data) or on describing the fungi that cause canker diseases (Olexa and Freeman *1978*; Tattar et al. *1994*). Little, however, is known of the factors governing the distribution, incidence, or effects of fungi that infect leaves of mangrove species (McMillan *1964*; García López et al. *1989*; Suryanarayanan et al. *1998*). Foliar diseases have been shown to have significant effects on plant survival, growth, and fitness in natural ecosystems (Alexander and Burdon *1984*; Krause and Raffa *1992*; Lively et al. *1995*). Our observation that *Rhizophora* suffers greater pressure from foliar diseases than do *Laguncularia* and *Avicennia* is supported by the fungal host index of Farr et al. (*1989*), who list ten known foliar diseases of *Rhizophora*, but only three for *Laguncularia* and one for *Avicennia*.

The apparently greater susceptibility of *Rhizophora* to foliar diseases is somewhat surprising given the documented resistance of its leaves and stems to microbial attack - *Rhizophora* leaves decompose much more slowly than do leaves of *Avicennia* (Robertson *1988*), and seedlings are protected against canker disease by polyphenol oxidase activity (Tattar et al. *1994*). Additionally, leaves of *Rhizophora* seedlings grown under low light levels (similar to those of the mangrove understory in our study) have been shown to have higher levels of

phenolics (principally condensed tannins) than do leaves of *Laguncularia* (mostly gallotannins) (McKee *1995*). *Avicennia* leaves have very low levels of phenolics (McKee *1995*), although anti-herbivore compounds such as iridoid glucosides may be present (Fauvel et al. *1995*). Because development of foliar diseases in the tropics is very strongly associated with insect damage (Garcia-Guzman and Dirzo *2001*), we would predict that species like *R. mangle*, with high levels of anti-herbivore phenolic compounds, would also suffer less foliar disease damage. Why then, do *Avicennia* and *Laguncularia*, growing at high densities in a species-poor environment, suffer so much less foliar disease than does *Rhizophora*?

Importantly, different mangrove species have developed distinct mechanisms for dealing with the salt stress from exposure to tidal flooding by seawater. We hypothesized that one strategy - excreting salt through the leaves - may also serve as an effective mechanism reducing fungal infection and subsequent disease development. The largely disease-free *Avicennia* take up salt in the transpiration stream and excrete salt through glands in their leaves (Tomlinson *1986*). Through evaporation, the salt crystallizes on the leaf surface, where it then falls or washes off. *Laguncularia* also accumulate salt in their leaves, but it is not clear that the observed glandular structures function in the excretion of salt (Tomlinson *1986*). In contrast, *Rhizophora* filter salt from the water as it passes into their roots, so that little salt passes into the plant and up to the leaves (Tomlinson *1986*). In mangrove forests in Panama, we compared disease severity and incidence, fungal infection, and the structure of associated fungal assemblages among three mangrove species that vary in their mechanisms for living in high-salt environments. Specifically, we explore the hypothesis that the high salt concentration in and on leaves of *A. germinans* and *L. racemosa* may effectively protect them from foliar diseases, compared to *R. mangle*.

Materials and methods

Sites and host species

This study was conducted between late 1995 and early 1998 at two locations: in a

Caribbean mangrove forest near the Smithsonian Tropical Research Institute's Galeta Marine Laboratory (9°24′18" N, 79°51′49" W) at Punta Galeta, Colón province (Garrity et al. *1994*; Sousa and Mitchell *1999*), and in a Pacific mangrove forest in the Ensenada de La Claridad, Punta Chame (8°38′8" N, 79°43′11" W), Republic of Panama (Castillo *1996*). Both forests were strongly dominated by the same three mangrove species, *Rhizophora mangle*, *Avicennia germinans*, and *Laguncularia racemosa*, hereafter referred to by genus name. In both locations we chose study sites where the three mangrove species occur sympatrically: three sites at Punta Galeta and one area at Punta Chame. At Punta Galeta, we selected sites separated by at least 1 km, all located within the Margarita and Western Bahía Las Minas sections of Punta Galeta (Duke et al. *1997*; Sousa and Mitchell *1999*).

Disease severity and incidence

To determine patterns in the incidence and severity of foliar diseases, we censused 100 juveniles of each of the three mangrove species at each of the three sites at Punta Galeta (300 plants per species, all between 30 and 100 cm tall). On each plant we determined how many leaves showed symptoms of any fungal diseases and how many were apparently healthy. Disease symptoms and signs included necrotic tissue characteristic of disease, associated chlorosis, and direct observation of fungal reproductive structures. For *Rhizophora* and *Laguncularia* we counted all leaves on a plant, but because even juvenile *Avicennia* had many leaves, we selected one branch haphazardly and counted all leaves on the branch; this provided us with comparable numbers of leaves per plant across the species (Table 1). For each site we determined the proportion of leaves that were symptomatic for any foliar disease (disease severity), and the percentage of plants with one or more symptomatic leaves (disease incidence). To test for differences in disease severity, we used ANOVA (SAS 6.12, 1996) to compare the proportion of leaves diseased among species. Data were arcsine-square-root transformed prior to analysis, and we conducted the analysis first treating each site as a block and then for each site independently. To assess differences between species in disease incidence we used chi-square contingency table analysis (SAS 6.12, 1996)

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on presence and absence of disease on individual plants for all sites combined.

