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Initial Fungus Attack on Tropical Woods in Panamanian Estuarine Waters

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ABSTRACT

Eight species of tropical timber woods (*Chrysophyllum cainito*, *Ocotea rodiaei*, *Pinus caribaea*, *Pouteria chiricana*, *Prioria copaifera*, *Rhizophora mangle*, *Symphonia globulifers*, and *Tabebuia pentaphylla*), were submerged in estuaries near the entrances of the Panama Canal. These species were chosen according to their susceptibility to attack by marine borers and were invaded by salt-tolerant fungi, mostly imperfect species. Fungus infestation varied from almost none to very heavy in less than six weeks, but the amount of mycelium in the woods bore no apparent relation to either animal attack or salinity. *Ocotea rodiaei* (greenheart) and *Rhizophora mangle* (red mangrove) were practically fungus-free at the end of the test. *Prioria copaifera* (cativo) was filled with mycelium, and the others were invaded by scattered hyphae.

PROBLEM STATUS

This is an interim report; work on this problem is continuing.

AUTHORIZATION

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INITIAL FUNGUS ATTACK ON TROPICAL TIMBERS IN PANAMANIAN ESTUARINE WATERS

INTRODUCTION

Many factors affect the ability of fungi to attack wood. The obvious environmental factors are temperature, pH, the relative amount of water present, oxygen supply, and sometimes light. The morphology of the fungus and its enzymatic composition, that is its genetic capabilities, are also critical. Equally important are the characteristics of the wood: its physical hardness, its content of required nutrients, and the possible presence of inhibitory compounds. All wood is potentially open to attack by fungi, mainly the cellulolytic fungi.

Animals, too, attack wood. The requirements of animals are generally similar but not identical to those of fungi. A series of trials has been made in an effort to compare the activities of some animals, notably marine borers, with those of some salt-tolerant fungi.

Some woods are more resistant than others to infestations by marine borers. This resistance is known to be related to the chemical composition rather than to the hardness of the tissues, although the compounds responsible for the resistance are not known. In an extensive series of tests, Southwell et al. (1) exposed 114 species of tropical woods for fourteen months in a variety of sites, including waters of different salinities, and tropical soils. The tests established the resistance of a number of species of wood to shipworms (Teredinidae), to other wood-boring invertebrates (Limnoria and pholads), and, to some extent, to termites and terrestrial fungi.

The possibility of combined attack by marine borers and salt-tolerant fungi has been considered. Some observations indicate that fungi facilitate the activity of borers (2). Other experiments seem to discount this (3), but the matter is not unequivocally settled.

This combination of facts, suppositions, and knowledge gaps, prompted the inception of these present experiments. The intent was to determine whether those species of wood which are susceptible to borer attack are also susceptible to fungus attack, and to find the effect that temperature and salinity may have on the invasion of wood by salt-water-inhabiting fungi.

PROCEDURE

Choice and Exposure of Samples

From the species tested by the Southwell group, eight were chosen as representing a range of resistance to borers. None was completely resistant to borers in the brackish waters of Miraflores Lake in the Canal Zone, Panama (average salinity, 0.74 parts per thousand), but several were resistant in the saltier waters of the nearby oceans, bays, and estuaries. The species of woods, selected for exposure to marine fungi, listed with their resistance to borer attack, are these:

Chrysophyllum cainito L. (caimito, star apple) - resistant
Pouteria chiricana Standley (nispero de monte) - resistant

Ocotea rodiaei (Schomb.) Mez. (greenheart) - resistant
Tabebuia pentaphylla (L.) Hemsl. (roble de sabana) - moderately resistant
Pinus caribaea Mor. (Nicaraguan pine) - susceptible
Prioria copaifera Gris. (cativo) - susceptible
Rhizophora mangle L. (red mangrove) - susceptible
Symphonia globulifera L. f. (cerillo) - susceptible

The samples, which had been dried for three years in air-conditioned storage, were cut into strips 100 by 6 by 6 mm. These were suspended in the water by Nichrome wires so that even at low tide they were covered. The exposure sites were chosen for as much variety as expediency permitted. All the sites were located in the Canal Zone near the entrances to the Panama Canal; however, most of the sites were on the Atlantic side. Their arbitrarily assigned numbers, representative temperature readings, and exposure periods are given in Table 1.

