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On Water-Stored Oak Timber and its Decay by Fungi and Bacteria

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With 8 Figures in the Text

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The interest in the decay of wood in water has greatly increased since the cause of the so called "soft rot" has been better known. This type of rot is found on the surface of wood which has been in contact with water for a long time. Soft rot is a fungal decay caused by other microorganisms than *Basidiomycetes*. The fungi involved in this type of decay are certain *Ascomycetes* and *Fungi Imperfecti*. Soft rot decay is characterized by predominantly longitudinal growth of the fungi within the secondary cell-wall. The hyphae of rot causing Basidiomycete fungi, on the other hand, penetrate the cell-wall rather transversely through pits or bore holes and they are frequently visible in the cell-lumen. For a more detailed description of soft rot we refer to the work of FINDLAY and SAVORY (1950), SAVORY (1954), and SAVORY and PINION (1958). For the authors of this paper the salvage of the wreck of H.M.S. "Wasa" and the problems connected with its preservation were an extra incentive to try to gain more knowledge about wood-decaying micro-organisms in water. The oak wood investigated comes from "Riksäpplet" and "Gröne Jägaren" two Swedish ships which sank in the neighbourhood of Dalarö (east coast of Sweden, about 25 km south of Stockholm) at the beginning of the 17th century. The depth of the Baltic at the places where the finds were made is about 25 m and the salinity is about 0.6 %.

Oak wood which has been lying in water for a long time seems to be more liable to decay during the drying period than other oak wood. Fig. 1 shows a cross section of a plank from "Riksäpplet" with three different zones of decay. This wood was brought ashore about a year before it was investigated. Fig. 2 shows the damage in the microscopic structure of the wood in zone 2 (see also Fig. 1). Very few hyphae were observed in zone 2 (Fig. 2). Zone 3, however, which probably represents a type of deterioration similar to soft rot, contained somewhat larger amounts of visible hyphae. In the "charred" zone 1, no hyphae at all could be detected. In this part nothing more than middle lamellae and

some doubtful remains of the primary wall were left. The infrequent occurrence of hyphae might be due to autolysis but it may also depend on the fact that bacteria are involved in the deterioration.

Fig. 1. Plank from the "Riksäpplet" in cross section with three zones of decay

Oxygen is restricted as the sea bottom but it is not certain that such a situation hinders the fungi from migrating inwards in the logs (THACKER and GOOD 1952). However, deterioration by soft rot occurs not more than a few millimeters from the wood surface. Reports on decay of wood by bacteria are rare (ELLWOOD and ECKLUND 1959; KNUTH and MCCOY 1962). Therefore it was decided to investigate how deep in the timber of the wrecks fungi and bacteria occurred and to what degree and under what circumstances they might develop and decay wood.

The experimental work can be subdivided as follows: 1. Isolation of fungi taken from different places in cross sections of logs from "Gröne Jägaren" and "Riksäpplet." 2. Investigation of the wood-decaying effect under aerobic conditions of some of these fungi. 3. Investigations of the wood-decaying effect of bacteria and fungi in near anaerobic culture solutions inoculated with scrapings from timber of the "Gröne Jägaren."

Experimental

Isolation of fungi

The first investigations were performed on logs which had been soaked in water after removal from the Baltic. As there was a risk in these cases of obtaining false results by secondary infections other isolations were made on material which immediately after removal from the sea was wrapped in sterile aluminium foil and polyethylene sheets. They were stored at $+4°C$ until the investigation was made.

The pieces of timber had an approximate size of $20 \times 20 \times 70$ cm. A 5 cm thick section was taken out of the middle of each piece. This section was cut under

sterile conditions 10 cm, 5 cm, 2 cm, 0.5 cm inwards and just beneath the surface. Scrapings were taken from these cuttings. The scrapings were suspended in sterile, distilled water and subsequent dilutions were made. Malt agar plates were seeded with these dilutions. Other scrapings were placed directly on malt agar. The plates were incubated at 26° C and inspected after about a week and if necessary the colonies were further purified.