Table 1. Percentage of leaves of three mangrove species (± SD) that showed fungal disease symptoms in each of three sites. Means within a row followed by the same letter are not significantly different ($P \leq 0.05$, Fisher's protected LSD)

	Percent of mangrove leaves symptomatic[a]				
Site	*Avicennia*	*Laguncularia*	*Rhizophora*	F[b]	P value
Combined	3.8±1.4 a	6.6±5.4 a	27.3±6.6 b	254.5	0.0001
1	2.2±5.2 a	2.4±7.5 a	21.3±18.2 b	97.6	0.0001
2	4.1±6.6 a	4.6±8.0 a	34.4±20.9 b	142.6	0.0001
3	5.0±8.0 a	12.7±16.5 b	26.3±18.9 c	49.4	0.0001

[a]Mean ± SD number of leaves examined per plant was 7.2±0.9 for *Rhizophora*, 5.7±0.7 for *Laguncularia*, and 7.8±0.8 leaves per *Avicennia*, as described in Materials and methods

[b]For combined analysis, site effect was significant (df=2, 895, F=20.5, $P \leq .0001$) and for main effect shown, df =2, 895. For each of sites 1, 2, and 3, df =2, 297

Fungal abundance, diversity, and specificity

To assess the infection rates, diversity, and specificity of the leaf-inhabiting fungi, we isolated fungi from recently matured, healthy leaves of each of the three mangrove species at Punta Chame. Based on preliminary studies, leaves of *Avicennia* and *Laguncularia* reach mature size after 2 months, whereas *Rhizophora* leaves require 4 months to mature. Three leaves were collected from each of ten individuals per species, and processed within 24 h. The median portion of each leaf was cut into many small segments (3 mm^2) and then surface sterilized in 70% ethanol (1 min) and 0.5% sodium hypochlorite (3 min) solutions, followed by a 30-s rinse in sterile water. After sterilization, 24

randomly selected segments per leaf were divided between two Petri plates with 1.5% malt extract agar (MEA), and incubated at 22°C for 15 days. All fungi growing from the different segments were isolated at 3, 7, and 15 days. The portion of the Petri plate from which it came was discarded after isolation to prevent overgrowth of cultures. Six weeks after isolation, the strains were classified to morphotypes based on macroscopic and microscopic morphological traits, including colony color, texture, growth pattern, hyphal structure, reproductive structures, and spore morphology (Arnold et al. *2000*). Because multiple isolations of the same morphotype within a leaf may overestimate the rate of fungal infection, we included only one isolate per morphotype per leaf in all analyses. This yields a conservative measure of infection rate and fungal diversity because it does not include multiple infection of a given host plant by the same fungal morphotype. We used a nested ANOVA (SAS 6.12, 1996) to determine if the frequency of fungal infection differed among the three mangrove species, and to partition variation among leaves, individuals, and species. A one-way ANOVA, with Tukey's HSD comparison of means (Systat, 1998), was used to compare the number of fungal morphotypes per leaf among host species.

Differences in fungal diversity (measured as species richness) across mangrove species could be due to differences in susceptibility to particular fungal species (host preferences effect). Alternatively, if certain mangrove species reduce infection against all fungal species equally (say, by reducing germination of spores of all species by 40%), the lower overall infection rate would result in a smaller sample of the total fungal community. As a consequence, rare fungi would by chance be excluded from the sample, reducing total fungal diversity (sampling effect). To test if there was evidence for host preferences beyond a simple random sampling effect, we used Monte Carlo simulations in two ways. First, to ask whether the differences in fungal morphotype richness observed across host species was consistent with random samples of different sizes of a common pool of fungi, we took 999 random samples of the number of isolates actually collected from each host, using the complete set of 845 isolates. After each sample, we calculated the total number of fungal morphotypes found in the sample. The second analysis was designed to determine the probability that a morphotype of a given global frequency should be restricted to just one or two

host species (host preference), given random colonization (no host preferences). Analysis was limited to morphotypes of frequency 3 or more, because only for those was it numerically possible for a morphotype to be found on all three host species. We randomized all fungal isolates across the three host species, retaining the original number of isolates per host species. We determined whether each morphotype was originally on one, two, or three host species, and compared the number of morphotypes with a given host range to that expected under random colonization (with 95% confidence intervals), based on 999 runs.