Table 1
Exposure Sites of Wood Samples

No.	Station	Exposure Period	Water Temperature (°C)
0	Coco Solo Pier 1	7/23 - 9/3	27.2
1	Galeta Point	7/23 - 9/5	27.8
2	Coco Solo Pier 1	7/23 - 9/3	27.2
3	Coco Solo ramp	7/23 - 8/30	27.8
4	Fort Sherman ferry slip	7/24 - 8/27	29.1
5	Four-hundred foot hill	7/23 - 8/26	30.0
6	Four-hundred foot hill	7/28 - 8/28	30.0
7	Galeta Point	7/23 - 9/5	27.8
8	Fort Sherman Light	7/24 - 8/27	32.9
10	Galeta Radio Station	7/23 - 8/23	28.3
11	Coco Solo ramp	7/23 - 8/30	27.8
13	Fort Amador pier	7/25 - 8/23	26.7

Salinities were determined by the chromate-silver-nitrate method of Harvey (4). Temperatures were relatively constant, with a minimum of 26.7 °C at Fort Amador (Pacific side) and a high of 32.9 °C at the Fort Sherman Light (Atlantic side). Salinity varied because of runoff from rains, which, on the Atlantic side measured 15.37 in. during July 1963, and 22.18 in. during August 1963.

Fungus Cultures and Evaluation of Samples

When lifted from the water, wood samples were dropped into individual, sterile containers. In the laboratory, each sample was divided aseptically into three pieces. One portion was air-dried and stored, one portion was fixed in formaldehyde, ethanol, and acetic acid, and one portion was used for isolation of fungi.

The fixed samples were used to evaluate the amount of hyphal invasion. Soaking in glycerol-ethanol softened the wood before it was cut longitudinally on a sliding microtome at 25 microns. The method of Hubert (5) uses methyl violet and Bismarck brown to differentiate lignified walls, which stain orange to red; cellulose walls, which stain light purple; and fungus mycelium, which stain dark purple.

Evaluation of the amount of fungus infestation was made by inspecting five fields of view in each of five different sections. Each field of view, as seen with a 4-mm microscope objective, was scored on a five-point scale from 0 to 4 (field crowded with hyphae). Twenty-five readings were made on each wood sample at each exposure site. Subjective bias was avoided by evaluating the first field to come into focus, then throwing the section out of focus while the slide was being moved to a new position, refocusing, and evaluating the new field where the slide stopped. The evaluations expressed on the 0-to-4 scale, were averaged for each species at any one site; then the averages were averaged for the species at all sites.

To identify the fungi, cultures were grown from teased slivers of wood which were placed on the surface of agar made with sea water, 0.5% glucose, and 0.1% yeast extract.

RESULTS

On a basis of the amount of visible mycelial infestation in the sections, the eight species of woods could be grouped into three classes, with clear gaps in the susceptibility spectrum. With a score of 0 representing total resistance, and 4.0 representing massed hyphae in every field of view examined, the species were rated as shown in Table 2. *Rhizophora mangle* (red mangrove) and *Ocotea rodiaei* (greenheart) are in a class by themselves with respect to freedom from fungus invasion. *Rhizophora mangle* was practically fungus-free in all the exposure sites, but one *Ocotea rodiaei* sample from Galeta Point did have some hyphae in a few wood cells. Most of the other species showed some scattered mycelium in most fields and in many sections. Their ratings were bunched in a close sequence from 0.4 to 0.8, with no conspicuous gaps.

Prioria copaifera (cativo) was alone in its susceptibility, achieving a rating of 1.78 after submersion of only four to six weeks. Some of the individual samples, which contributed to the average rating, scored as high as 2.7 (at the Fort Sherman ferry slip) and 3.0 (at the Coco Solo Pier 1). The numerical ratings indicate only that fungus infestation was common, but they do not show the vigor of the invading hyphae, as evidenced by their large size and rich branching which was greater than in other samples. One species of *Pestalotia*, present in several samples, was unusually active and had a unique method of growing from cell to cell (6). The appearance of wood tissue being attacked by fungi is shown in Figs. 1 through 3, with apparently naked extensions of hyphae advancing to and through the walls of the host tissue.

