

The Inhibitory Effects of Obtusaquinone on the Growth and Reproduction of Two Marine Fungi

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ABSTRACT

This report describes a stage in the search for improved means of protecting wood used in the sea from deterioration caused by marine borers and fungi. In previous studies of 113 tropical species, the heartwood of *Dalbergia retusa* Hemsl. (Cocobolo) was found to be the most resistant to such organisms, and a pigment, obtusaquinone, was isolated from it. The fungistatic effects of this compound on two wood-inhabiting marine fungi, *Pestalotia oxanthi* Thumen and *Chaetomium olivaceum* Ames, have been determined at five solute concentrations. The minimum concentration of obtusaquinone necessary to produce a substantial inhibitory effect upon vegetative growth rate was about 200 ppm for both organisms. Reproduction of *Chaetomium* was inhibited at 125 ppm, that of *Pestalotia* at 200 ppm.

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INTRODUCTION

Wood is the material of choice for much marine construction, being reasonably hard, abrasion-resistant, strong, flexible, and lower in cost than competing materials. Because of these qualities the Navy uses wood in about 1000 piers and wharves and some 180 miles of fender systems. However, wood can be damaged by marine organisms, especially the boring animals such as teredos (shipworms), pholads, and limnoria (gribble), and also by wood-inhabiting marine fungi. These latter organisms not only damage wood directly but, more importantly, may contribute to borer infestation by making the wood surface more hospitable to the larvae of borers. Destruction of an installation can sometimes occur within weeks after exposure to these invaders.

At present the most effective protection for wood against marine biodeterioration is whole creosote which is forced under pressure into the interstices of the woody tissue. However this treatment is not reliable in all waters, particularly in subtropical or tropical sites. Thus it is imperative that the Navy seek other means of assuring long service life for its wooden marine installations. Possibly, better synthetic chemical wood preservatives could be developed, but to be effective such agents must possess repellent or toxic qualities which might make them unacceptable for environmental reasons. An alternative is the use of naturally borer resistant woods whose resistance is due to physical properties of the cell walls or to the presence of toxic or repellent compounds synthesized by the living tree.

The Naval Research Laboratory conducted an extensive search for such woods [1,2]. Of the 113 tropical species evaluated for marine borer resistance, only a few were resistant to more than one of the boring organisms mentioned above. The most resistant wood was *Dalbergia retusa* Hemsl., a hardwood known as Cocobolo throughout its Central American range. This species was also completely resistant to attack by marine fungi. Unfortunately, there are several reasons why *D. retusa* cannot serve directly as a source of timber for marine situations. The tree does not produce logs of sufficient size and regularity to make usable pilings or fenders; the resistant, slow-forming heartwood is scanty in the younger, sounder trees in proportion to the nonresistant sapwood; the living heartwood tends to deteriorate physically with advancing age [3]; and the scattered occurrence of the tree makes harvesting difficult and expensive.

Since the wood of *D. retusa* is largely immune to borer infiltration and fungal infection, efforts have been made to extract, separate, and characterize the compounds responsible for this immunity. A preliminary report on the removal of an active component from the wood by solvent extraction has been issued [4]. Subsequently, several components have been isolated from the heartwood and characterized [5]. One of these compounds is obtusaquinone, an orange pigment comprising 3% of the weight of the heartwood.

Table 1
Composition of the medium used to
prepare the assay plates

Component	Grams/Liter
Glucose	5.0
Yeast extract	1.0
Agar	15.0
MgSO ₄	0.5
FeSO ₄ 7H ₂ O	0.01
NaNO ₃	6.0
KCl	0.50

The fungal colony on each plate formed a nearly circular disk growing outward from the center. The minimum distance across each colony was the criterion of growth and was measured daily beginning 3 days after inoculation and continuing until the control colonies had reached the outer edges of the plates. For the fast-growing *Pestalotia* this limit was reached seven days after inoculation; for the slow-growing *Chaetomium*, measurements were continued until the 14th day after inoculation. After growth measurements were discontinued, plates were kept under observation to determine the reproductive activity of the fungi.

RESULTS AND DISCUSSION

The day-by-day colony diameter (averaged from three identical culture plates) for each concentration of obtusaquinone is presented graphically for *Pestalotia* in Fig. 1 and for *Chaetomium* in Fig. 2. The average growth rate for *Pestalotia* is presented in Fig. 3, and that for *Chaetomium* in Fig. 4. On Figs. 1 and 2 the control and solvent control curves are quite similar, the differences being within the limits of experimental error. This fact indicates that trace amounts of residual acetone in the media did not significantly affect the growth of either of the two organisms.

Growth Rates

Obtusaquinone had an inhibitory effect upon the vegetative growth of both fungi, even at the lowest concentrations. Generally, the growth rate was reduced as the concentration of obtusaquinone in the medium increased until a threshold value of about 200 ppm was reached for *Pestalotia*; beyond this an increase in toxicant concentration did not significantly increase inhibition throughout the duration of the experiment. A similar threshold concentration of about 200 ppm obtusaquinone also occurred for *Chaetomium*. Two minor inconsistencies were the growth of *Pestalotia* on the media containing the two highest concentrations of obtusaquinone and the growth of *Chaetomium* on the media containing the two lowest concentrations. Nonhomogeneous dispersion of the compound throughout the media may have contributed to these anomalies, but it was not possible to determine precisely what caused them.

