Reports

Possible Relation between Marine Fungi and Limnoria Attack on Submerged Wood

Abstract. Wood submerged in the sea at Friday Harbor and at Naples contained only occasional hyphae, which showed no relationship to *Limnoria* burrows. Cultures of *Limnoria* established in "unconditioned" wood and in autoclaved wood were maintained in the absence of fungi. These results indicate that marine fungi have no significance for the activities of *Limnoria*.

In the 8 Nov. 1957 issue of *Science*, Meyers and Reynolds (*1*) reported that wood submerged in the sea is soon infested with marine fungi. Believing that fungal infection always occurs prior to attack by marine wood borers, especially *Limnoria*, they suggested that there might be a relationship between fungi and wood-destroying animals. Becker, Kampf, and Kohlmeyer (*2*), at about the same time, published the results of extensive observations and experiments that had led them to the same conclusion. Further work in support of this hypothesis was reported by Reynolds and Meyers (*3*) and by Schafer and Lane (*4*). In each case it was stated, suggested, or implied that marine wood-boring animals do not attack wood or become established in it unless the wood is first invaded and "conditioned" by marine fungi.

Because we believe that the evidence so far presented is insufficient to build a sound case, we undertook to examine the problem (*5*). From our studies, carried out at the Friday Harbor Laboratories of the University of Washington and at the Stazione Zoologica di Napoli, we have been unable to obtain results similar to those reported in the publications cited above. Since the suggestion that marine fungi may have a primary role in wood deterioration in the sea has serious economic implications, we feel that it is necessary to make our findings a matter of record.

The question of a possible relationship between marine fungi and marine wood borers was raised and discussed at the Friday Harbor Symposium on Marine Boring and Fouling Organisms, and much of the supporting evidence was published in the proceedings of that conference (*6*). The hypothesis rests mainly upon the following observations: (i) that wood when submerged in the sea is universally and rapidly attacked by marine fungi; (ii) that wood is not attacked by *Limnoria* until after it has been submerged for a period of time sufficient to "condition" the surface layers; (iii) that *Limnoria* is unable to survive in wood that has been sterilized by autoclaving.

Concerning the first observation, it was reported (*1, 3*) that fungi appeared on wooden test panels exposed in more than 63 stations in the Western Hemisphere, and that at Biscayne Bay the infection may be extensive in less than a week and vigorous sporulation by ascomycetous fungi may occur after 2 to 3 weeks' submergence. Working with laboratory cultures, Becker (*2*) recorded that all samples of both softwood and hardwood contained fungal mycelium in the surface layers and in the vicinity of *Limnoria* burrows after a submergence period of several weeks. It was also reported that wood samples collected from the North Sea, the Mediterranean, and the Indian Ocean regularly contained fungi.

We have examined, both at Friday Harbor and at Naples, wood that was collected at random from the sea, including pieces with and without *Limnoria* burrows, samples that appear to be relatively fresh, some that are water-soaked from long submergence, and others that are soft, spongy, and extensively deteriorated. We have also, again in both locations, placed blocks of fresh, fungus-free Douglas fir and western yellow pine into the sea at intervals so that they could be studied after known periods of submergence (up to 8 months at Friday Harbor; up to 5 months in the Bay of Naples). The procedure has been to make microscopic examination of thin freehand sections cut from the wood immediately upon its removal from the natural environment. Sections are cut parallel to the surface, tangential to the surface, and wherever possible, along the length of *Limnoria* burrows. In contrast to the authors of the previous reports, we do not find that marine fungi are universal inhabitants of submerged or floating wood; on the other hand, bacteria, some of which are undoubtedly cellulolytic, are nearly always present in the superficial wood fibers. That marine fungi do occasionally occur cannot be denied, but the presence of recognizable mycelium in wood freshly removed from the sea is uncommon indeed. Further, when fungi are present we find no evidence for a topographical relationship with *Limnoria* burrows. No significant differences could be seen between the cold temperate environment of the North Pacific and the warm subtropical conditions of the Mediterranean. It is our opinion that when a marine fungus does invade wood its presence is fortuitous so far as any relationship with *Limnoria* is concerned, and that these fungi have no significance for the activities of *Limnoria*.