Table 2
Rating of Wood Species with Respect to Fungal Invasion in Estuarine Waters from (Total Resistance) (Complete Infestation)

Species	Rating
Rhizophora mangle	0.1 ± 0.1
Ocotea rodiaei	0.1 ± 0.2*
Chrysophyllum cainito	0.4 ± 0.4
Pouteria chiricana	0.5 ± 0.3
Pinus caribaea	0.5 ± 0.4
Tabebuia pentaphylla	0.6 ± 0.7*
Symphonia globulifera	0.8 ± 0.5
Prioria copaifera	1.8 ± 0.6

*Disregard negative ratings.

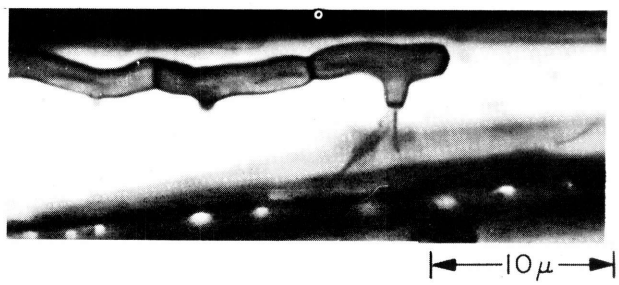

Fig. 1 - Thin extrusion of hypha extending toward vessel wall in sections of *Prioria copaifera*, 300X

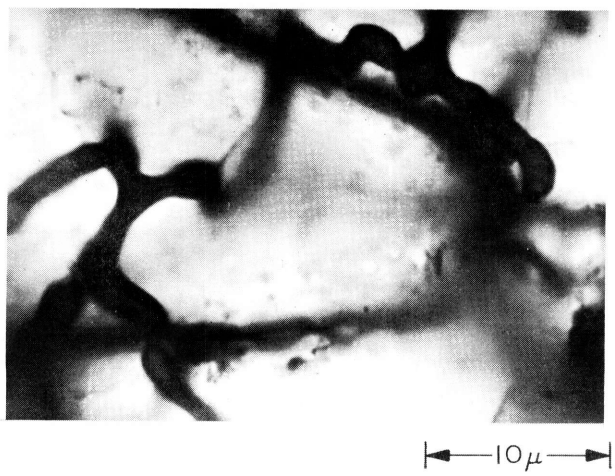

Fig. 2 - Hyphae passing (lower left) or about to pass (upper right) through pits in parenchyma cell of *Prioria copaifera*, 300X

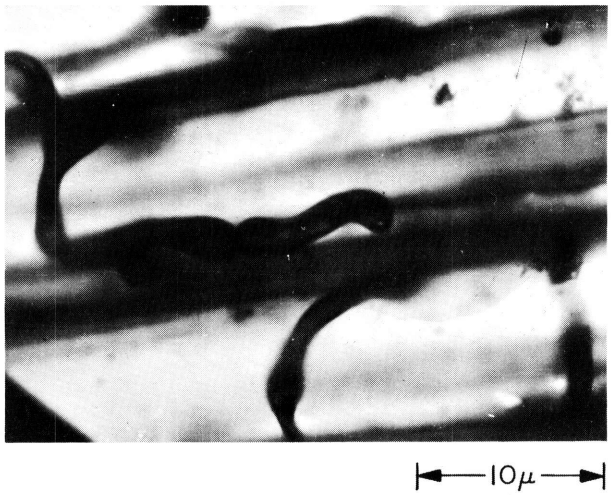

Fig. 3 - Branching mycelium in vessel and pore at end of hypha (right of center) from which penetrating hypha will extend into *Prioria copafera*, 300X

The fungi isolated from the samples are shown in Table 3. Many of them are obviously terrestrial molds which have sufficient tolerance to grow in sea water but they grow better in dilute media. Infestation was in general neither better nor worse in the less saline exposure sites where outflow from fresh creeks, especially after heavy rains, diluted the in-shore water. The amount of fungus growth in the nine sites which yielded the fullest data (some samples were lost from some sites) is compared in Table 4.