Spores and hyphae

To measure the density of spores and fungal colonization on the surface of leaves, we made impressions of the upper surface of healthy leaves of the three mangrove species by pressing double-sided cellophane tape onto the leaf blade and then carefully removing it (modified from Langvad *1980*). The leaf cuticle, along with fungal spores and hyphae on the leaf surface, stuck to the tape. We stained the preparations with 0.1% methyl blue, and counted the number of fungal spores per 0.7 mm^2 (5 haphazardly selected fields of view at $\times$400), and the number of fungal hyphae that crossed a line 1 mm long. Data for both spores and hyphae were log-normally distributed, so data were log-transformed [$\log_{10}$(count+1)] before analysis. We compared hyphal densities across species using one-way ANOVA, and assessed the relationship between spore density and hyphal density through linear regression of the transformed data (SAS 6.12, 1996).

Analysis of salt in and on leaves

To measure salinity of mangrove leaves, we collected one mature leaf from each of five haphazardly selected plants from each of five sites. In order to include the natural variation in soil salinity and flooding conditions, we collected the samples across the range of mangrove habitat, from the edge of the ocean to the edge of dry upland hills. Sites were selected subjectively from areas where all three mangrove species grew in close proximity. To determine total sodium content in the leaves, we ground oven-dried (60°C) leaves to a fine powder in a mortar and

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pestle. Samples were then analyzed using atomic absorption spectrophotometry at the Soil and Plant Analysis Laboratory of the Instituto de Investigaciones Agropecuarias (IDIAP) of the Republic of Panama. Mean sodium content was compared across species by ANOVA (SAS 6.12, 1996).

Additionally, to estimate the salt concentrations a germinating fungal spore might encounter on the moist leaf surface, we used 100-µl pipettes to collect the water left on the upper leaf surface of 16-17 leaves of each species, 15-60 min after rain. Samples were collected from the same area as the "spores and hyphae" study, above. The salinity of the liquid was determined using a Wescor 5500 Vapor Pressure Osmometer (Logan, Utah), comparing the samples to NaCl solution standards. Salinity levels were compared across species by ANOVA (SAS 6.12, 1996).

Germination in salt

We conducted two experiments to assess the effect of salt concentrations on germination and hyphal growth of fungi, because these are key life stages of fungi for successful infection of plants. The first experiment was conducted to determine the effect of salt on colony establishment by fungi whose spores were floating in the air of the Punta Galeta mangrove forest. We filled Petri plates with 2% MEA amended with NaCl to 0%, 1%, 2%, or 4% final concentration (sea water salinity is approximately 3.5%). Five replicate plates of each concentration were arranged randomly on a tray and left open in the forest for 40 min, at a height of 50 cm above the forest floor (a similar height to the plants in the disease survey). Plates were exposed to the air in areas of the forest inhabited by all three mangrove species. The experiment was conducted in two separate locations. Plates were sealed with parafilm and incubated under laboratory conditions (22°C) for 48 h. At that time we counted the number of germinating spores visible with a stereomicroscope in a 16-cm^2 area in the center of each plate. Data were transformed to indicate the rate of deposition of colony forming units (cfu cm^{-2} h^{-1}) and analyzed by regression analysis (SAS 6.12, 1996).

In the second experiment we tested whether spores of fungi isolated from

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mangrove leaves or from the air in mangrove forest at Punta Galeta could germinate in liquid at salinities similar to those encountered on leaf surfaces after a rain. Sixteen independent strains of fungi collected from leaves and air in forests in Panama were grown on MEA for 2-4 weeks, and spores were collected and suspended in a small amount of 0.5% malt extract broth. These strains included *Pestalotiopsis* spp., *Colletotrichum* sp., *Cladosporium* sp., *Graphium* sp., *Penicillium* spp., *Aspergillus* sp., *Trichoderma* sp., and an unknown hyphomycete. In wells of a 96-well microtiter tissue-culture plate, we mixed 25 µl of the spore suspension with 25 µl of distilled water, or with NaCl solutions to achieve nine final NaCl concentrations ranging from 0% to 0.3%. The spores were incubated for 24 h under laboratory conditions (22°C) and then observed at ×200, using an inverted microscope. We determined the proportion of spores that had germinated by counting all germinated and non-germinated spores in a transect across the middle of the well (usually 100-200 spores). Spores with germ tubes at least as long as the diameter of the spore were considered germinated. The mean among all strains of the relative germination response (relative to germination in 0% NaCl for each strain) was compared to measured concentrations of salt on leaves of the three mangrove species.

To measure whether salt concentration would inhibit mycelial growth of fungi, we placed a mycelial plug of actively growing cultures of 40 independent fungal isolates from leaves of *Rhizophora* (14 isolates), *Laguncularia* (12 isolates), and *Avicennia* (14 isolates) at the edge of a Petri plate with MEA amended with NaCl to 0%, 2%, or 4% final concentration. Linear mycelial growth was measured after 1 week of growth at 23°C. Fungi were not identified to species.