Laboratory cultures of *Limnoria*, set up for breeding experiments, have been kept for 10 months in the sea-water system at Friday Harbor and show only four cases of fungal infestation out of 50 cultures of animals living in Douglas fir and western yellow pine. In an effort to obtain fungi in laboratory cultures, 10 Kolle flasks containing sterile sea water and autoclaved wood were each inoculated with 25 specimens of *Limnoria*, and to half of these a solution of penicillin-streptomycin was added. In the latter, bacteria have been suppressed, fungi have grown, and very few *Limnoria* have survived; in the controls there is no evidence of fungal infection, and the animals are healthy and vigorous and have established growing populations. These cultures have been maintained for 8 months.

The idea (observation ii) that *Limnoria* will not attack wood until its surface has been "conditioned" is based upon field observations (*1–3*) that there is a lag of some days, weeks, or even months between the time that wood is placed in the sea and the time when wood borers begin their invasion. During this interval microorganisms, especially bacteria, do indeed appear in the superficial wood fibers and may begin to deteriorate them; this is apparently what is meant by "conditioning." But these facts do not establish that *Limnoria* is unable to attack fresh wood or that such surface softening enhances the likelihood of *Limnoria* attack. It is not an unexpected finding that, in the natural environment, time should elapse before wood borers appear, since these animals are not constantly present, swimming

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

freely in the sea water and in a position to exploit a new food supply. Whether attack on fresh wood is possible can be tested under controlled laboratory conditions. Accordingly, dry "unconditioned" blocks of lumber, including Douglas fir, elm, hemlock, redwood, western red cedar, and western yellow pine, were exposed to healthy *Limnoria*. Each of these wood species was attacked within 24 hours at Friday Harbor, and within 2 to 3 hours at Naples. If "conditioned" wood is unnecessary in laboratory cultures, it is unlikely to be essential in the sea.

Finally, with reference to observation (iii), it was reported that *Limnoria* is unable to attack sterilized wood (*3*) and that animals living in sterilized wood survive no longer than controls kept without a food source (*2*). We find that healthy animals are quite capable of attacking and living in wood sterilized by autoclaving. In these tests the same six species of wood were used and the animals attacked them all within the same period of time (24 hours at Friday Harbor; about 2 hours at Naples). In all of these cases, growing populations were established in the absence of marine fungi (*7*).

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References and Notes

1. S. P. Meyers and E. S. Reynolds, *Science* 126, 969 (1957).
2. G. Becker, W.-D. Kampf, J. Kohlmeyer, *Naturwissenschaften* 17, 473 (1957).
3. E. S. Reynolds and S. P. Meyers, *Office Naval Research, Research Revs.* (Dec. 1957), pp. 6–11.
4. R. D. Schafer and C. E. Lane, *Bull. Marine Sci. Gulf and Caribbean* 7, 289 (1957).
5. These studies were aided by a contract (NR 104-142) between the Office of Naval Research, Department of the Navy, and the University of Washington.
6. "Marine Boring and Fouling Organisms," *Proc. Friday Harbor Symposia in Marine Biology* (Univ. of Washington Press, 1958).
7. A full report on this whole problem, including consideration of marine wood-inhabiting bacteria and a discussion of the suggestion made by Becker and by Schafer and Lane that fungi might contribute to the nutrition of *Limnoria*, is in preparation.

8 September 1958

Zinc-65 in Foods and People

Abstract. Disposal of trace amounts of Zn^{65} is made in the Columbia River via Hanford reactor effluent water. The subsequent utilization of river water for irrigation permits the concentration of this radioisotope in farm produce and its eventual deposition in man. The Zn^{65} in irrigation water, in farm produce, and in individuals utilizing these materials has been measured.