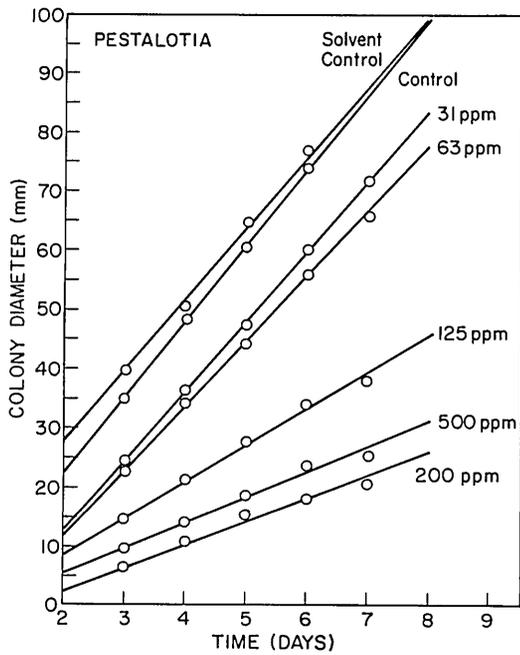


Fig. 1 — Average colony diameter (minimum distance across each colony) as a function of colony age for *Pestalotia oxanthi*

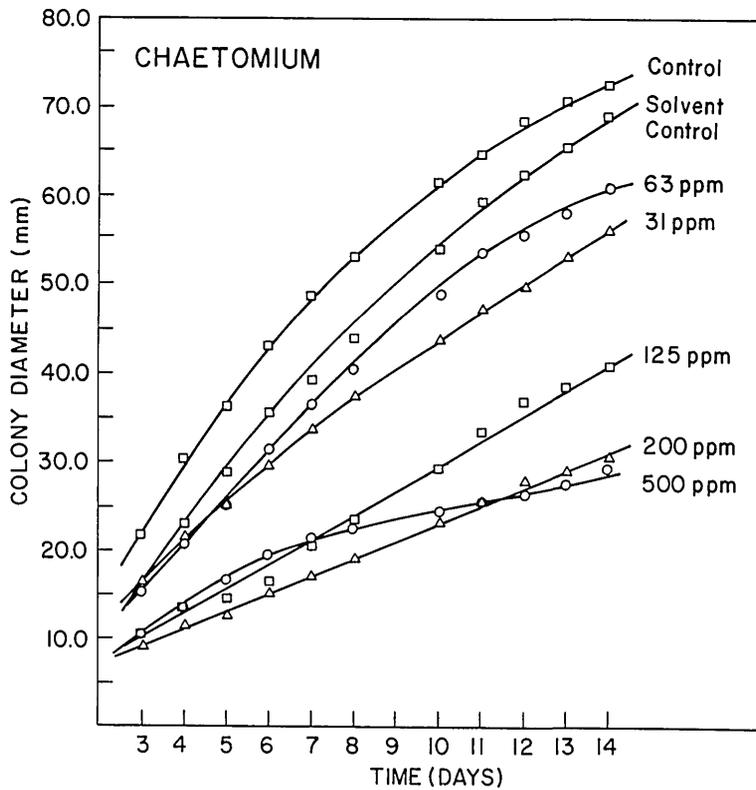


Fig. 2 — Average colony diameter (minimum distance across each colony) as a function of colony age for *Chaetomium olivaceum*