Results

Disease severity and incidence

In a test of severity among species, *Rhizophora* suffered a great deal of foliar damage from fungal pathogens; in contrast, *Laguncularia* and *Avicennia* showed much lower levels of disease (Table 1). Across the three sites at Punta Galeta

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combined, *Rhizophora* had 4 times more diseased leaves than *Laguncularia*, and 7 times more than *Avicennia*. There was no significant difference between *Avicennia* and *Laguncularia* for all sites combined. Because of the significant block effect, we analyzed each site separately. Results from sites 1 and 2 were consistent with the combined analysis, whereas at site 3 *Laguncularia* had significantly more disease than *Avicennia*, and less than *Rhizophora*. In a comparison of disease incidence among species, *Rhizophora* showed a far greater percentage of individuals with symptoms (80.3%) compared with *Laguncularia* (27.3%) and *Avicennia* (26.7%) (χ^2=230.1, *df*=2, $P \leq 0.001$). Species of the pathogenic coelomycete fungi *Pestalotiopsis* and *Colletotrichum* were most often associated with disease symptoms, but we did not carry out proof of pathogenicity tests.

Fungal abundance, diversity, and host specificity

Fungi were isolated from 20%, 92%, and 82% of leaf pieces of *Avicennia*, *Laguncularia*, and *Rhizophora*, respectively. Allowing an individual fungal morphotype to be counted only once per leaf as a conservative estimate of the number of distinct infections, 845 strains from 293 different morphotypes were isolated from the 90 leaves sampled (Table 2). Using this more conservative count there were three fold fewer fungal infections in leaves of *Avicennia* than in *Rhizophora*, with an intermediate number in *Laguncularia*.

Table 2. Total number of isolates and morphotypes, and number of morphotypes per leaf on three mangrove species. For morphotypes per leaf, means followed by different letters are significantly different (Tukey's HSD, $P \leq 0.001$)

	Avicennia	*Laguncularia*	*Rhizophora*	Total
Number of isolates[a]	111	340	394	845
Number of morphotypes	64	106	183	293
Morphotypes per leaf (*n*=30)	4.1±2.9 a	11.3±2.1 b	13.1±3.4 c	

[a]Includes only one isolate of each morphotype per leaf

The highest overall fungal richness (total number of morphotypes) and highest number of morphotypes per leaf were found in leaves of *Rhizophora*, followed by *Laguncularia* and *Avicennia* (Table 2). Most morphotypes (62.4%) were rare, found only once in the entire collection (26 on *Avicennia*, 48 on *Laguncularia*, and 109 on *Rhizophora*, representing 41%, 45%, and 60% of all morphotypes found on each host, respectively). In communities dominated by rare taxa, sample size will have a large effect on estimates of community structure and composition. Sample effort was equal among host species for number of leaves sampled, but the 3-fold variance in number of fungi inhabiting those leaves affects estimates of fungal richness per isolate. It appears that the per-isolate morphotype diversity is greater in *Avicennia* (0.58 morphtypes per isolate) than in *Rhizophora* (0.46 morphotypes/isolate) or *Laguncularia* (0.31 morphotypes/isolate). However, the number of morphotypes found per host species was consistent with a random sample from the overall pool of fungi in the collection for *Avicennia* (expected mean 71 morphotypes, 95% C.I. 62-79) and *Rhizophora* (expected mean 178 morphotypes, 95% C.I. 165-170) indicating that the higher morphotype/isolate ratio is a function of all fungal morphotypes appearing rare at the smaller sample size of *Avicennia*. On the other hand, the 106 morphotypes found on *Laguncularia* fell significantly below the expected richness (expected mean 160 morphotypes, 95% C.I. 148-173), suggesting selection against colonization by particular fungi.

Among the 64 most common morphotypes (those found three or more times), 17% were found on all three host species, significantly fewer (P=0.001) than expected if the fungi had no host preferences (Table 3). At the same time, for each of the three host species there were significantly more ($P \leq 0.006$) fungal morphotypes restricted to that single host than expected without host preferences (Table 3). Both *Laguncularia* and *Rhizophora* shared significantly more morphotypes exclusively with *Avicennia* than expected, and significantly fewer than expected exclusively with each other ($P \leq 0.05$, Table 3). Sharing of common fungal morphotypes was strongly asymmetrical; for both *Laguncularia* and

Avicennia 70% of their common fungal morphotypes were shared with *Rhizophora*, but only 38% and 58% of common *Rhizophora* fungal morphotypes were also found on *Avicennia* or *Laguncularia*, respectively. These results indicate that the fungi associated with *Avicennia* and *Laguncularia* were largely a subset of those associated with *Rhizophora*, complemented by a number of fungi showing strong host preferences.

