A NOTE ON LIGHT AND ELECTRON MICROSCOPY STUDIES OF WHITE PINE EXPOSED TO MARINE BORER DAMAGE

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(Received 4 November 1974)

ABSTRACT

A sample of white pine exposed to marine borer attack during an 11-month period in 11 meters depth, off Hokkaido, Japan, was used for anatomical studies. The sample received had been damaged by *Limnoria, Bankia setacea,* and *Teredo navalis.* In addition to damage due to the above, degraduation of parenchyma cells indicates possible bacterial action. With the exception of loss of parenchyma, anatomical integrity of the sample had been maintained; there was no evidence of fungal attack. Analyses of wood extractives, holocellulose content, lignin content, and selected inorganics were also undertaken. Both lignin and holocellulose content were below normal, indicating that the lower molecular weight fractions of these components were increased during the exposure period. Interactions of sea water, borer damage, and possible presence of microorganisms (bacteria) on wood structure are discussed along with the use of microscopy as a supporting tool in studies of marine borer damage to wood.

Additional keywords: Pinus sp., SEM, marine borers, wood structure, biodegradation.

INTRODUCTION

Sources of information on wood damage due to marine borers generally lack any information on anatomical changes of the wood at the microscopic level (Findlay 1953; Hunt and Garratt 1957; Hochman 1973; Panshin et al. 1964; and St. George 1950). Actually, the use of light microscopy and SEM studies may provide useful information to supplement chemical and other analyses of the complex, interrelated happenings during exposure of wood to marine conditions. The SEM is a useful tool for study of fragile and refractory materials and thus may provide additional insight to studies of wood degraded in service where light microscopy is limited because of problems in sample preparation for observation and/or limited depth of focus and magnification ranges. This study was initiated to explore results of marine borer damage at the microscopic level, and, hopefully, to indicate that such analyses would provide an additional dimension in future research studies of marine borer damage to wood.

MATERIALS AND METHODS

A white pine board (approximately 15 × 31 × 2 cm) exposed to a variety of types of marine borer attack for an 11-month period in the sea just off the bottom in 11 meters depth off Hokkaido, Japan, was obtained for microscopic analyses through the courtesy of the U. S. Navy.[1] The organisms that had attacked this sample were *Limnoria, Bankia setacea,* and *Teredo navalis.* Both face and end views of the sample were photographed prior to division for microscopic and chemical analyses; these macroscopic views are shown in Fig. 1.

For light microscopic analyses, conventional paraffin-embedding techniques proved to be futile. A nitrocellulose method (Humason 1962) was found acceptable; and cross, radial, and tangential sections approximately 30 μm thick were cut and stained in Safranin and Fast Green and mounted in Permount. Blocks approxi-

[1] DePalma, J. R. 1973. Personal Communication. U.S. Naval Office, Washington, D.C.