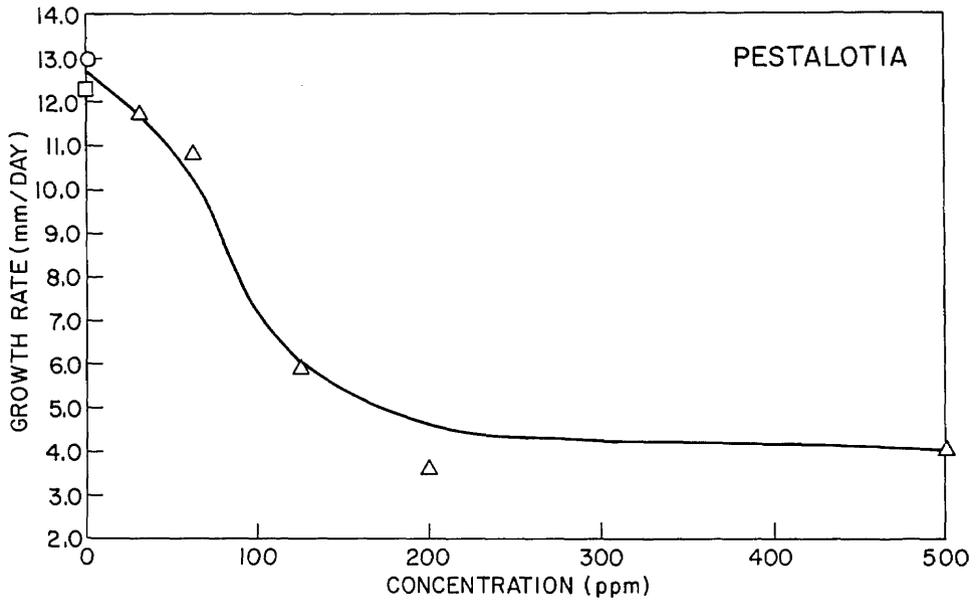


Fig. 3 — Growth rate of the *Pestalotia oxanthi* colonies as a function of obtusaquinone concentration: ○ = normal control; □ = solvent control

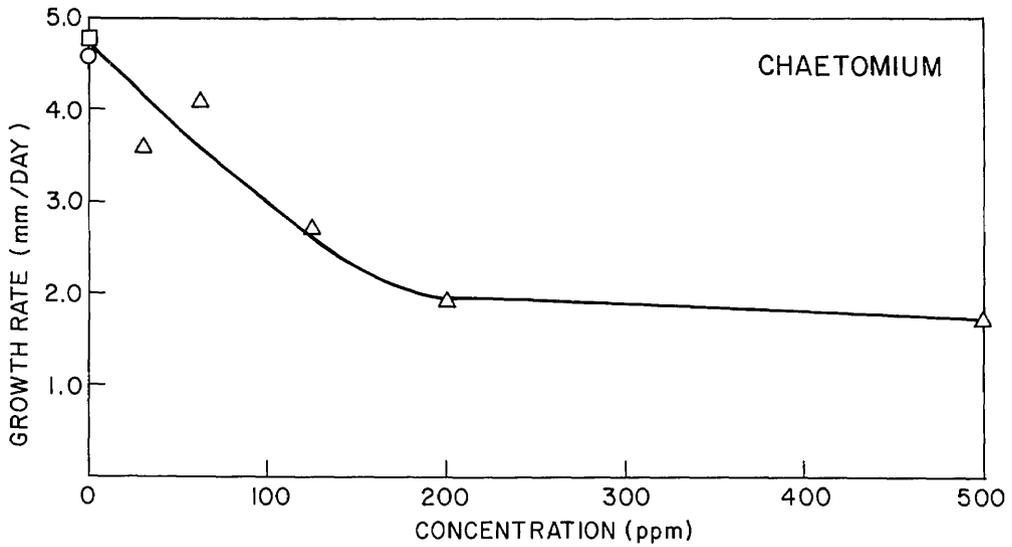


Fig. 4 — Growth rate of *Chaetomium olivaceum* colonies as a function of obtusaquinone concentration: ○ = normal control, □ = solvent control

All final pH measurements were made four weeks after inoculation of the plates. At this time the average pH of all the *Pestalotia* plates was about 7.0. The average pH of the *Chaetomium* plates at the lower concentrations of obtusaquinone was 8.0; that for the higher concentrations was 6.0. Since *Chaetomium* grows best in acid media, an increase in pH contributed to a decrease in growth rate with time. Also, the slow growth of *Chaetomium* allowed time for the accumulation of the usual growth-inhibiting factors associated with fungus cultures in the laboratory, i.e., drying of the agar, formation of metabolic by-products, and relative reduction of living space. These factors were not apparent and did not significantly affect the more rapidly growing *Pestalotia* cultures.

Reproduction

At the highest concentrations obtusaquinone in the culture media had a retarding effect on the reproduction of the tested fungi. Within a week after inoculation, *Pestalotia* was producing masses of black pycnidia and conidia on all plates except those containing 200 and 500 ppm obtusaquinone. At these higher concentrations, pycnidia and conidia appeared only on the tiny agar cubes which were used for inoculating the plates. Even at the end of three and a half weeks no reproductive structures were produced except on the cubes. Conidium production was only slightly reduced at 125 ppm obtusaquinone, and at the lower concentrations it was apparently unaffected. In those cultures of *Chaetomium* containing obtusaquinone at concentrations of 31 and 63 ppm, perithecia were present after 17 days. At concentrations of 125, 200, and 500 ppm, perithecia appeared on the inoculating cubes, but in much smaller numbers than on cubes in the other cultures of this organism. Possibly enough of the inhibitor diffused into the inoculating cubes to affect perithecium production. At the end of three and a half weeks there were still no perithecia on the medium beyond the cubes.

SUMMARY

Obtusaquinone has an inhibitory effect upon the growth rate and reproduction of two wood-inhabiting marine fungi: *Chaetomium olivaceum* Ames and a species morphologically similar to *Pestalotia oxanthi* Thumen.

The concentration of obtusaquinone required to produce a near-maximum inhibitory effect upon the vegetative growth rate of *Pestalotia* and *Chaetomium* was 200 ppm.

Obtusaquinone inhibits reproduction in *Chaetomium* at a concentration of 125 ppm, and in *Pestalotia* at 200 ppm.

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