Table 3. Observed host distribution of the 64 most common fungal morphotypes (those found three or more times) in leaves of the mangroves *Avicennia germinans*, *Laguncularia racemosa*, and *Rhizophora mangle*, and the expected distribution if fungi had no host preferences

Host species	Percent of common morphotypes	Observed no. of morphotypes	Expected no. of morphotypes (95% confidence limits)	Probability of as extreme or more a no. of morphotypes
Avic./Lagun./Rhiz.	17.2	11	28.8 (24-34)	0.001
Avic./Lagun. only	9.4	6	2.8 (1-6)	0.050
Avic./ Rhiz. only	12.5	8	3.8 (1-7)	0.033
Lagun./ Rhiz. only	28.1	18	23.8 (18-29)	0.050
Avicennia only	3.1	2	0.1 (0-1)	0.003
Laguncularia only	9.4	6	1.8 (0-4)	0.006
Rhizophora only	20.3	13	3.0 (1-6)	0.001

Salt content of leaves and mycelial growth

We hypothesized that the much lower levels of foliar disease on *Avicennia* and *Laguncularia* (compared to *Rhizophora*) might be related to the high levels of salt in the leaves of these two salt-excreting species. The total sodium content in the

salt-excluding *Rhizophora* was 7- to 9-fold less than in the leaves of salt-excreting *Avicennia* and *Laguncularia* (Table 4). Consequently we tested for the effect of salt on radial growth of mangrove-associated fungi on MEA plates, finding that a minimum of 4% NaCl was required to significantly decrease mycelial growth of established colonies of a variety of mangrove-associated fungi (data not shown). The bulk salt concentration in mangrove leaves did not exceed 1% NaCl (dry weight), and thus is unlikely to reduce disease levels by directly inhibiting mycelial growth in the leaves. In contrast, we did find a strong reduction in fungal colony formation with the addition of as little as 1% NaCl to MEA plates exposed to the air spora of a mangrove forest (Fig. 1). This suggests that the germination and early growth phases may be more susceptible to salt than is the growth of established mycelium.

Table 4. Analysis of salt content of bulk leaves and of rainwater remaining on leaf surface after light rain, for three species of mangrove. Means within a column followed by the same letter are not statistically different (Fisher's protected LSD, $P \leq 0.05$). For bulk leaf measurements, $F_{2, 12}=10.7$, $P=0.002$. For surface rainwater, $F_{2, 46}=9.10$, $P=0.0005$

	Percent sodium±SD	
Mangrove species	Bulk leaf	Surface rainwater
Avicennia	0.68±0.34 a	0.24±0.12 a
Laguncularia	0.92±0.36 a	0.14±0.10 b
Rhizophora	0.10±0.06 b	0.10±0.08 b

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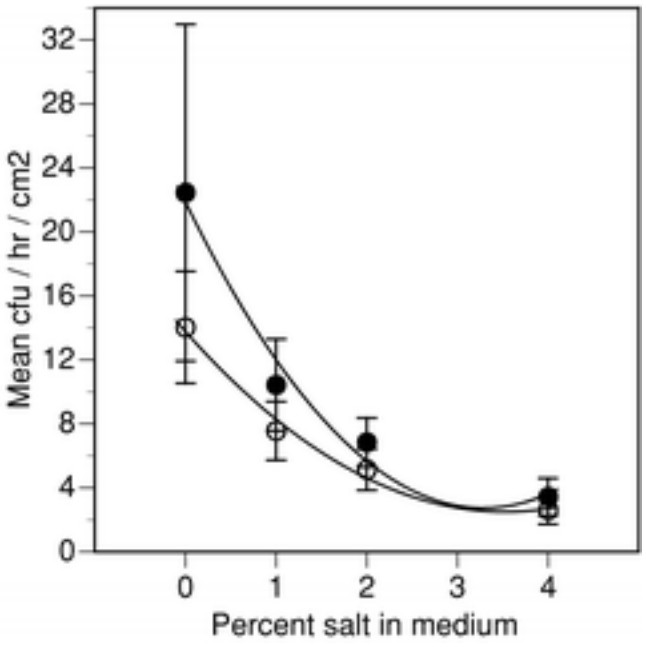

Fig. 1. Fungal colony forming units (*cfu*) developing on agar plates exposed to the mangrove environment in two separate experiments at Punta Galeta (represented by *open and filled circles*), with increasing concentrations of salt in the medium. Curve for the *filled circles* is (cfu h^{-1} cm^{-2})=1.8 (% NaCl)^2-11.6 (%NaCl)+21.9, and for *open circles* (cfu h^{-1} cm^{-2})=0.9 (% NaCl)^2-6.4 (%NaCl)+13.8. Partial R^2 values for all terms were greater than 0.53. *Error bars* are standard deviations

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Salt concentration and fungal growth on leaf surfaces

There were no significant differences among mangrove species in density of spores on the leaf surface ($F_{2, 22}$=2.46, P=0.09), but there was considerable variation in spore density among leaves within a species (*Avicennia* 10.5±14.0, *Laguncularia* 9.3±10.5, *Rhizophora* 25.2±49.8 spores/mm^2). *Avicennia* had much lower hyphal densities on the upper leaf surface than did *Rhizophora* and *Laguncularia* ($F_{2, 82}$=6.8, P=0.002; Fig. 2); there was more than three times the number of hyphae per millimeter on *Rhizophora* leaves as on *Avicennia*. Because hyphal growth on the leaf surface usually initiates from germinating spores, we expected a correlation between spore and hyphal density on a leaf surface. For *Rhizophora* and *Laguncularia* there were significant, positive linear relationships between spore and hyphal density, but hyphal density on *Avicennia* leaves was

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low across the range of spore densities (Fig. 3). This indicates that hyphal density in that species was determined not by a limitation in spore availability, but most likely by subsequent physiological restriction of spore germination or hyphal growth. We therefore investigated whether the concentration of salt in remnant rainwater on mangrove leaves is sufficient to affect the germination and growth of fungi that immigrate to the leaf surface.