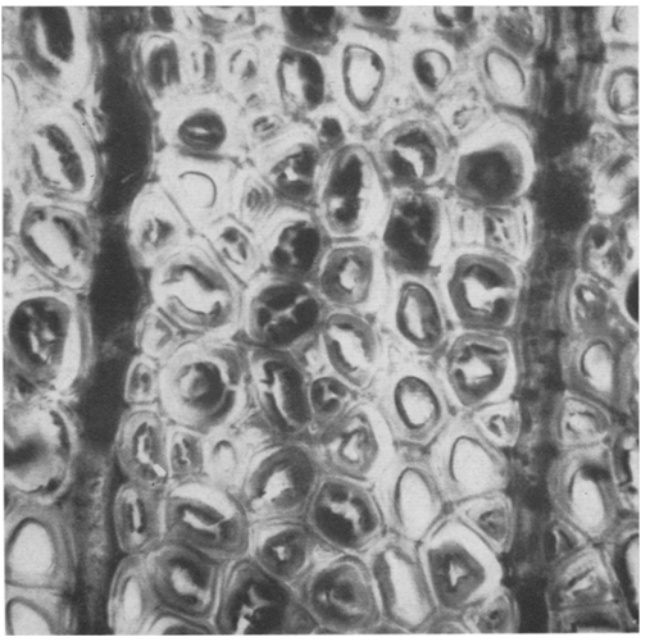

Fig. 2. Fragment of a microtome section of wood from zone II (see Fig. 1) with decayed cells

Aerobic wood-decaying capacity

Shavings of sound oak wood were dried at 100° C. Previously weighed amounts (Table 3) of the shavings were put in flasks together with a culture solution. The flasks were sterilized at 120° C for 20 min. The composition of the solution was 2.0 g $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g KCl, 0.2 g $MgSO_4$, 0.1 g $CaCl_2$, 0.5 g yeast, 1 mg aneurin per litre water. The flasks were inoculated with those cultures mentioned in Table 3 (among them a species of *Phoma*). After shaking at + 26° C for two months the shavings were separated by filtration, dried and weighed. Shavings decayed by the species of *Phoma* and controls were microscopically investigated.

Cultivation under conditions of oxygen restriction

Two types of culture solutions were used. One of them (Imschenezki 1959) contains only peptone and $CaCO_3$. The other (Enebo 1954) is more complex (base solution + standard medium I according to Enebo's terminology). Test tubes (1 × 18 cm) are filled with 10 ml of these solutions. Cellulose powder or sound oak wood pieces are suspended in the culture solutions. After sterilisation the tubes were inoculated with scrapings from "Gröne Jägaren" taken from different depths in the logs (see before). In order to get anaerobic conditions the test tubes

were placed in a vacuum desiccator and the air was replaced by carbon dioxide. Subsequently the test tubes were taken out and sealed immediately by melting. An initial oxygen content of less than 1 mg per l was determined by the Winkler method (Standard methods 1960). As the Winkler method can give false results when mercapto groups are present the initial oxygen determinations were performed on test tubes containing water instead of culture solution. Some of the tubes were stored at +4°C and others at +37°C. They were inspected now and then. After 19 months storage they were opened and the solutions and the oak pieces were investigated microscopically.

Sectioning of incubated wood

The pieces of oak were sectioned in an ordinary sliding microtome. Most pieces were embedded in Durcupan and sectioned with glass knives (BOUTELJE and ISHIDA 1963).

Results and Discussion

Most of the fungi isolated appeared to be species of *Penicillium*. Table 1 and 2 show that fungi were found through the whole timber.

Table 1. *Isolation of fungi from the "Gröne Jägaren"*
The wood had not been soaked in water after removal from the Baltic

Place where scrapings were taken	Dilution			
	1:1	1:10	1:100	1:1000
Beneath surface	Greasy fungus	White fluffy fungus	—	—
0,5 cm beneath surface	—	*Penicillium* sp. *Phialophora* sp.	*Penicillium* sp. *Phialophora* sp.	*Penicillium* sp.
2 cm beneath surface	*Penicillium* sp. White fungus	*Penicillium* sp.	*Penicillium* sp.	—
5 cm beneath surface	*Penicillium* sp.	—	*Penicillium* sp. *Phialophora fastigiata*, *Phialophora* sp.	—
10 cm beneath surface	*Penicillium* sp.	*Penicillium* sp.	*Penicillium* sp.	*Penicillium* sp. *Oidiodendrum griseum*

If no synonyms are overlooked, *Phialophora fastigiata*, *Oidiodendron griseum*, *Penicillium funiculosum* and *Verticillium malthousei* are not recorded earlier from marine sources.