Water from the Columbia River is used as a coolant for the Hanford reactors. The subsequent disposal of this water in the river introduces trace amounts of several induced radioisotopes, most of which have half-lives of the order of minutes to a few hours; however, the half-lives of some of these isotopes are sufficiently long to permit tracing the distribution of the isotopes into the food chains of the aquatic life in the river (*1*). Zinc-65 is the major long-lived radioisotope introduced into the river, and although it is present at a concentration far below the most conservative permissible limits, it exists in sufficient amounts to serve as a tracer; it is thus possible to follow its path from irrigation water through plants and animals to man.

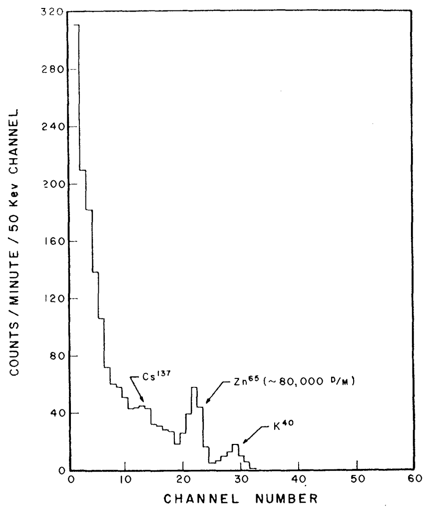

Fig. 1. Gamma-ray spectrum of an individual containing Zn^{65}.

Only a small fraction of the Columbia River water used for irrigation is obtained downstream from the Hanford project. The farm-produce and animal samples considered here were obtained from an irrigation project about 30 miles downstream from the Hanford reactors. By means of gamma-ray spectrometric techniques, measurable amounts of Zn^{65} were found in all the farm produce sampled from this location. The Zn^{65} concentrations found in milk, beef, and the various types of vegetables from this land are shown in Table 1. The concentration factor (Zn^{65} concentration in the sample/Zn^{65} concentration in the irrigation water) for each sample is also included.

With the exception of the beef, all of these samples were obtained during July and August 1957. The beef was obtained from an animal slaughtered in January 1957 after it had lived 1 year on the irrigation project. The fact that the pasture grass contained a relatively high Zn^{65} concentration as compared with the vegetables is probably related to both the manner and amount of irrigation as well as to the fact that some difference in uptake between the leaf and fruit portion of plants would be expected. The pasture grass was irrigated almost continuously, while the vegetables were irrigated only a few times during their growing season. In addition, the Zn^{65} may enter the grass by foliate absorption during irrigation as well as through the soil.

The relatively high Zn^{65} concentration in milk as compared with that in the pasture grass indicated that a large amount of Zn^{65} is taken from the feed into the blood stream of the cow and translocated into the milk. The low Zn^{65} concentration found in the beef samples (Table 1) may be explained by the fact that the animal was slaughtered in late winter and had been fed on essentially Zn^{65}-free foodstuffs for 3 to 4 months prior to that time. Measurements of Zn^{65} in the same milk supply during January and February of 1958 showed about 10 percent of the value listed in Table 1. This again can be explained by the animals' relatively Zn^{65}-free diet during the winter months.

A second animal which had spent its entire life in the same location was slaughtered in March of 1958 and was

Table 1. Concentrations of Zn^{65} in farm produce.

Sample	Concentration ($\mu\mu c/g$)	Concentration factor (produce/water)
Pasture grass	82.9	440
Beef, flesh	5.23	28
Beef, fat	1.48	7.9
Beef, bone	5.80	31
Milk (cow)	4.88	26
Black-eyed peas	0.55	2.9
Tomatoes	0.46	2.4
Okra	0.39	2.1
String beans	0.29	1.5
Corn	0.16	0.83
Grapes	0.089	0.47
Irrigation water	0.188	

Table 2. Concentrations of Zn^{65} observed in the various organs of a beef animal.

Sample	Concentration ($\mu\mu c/g$)
Flesh	10.7
Fat	2.22
Bone	13.4
Ovaries	4.07
Hide	3.91
Kidney	5.98
Lung	5.11
Brain	2.74
Pancreas	7.27
Blood	0.86
Hair	28.6
Thymus	3.79
Liver	11.5
Horn	3.59
Hoof	2.59