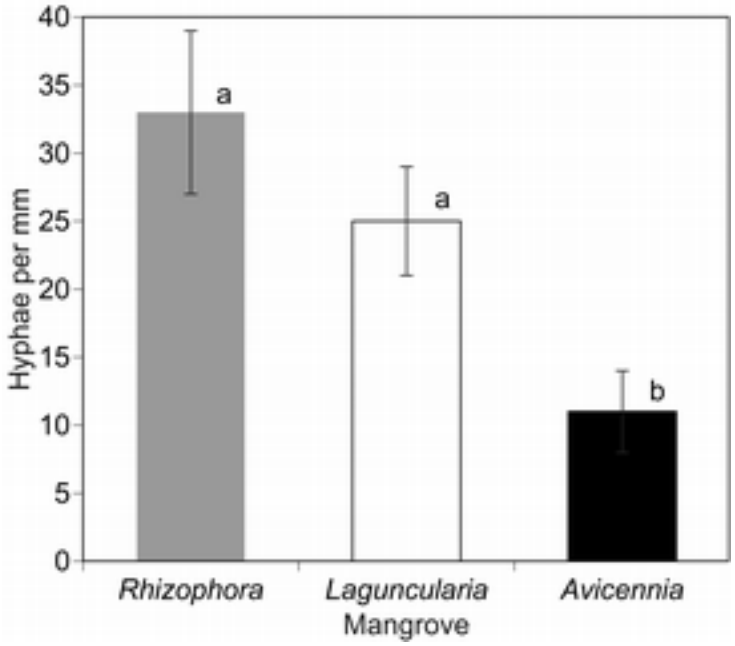

Fig. 2. Mean density of fungal hyphae on upper surface of leaves of three mangrove species. Means with the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$). *Error bars* are standard deviations

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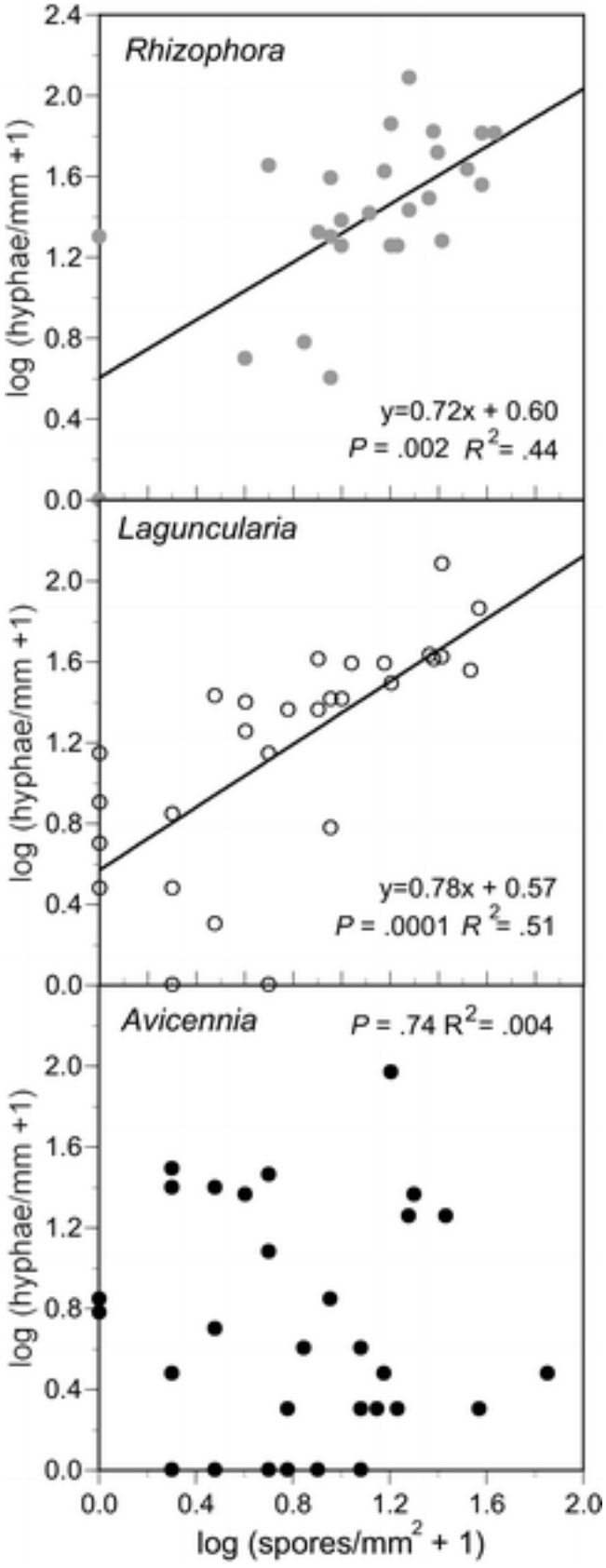

Fig. 3. Relationship between the density of spores and hyphae on leaf surfaces of three mangrove species

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Salt concentration in remnant rainwater on *Avicennia* leaves was significantly greater than on *Rhizophora* or *Laguncularia* (Table 4). Spore germination of most mangrove-associated fungi tested was strongly inhibited by salt at the concentrations found on black mangroves, but not at the levels associated with *Rhizophora* or *Laguncularia* leaves (Fig. 4).