Other samples of timber from the "Gröne Jägaren" and "Riksäpplet" showed a similar fungal distribution, i.e. fungi were found throughout the whole log. From one of these series *Penicillium lilacinum*, *Penicillium*

Table 2. *Isolation of fungi from the "Gröne Jägaren"*
The timber had not been soaked in water after removal from the Baltic. Scrapings were placed directly on the agar plates

Place where scrapings were taken	Fungus
At the surface	Grey and white fluffy fungus
0,5 cm beneath surface	*Verticillium malthousei, Penicillium* sp.
2 cm beneath surface	*Penicillium* sp., pinkish fungus
5 cm beneath surface	*Penicillium* sp., yellow fungus
10 cm beneath surface	*Penicillium* sp., grey fungus, yellow fungus

Table 3. *Deterioration of oak shavings in shake cultures*
The fungi were isolated from logs which had been soaked in water after removal from the Baltic

Source of inoculum	Weight of shavings before autoclaving g	Weight after 2 months cultivation g	Weight loss %
"Gröne Jägaren"	1.0380	0.9768	5.9
Penicillium lilacinum,	1.0272	0.8845	13.9
from 10 cm inwards from the surface of the timber	1.0170	0.9181	9.8
"Riksäpplet"	1.0190	0.8811	13.5
Penicillium lilacinum,	1.0246	0.8987	12.3
from the surface of the timber	0.9735	0.8807	9.5
"Riksäpplet"	1.0300	0.8963	13.0
Penicillium funiculosum,	1.0280	0.8732	13.1
from 5 cm inwards from the surface of the timber	1.0450	0.8742	16.4
"Gröne Jägaren"	1.0322	0.7621	21.3
Phoma sp., from 10 cm inwards	1.0056	0.7979	20.7
from the surface of the timber	1.0158	0.7654	24.8
Controls, no inoculation	1.0180	0.8640	15.2
	0.9964	0.8419	15.5
	1.0414	0.8890	14.7

funiculosum and a species of *Phoma* were isolated. Data about the wood-decaying capacity of these fungi are given in Table 3. The values indicate that *Phoma* possesses capacity for decaying oak wood. The other two species show no distinct decaying effect. Figs. 3 and 4 illustrate clearly that the above species of *Phoma* has cellulolytic properties. The fungus finally leaves only the middle lamella (Fig. 3). The typical longitudinal growth in the cell-walls resembles the growth described for soft rot (Fig. 4) but also transverse growth of hyphae can be detected. A cross section of an uninoculated control is shown in Fig. 5.

In the sealed test tubes no growth could be detected by visual inspection after two months. After 19 months slime had developed in some

tubes and often the cellulose powder or the oak pieces were stained a dark colour. Because the growth in those tubes containing the culture solution according to ENEBO (ENEBO 1954) seemed to be small, only those tubes containing the Imschenezki medium (IMSCHENEZKI 1959)

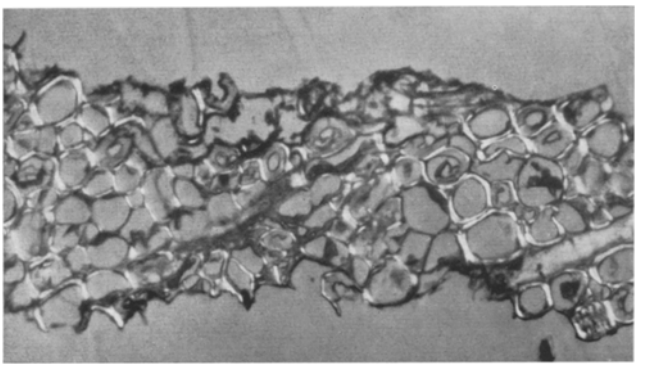

Fig.3. Cross section of oak shaving showing tissue severely decayed by *Phoma* sp. 280×