Table 3
Genera of Fungi Isolated From Wood Samples in Order of Frequency of Isolation

No. of Isolations	Genus
29	Fusarium
18	Tritirachium
15	Pestalotia
13	Phoma, Trichoderma
12	Sterile mycelia
9	Paecilomyces
8	Curvularia
6	Aspergillus
4	Penicillium
2	Geotrichum, Gliocladium, Phomopsis
1	Ascomycete (indet.), Candida, Cephalosporium, Coniothyrium, Epicoccum, Haplosporangium, Hormodendrum, Lulworthia, Mucor, Nigrospora, Oedocephalum, Sphaeropsis, Spicaria, Zygedesmus

Table 4
Relative Fungus Infestation of all Wood Species, Listed in Decreasing Order of Salinities

Site No.	Salinity range, (parts per thousand)	Rating
8	30.01 - 30.32	0.4
13	29.06 - 32.30	0.4
1	29.51 - 31.67	0.4
7	29.21 - 31.47	0.4
10	26.57 - 29.61	0.3
2	23.32 - 24.70	1.0
11	14.20 - 24.30	0.5
6	11.90 - 22.22	1.1
5	11.89 - 21.20	0.5

DISCUSSION

The way in which fungi invaded these particular species of woods, under the existing environmental conditions, brings up several notable points.

Fungus succession, as an ecological phenomenon, is poorly understood (7). Among marine and estuarine fungi, the pioneers in tropical as in temperate climates are usually the imperfects. Although ascomycetes are common in saline habitats, the current tests, being limited to six weeks or less, allowed time for only two species of ascomycetes to establish themselves. One of them, on *Chrysophyllum cainito* in Panama Bay, was never identified, and one was the ubiquitous *Lulworthia medusa* (Ell. and Ev.) Cribb and Cribb, which occurred on *Prioria copaifera*, also in Panama Bay. Ascomycetes are typically slower in the succession of fungi appearing in substrates exposed in salt water.

The growth of essentially terrestrial molds in saline habitats, where they find osmotic and ionic conditions less than optimal, is not surprising. The conditions which an experimenter decides are best as based on his laboratory experiments are not necessarily the best for a given strain of organism in nature, and the organism is not necessarily restricted to the habitat which in the opinion of an experimenter is optimal for the organism (8).

The wood species under observation in this experiment were rated previously for resistance to decay by bacteria and terrestrial fungi as a part of the marine borer screening program (8). In that project, sample stakes were driven partially into the soil, many of them under tropical rain forest conditions where warmth, moisture, and abundant nourishment are conducive to fungal growth. After 18 months, the samples were rated by macroscopic inspection for evidence of decay. When fungal activity in soil and in salt water is compared, only one species, *Ocotea rodiaei*, proved resistant in both habitats. *Rhizophora mangle*, which had the least mycelium of any species in the present trials, showed considerable terrestrial decay. As to susceptibility, again only

one species was consistent in soil and in salt water: *Prioria copaifera* (cativo). This species was also heavily damaged by marine and brackish-water borers. No clear relation between resistance to terrestrial and salt-water fungi appears, although parallels can be inferred in the case of *Ocotea rodiaei* and *Prioria copaifera*.

The uncertainty of a relation between fungus and borer destruction of wood in salt water still remains. A comparison of the data of Table 2 with those reported by Southwell et al. (1) shows that one species *Rhizophora mangle*, may be free from salt-water fungi, riddled by borers, and rotted by terrestrial fungi. Another, *Ocotea rodiaei*, may have general resistance to all three kinds of organisms; another *Pouteria chiricana*, may have moderate resistance to salt-water fungi, high resistance to borers, and low resistance to terrestrial decay. Only *Prioria copaifera* proved to be generally susceptible. Inasmuch as no pattern is discernible, a relation between fungi and wood-eating animals seems unlikely.

Although the relatively short exposure time of these trials limits generalization, the similarity of infestation in all the sites indicates that salinity was a weak factor in determining the rate and the amount of fungus growth (Table 4). An apparently considerable difference separates the ratings at sites 5 and 6 (ratings of 0.5 and 1.1 respectively); yet, their salinities were almost the same, and the sites were only a few feet apart. Sites 5 and 13, having similar amounts of infestation (ratings 0.5 and 0.4 respectively) were at the extremes of the salinity range, and were in different oceans.

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13. ABSTRACT

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