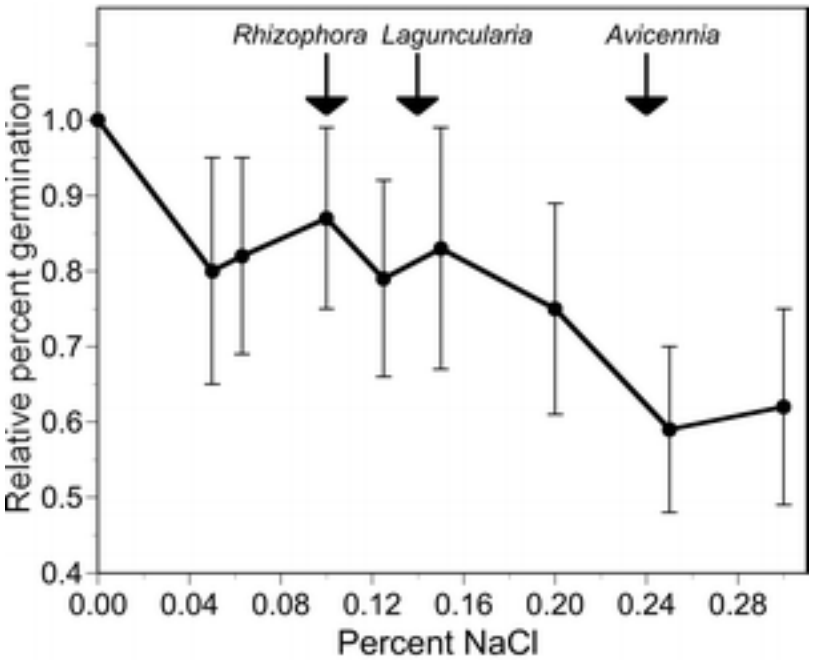

Fig. 4. Relative germination rates of spores of mangrove-associated fungi in dilute broth amended with increasing concentration of NaCl. Means and standard errors for 16 strains of fungi are shown. *Arrows* indicate the mean concentrations of NaCl found in remnant rainwater on the leaves of each of three mangrove species

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Discussion

It is unlikely that salt excretion evolved directly as a disease resistance mechanism, but in addition to providing a necessary mechanism for dealing with the physiological stresses of a saline environment, it may provide a complementary defense to more traditionally recognized mechanisms. Efficient defenses against pathogens may be of particular importance in natural

communities like mangrove forests, where host diversity is low and the density of individual host species high - ideal conditions for diseases to have strong effects on plant populations.

Leaves of *Rhizophora* commonly suffered leaf necrosis surrounded by a chlorotic halo; these symptoms were commonly associated with *Pestalotiopsis* spp., and sometimes with species of *Colletotrichum*. García-López et al. (*1989*) reported similar symptoms associated with two *Pestalotiopsis* species (one reported as *Pestalotia disseminata* = *Pestalotiopsis disseminata*) as the most common causes of leaf lesion in *R. mangle* in Cuba. *Laguncularia* suffered little from fungal diseases, but did suffer a great deal of insect herbivory (personal observation). The low levels of disease development in *Laguncularia*, despite the normally common association between leaf diseases and herbivory damage (García-Guzman and Dirzo *2001*), suggests strong resistance to microbial infection. With the high internal salt content in *Laguncularia* leaves, herbivory wounds would be highly saline, and may be an effective barrier to fungal infection. Although there are several reports of foliar diseases in naturally low-diversity tropical forests (McMillan *1964*; Farr et al. *1989*; García López et al. *1989*; Suryanarayanan et al. *1998*), to our knowledge none describe effects of diseases on host population dynamics. Such studies are much needed for understanding the role of diseases in the structure and dynamics of mangrove forests.

Germination and early growth of fungi seem to be more sensitive to saline environments than is later mycelial growth, as suggested by previous work. Amir et al. (1996) found that mycelial growth of *Fusarium oxysporum* was not inhibited by salinities less than 2%, and that 3% salt reduced mycelial growth by only 19%. In contrast, conidial spore germination was inhibited by as little as 0.5% salt, with up to 50% inhibition at 1%. These results are similar to ours; the regression describing the reduction in colony formation on Petri plates exposed to mangrove air with the addition of sodium chloride indicates an expected 50% reduction in colony formation at about 1.3% salt.