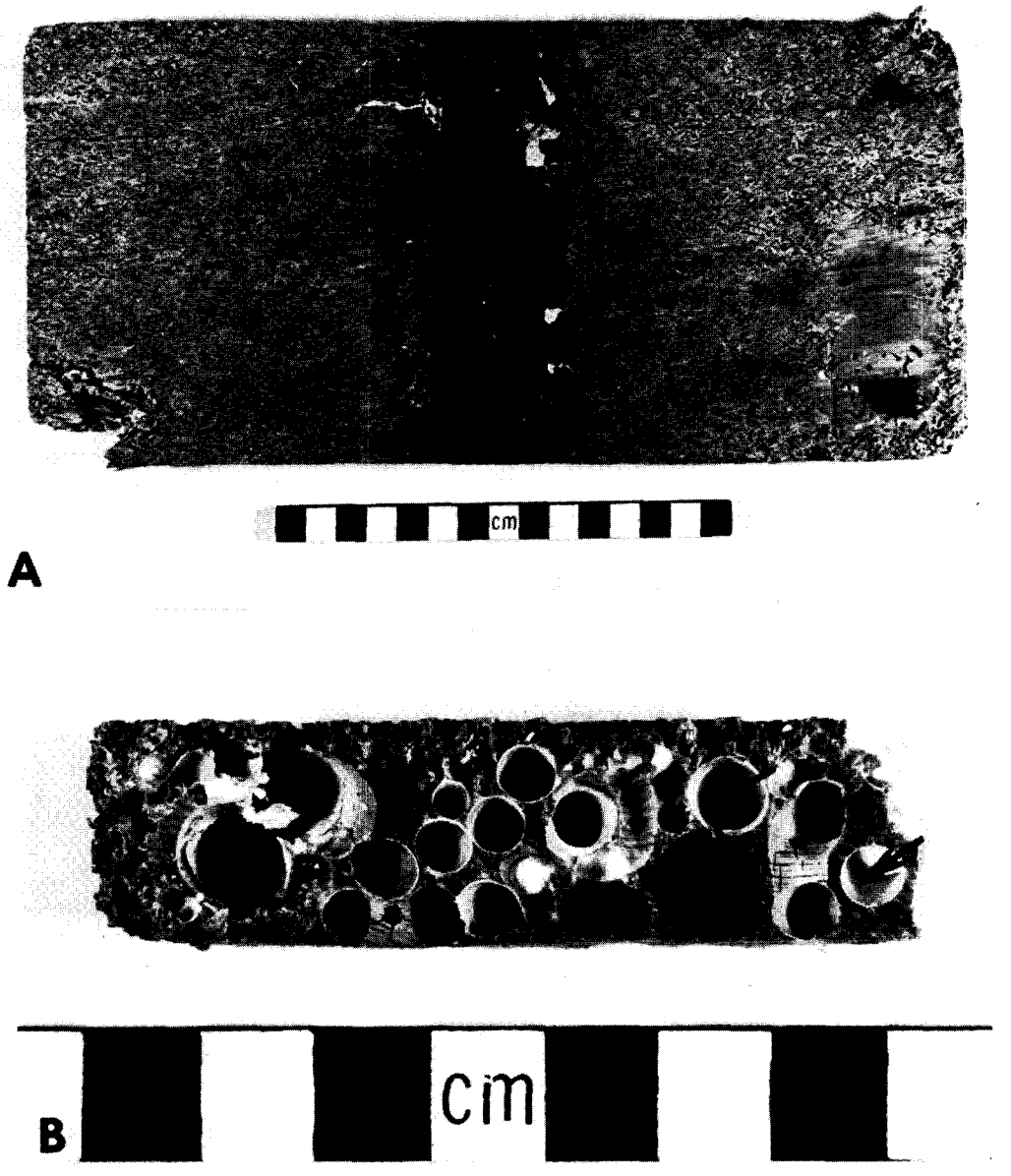


FIG. 1. Sample of white pine explosed to marine borer damage (see text). Top (**A**) shows surface damage due to *Limnoria*, while (**B**) is a cross-sectional view of the sample showing *Limnoria* damage plus damage due to *Bankia setacea* and *Teredo navalis*. "Limey burrows" are evident (arrows).

mately 1 × 2 × ½ cm were cut from the sample board for SEM studies. Samples were taken from both the exterior and interior surfaces of the board. These samples were dried over a desiccant, coated with gold, and examined with a JEOL instrument operating at 10 kV.