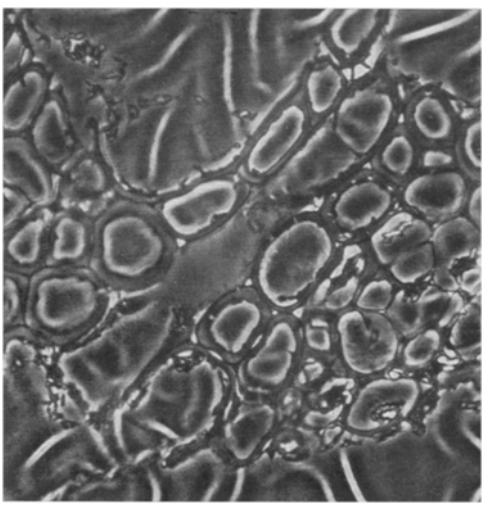

Fig. 4. Cross section of oak shaving decayed by *Phoma* sp. Holes in the cell-walls caused by longitudinal growth of hyphae. Striped appearance of background caused by folds in embedding agent. Phase contrast. 410×

were investigated. The solutions smelled distinctly of hydrogen sulfide. Bacteria could be detected in all tubes. Mostly they were short gram negative rod-shaped bacteria. In some tubes, stored at +4°C, fungi were found (Figs. 6 and 7). It seems odd to get fungi under near anaerobic conditions. There are, however, cases described when fungi grew in extremely low oxygen concentrations (THACKER and GOOD 1952; DENNY

1933). Apparently minimal traces of oxygen are sufficient for the fungus observed. Its brown, rather coarse, hyphae penetrated the wood like a

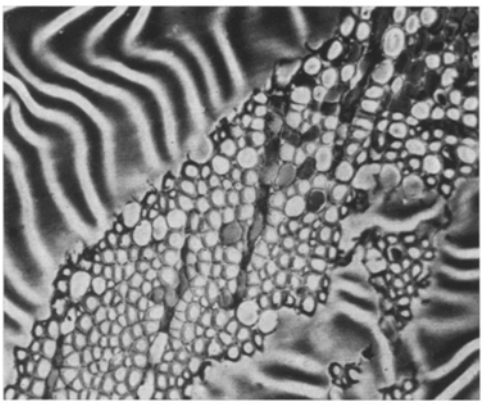

Fig. 5. Uninoculated control. Oak shaving subjected to same treatment as shavings in flasks inoculated with *Phoma* sp. Background of figure shows folds in the embedding substance. 140 ×

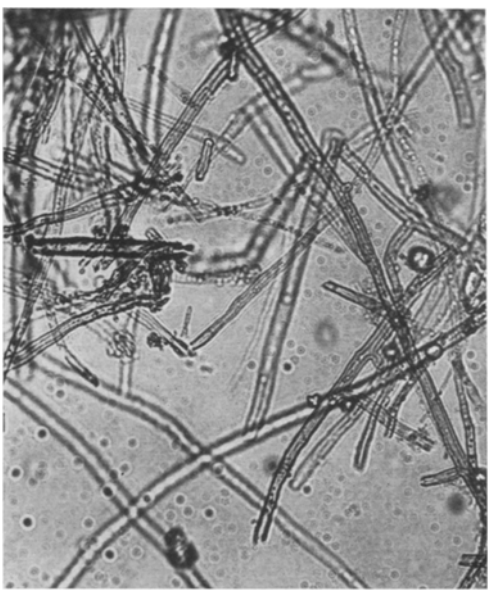

Fig. 6. Development of fungi in a near anaerobic culture solution at +4° C, prepared according to IMSCHENEZKI (see text). 400 ×

sap stain. Radial walls of the fibres were mainly penetrated through the pores. Often its hyphae were found in the parenchyma cells but they were also present in other tissue elements.