The salt concentrations we measured on leaf surfaces after rain should be regarded as a conservative lower bound of the salt concentrations that fungi may

encounter on the leaf surface. As the water evaporates, the salt concentration will increase, potentially exposing fungi to brief periods of very high salinity, which may have much greater toxicity that that reported here. Rainfall at the Punta Galeta site is very high [more than 2,600 mm per year, with 8 or more months of more than 100 mm precipitation (data from Smithsonian Tropical Research Institute - Marine Environmental Sciences Program)], so heavy rainfall that may wash away significant amounts of salt is the norm. In areas where light rain or dew is more dominant, fungi might experience even greater salt concentrations. Additional work on effects of short-term exposure to high salt concentrations and the dynamics of salinity on leaf surfaces may provide additional insights into the importance of salt on fungal colonization.

Our study suggests that although mangrove species growing in close proximity are likely exposed to the same density and composition of potential pathogens, disease development may be mediated by foliar salt concentrations. Variation in fungal colonization and disease development across species is not caused by differences in spore availability, since there were no significant differences in spore density across hosts. Both *Rhizophora* and *Laguncularia* showed the expected linear positive relationship between spore load and hyphal growth - increased availability of spores was associated with higher colonization by fungal hyphae. *Avicennia*, in contrast, showed no significant relationship between spore and hyphal density, suggesting that spore germination on this host is not just a uniform reduction in germination by all fungal morphotypes. If globally common morphotypes were more often sensitive to salt than were rarer morphotypes, it would explain both the overall reduction in infection rates of leaves of *Avicennia*, as well as patterns of apparent host specificity. Research on the relative salt tolerance of different mangrove-associated fungi is needed to further explore this possibility.

The fungal assemblage found on *Avicennia* appears to be a subset of the fungi that can grow on the comparatively less restrictive medium represented by *Rhizophora* and *Laguncularia* leaves. Fungi may be specific to *Rhizophora* and *Laguncularia* not because of positive selection for particular fungal taxa, but because they are excluded from growth on the more restrictive *Avicennia*. This asymmetrical

pattern of sharing of fungal morphotypes, as well as the uncoupling of spore load and hyphal growth, is consistent with selection against particular salt-intolerant fungal taxa, rather than a simple reduction in germination by all fungi. The effect of high leaf-surface salinity on spore germination could thus explain the restricted fungal colonization, low fungal diversity, and low levels of disease found in *Avicennia* leaves. Similarly, salt in *Laguncularia* leaves may select against infection by salt-intolerant fungi. With the lowest leaf salinity, *Rhizophora* consistently suffered more disease and supported greater fungal infection, fungal diversity, and hyphal growth. Salt concentration may be an important filter determining the outcome of plant-fungus encounters in mangrove forests. Of particular interest would be studies of the effect of leaf salinity in releasing salt-tolerant fungi from competition from aggressive salt-intolerant species.

Soil salinity has been previously implicated in reducing plant disease development, but to our knowledge not for foliar diseases. Amir et al. (*1996*) found that *Fusarium* wilt of date palm was suppressed on soils with higher salinity, but concluded that the effect of salinity was likely through altering the competitive ability of the *Fusarium* with other soil microbes, and was not directly responsible for inducing disease resistance.

Mangrove forests are often show striking species zonation; this pattern is determined by a complex set of environmental and biotic factors, but salinity of the soil water is thought to be a major determinant (MacNae *1966*). Similarly, the distribution of marine fungal species are apparently strongly affected by salinity, with a few species dominating under conditions of full salinity and other species, more common in brackish or freshwater habitats are absent from habitats with high salinity (Jones *2000*). While these fungi are seldom pathogenic, colonizing primarily submerged wood, leaf litter, and root systems, similar selection in fungi colonizing living leaves may occur along salinity gradients. Understanding how changes in salinity affect disease susceptibility of mangrove hosts could be important in understanding the striking spatial patterns associated with mangrove forests. If salt excretion is an important defense against fungal diseases, disease pressure may increase in less saline environments, reducing the fitness of the mangroves and playing an important role in limiting their distribution in intertidal

habitats. Measuring the disease pressure on each of the three mangroves experimentally grown in fresh water, but within the mangrove forest would be a useful test of this hypothesis.

Mangrove forests are unusual among tropical forests for their low tree diversity and associated high density of individual species. Mangrove species are unusual in their ability to grow in flooded, saline soils and for the array of mechanisms they have evolved to tolerate high salt concentrations. Some mangrove species may also be unusual in their escape from strong disease pressures even when growing at high densities, through the effects of high foliar salt concentration on fungal infection. Further exploration of both the role of microorganisms and host characteristics in the dynamics and stability of low-diversity tropical forests (Connell and Lowman *1989*; Torti et al. *2001*) and of the role of salt as a host defense in mangrove forests, salt marshes, and other intertidal plant communities may provide fruitful clues to mechanisms underlying patterns of plant distribution.

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