In wood sections prepared directly after the opening of the test tubes (stored at +4° C) it seemed that bacteria were concentrated in parenchyma cells. In other pieces of wood which were embedded in Durcupan before sectioning the microscopic structure appeared to be rather well preserved. In sections of wood (stored at +37°C) the fibres were damaged in some places in a peculiar way (Fig. 8). This damage was considered by us to be due to bacteria. Secondary walls of peripheral fibres of pieces of wood, kept at +4°C, showed in some cases a turbid appearance. This phenomenon might also be due to bacterial decay. Walls of parenchyma cells were damaged to some extent in a few sections. Since this damage was not found persistently and might have been due to sectioning it was not considered as convincing proof of bacterial attack on the walls of the parenchyma cells. As the parenchyma cells of the sapwood were almost completely empty the bacteria had probably metabolished their contents. For pine wood logs stored under anaerobic conditions in ponds it has been

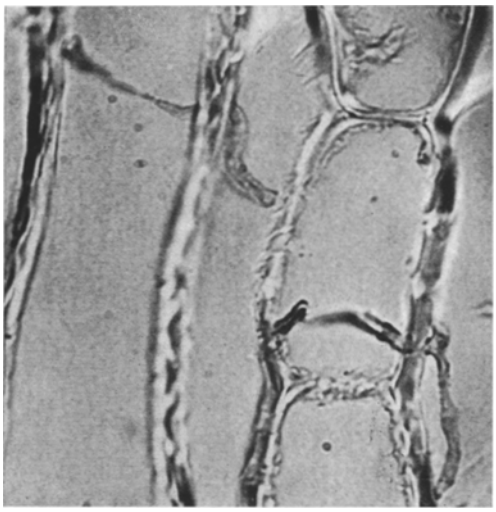

Fig. 7. Penetration of fungi into wood in a near anaerobic culture solution (see Fig. 6). 720×

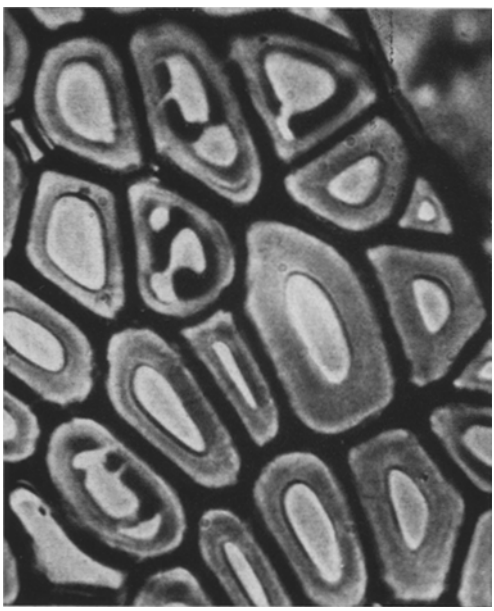

Fig. 8. Sapwood of oak deteriorated by bacteria in a near anaerobic culture solution at +37° C (see text). Stained with safranin. 1200×

shown (ELLWOOD and ECKLUND 1959; KNUTH and MCCOY 1962) that *Bacillus polymyxa* causes removal of the ray cell contents and in extreme cases destruction of the thin walls of the ray parenchyma cells.

The timber was once air-dry, thus when the ships sank the fungi and bacteria may have penetrated the wood via microscopic and macroscopic drying cracks. This would mean that if there is no exchange of micro-organisms at the bottom the isolated species have survived more than 300 years in the water-saturated wood. It would in this connection be interesting to get comparable data on fungi and bacteria in fresh submerged timber. Such data might give additional information on the penetration of wood by micro-organisms.

Phoma and *Phialophora* are often isolated from wood submerged in water (DUNCAN 1960; SIEPMANN and JOHNSON 1960; GOLD 1959). These genera contain species with considerable wood-decaying capacity (DUNCAN 1960) and are probably an important cause of soft rot in water-stored timber. Only a few fungi isolated from the submerged oak timber were investigated in respect of their capacity to decay wood. At least one of them (*Phoma* sp.) isolated from the interior of the timber has a distinct wood-decaying effect. It seems therefore that when circumstances become favourable decay may occur even if secondary infections are avoided. Since also slight deterioration was caused by bacteria it is recommendable also to take bacteria into consideration when the preservation of previously submerged wood is carried out.

Summary

A number of fungi were isolated from oak timber from two ships which sank in the Baltic at the beginning of the 17th century. Fungi were found throughout the timber. One of the isolates, a species of *Phoma* isolated from the central parts of the timber, showed a distinct wood-decaying capacity, causing a kind of rot resembling soft rot. Bacterial deterioration of oak wood in aerobic culture solutions inoculated with scrapings from the submerged oak timber was observed.

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