Extractives were determined in a Soxhlet

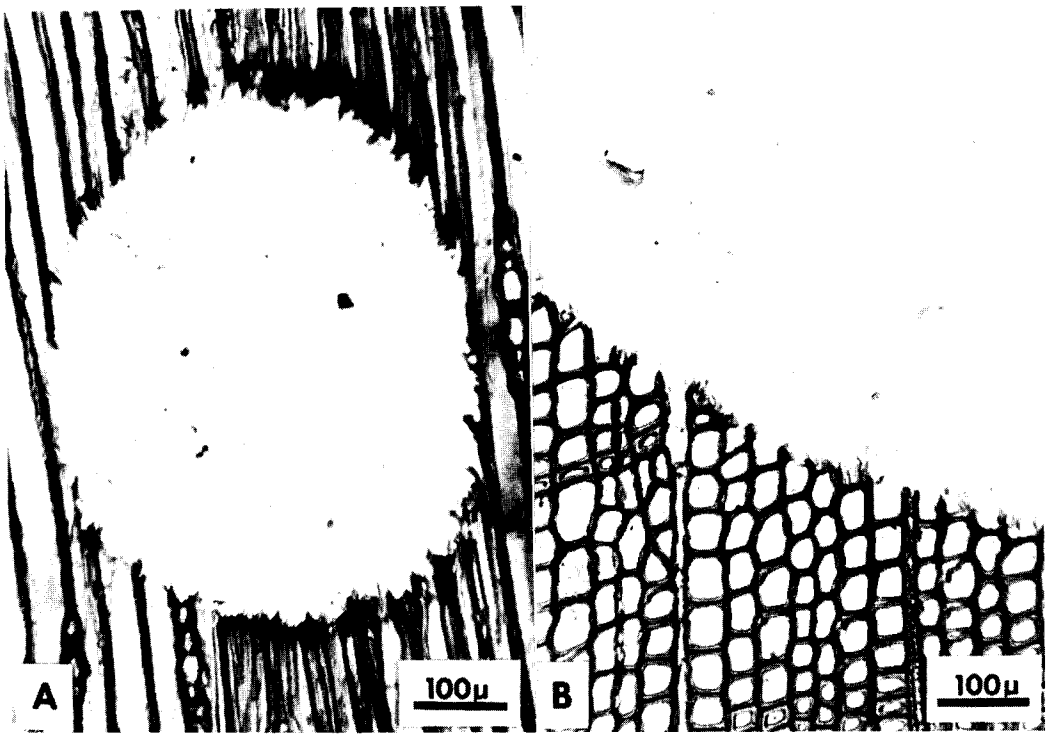


FIG. 2. Tangential section (**A**) showing circular hole made by gribbles; (**B**) cross-sectional view of larger diameter hole made by shipworms.

apparatus using water and then ethanol as extracting solvents. Extraction time was 4 h for each solvent. Holocellulose was determined using a chlorite procedure (Wise et al. 1946), and lignin content was determined by a standard 72% sulfuric acid method (Browning 1967). Selected inorganic concentrations were obtained by neutron activation analyses utilizing the university reactor facility that operates on an approximate flux of 8×10^{13} thermal neutrons per cm^2/second.

RESULTS AND DISCUSSION

Figure 1 shows damage due to both gribbles (lacelike surface effect) and shipworms (larger holes in cross-sectional view). Viewed with both light and scanning electron microscopes, gribble burrows are cylindrical in cross section (Fig. 2A and 3) as are shipworm holes (Fig. 2B) but lack incrustation buildup typically associated with shipworm damage (Fig. 4).

Ray tissue, particularly parenchyma cells, appears destroyed or in a badly degraded state throughout the sample as shown in Figs. 5 and 6. This destruction of ray tissue is similar to that reported for bacterial action on wood (Wilcox 1970), and chemical analyses alone would not demonstrate this point. Although bordered pit structure retains anatomical integrity (Fig. 7), SEM views of cell walls shown in Figs. 6 and 7 indicate some type of degradation since excessive amounts of exposed, torn lamellae present on split surfaces are atypical for normal wood of white pine species similarly prepared for microscopic examination. This degradation apparently was influenced by the solubilizing effect of sea water itself, possible influence of marine borers, and an additional factor, presumably microorganisms (bacteria), cannot be ruled out.

TABLE 1. *Selected chemical analyses of untreated white pine exposed to marine borer damage.*

Form of Sample	Type of Analysis						
	Organic Analyses						
	EtOH Extractives %		HOH Extractives %		Lignin[a] %		Holocellulose[a] %
40 mesh, entire sample	1.44		5.57		21.78		59.18
	Inorganic Analyses (ppm)[b]						
	Mg	Cl	Na	Ca	Mn	I	Br
whole sample, unextracted	1,940	3,370	2,817	7,579	30	59	50
outer wood (primarily gribble zone), unextracted	8,903	26,564	16,549	3,341	239	475	428
inner wood (primarily shipworm zone), unextracted	---	3,349	2,972	960	---	---	26
"Limey burrows," unextracted	21,459	705	2,891	246,904	---	---	14

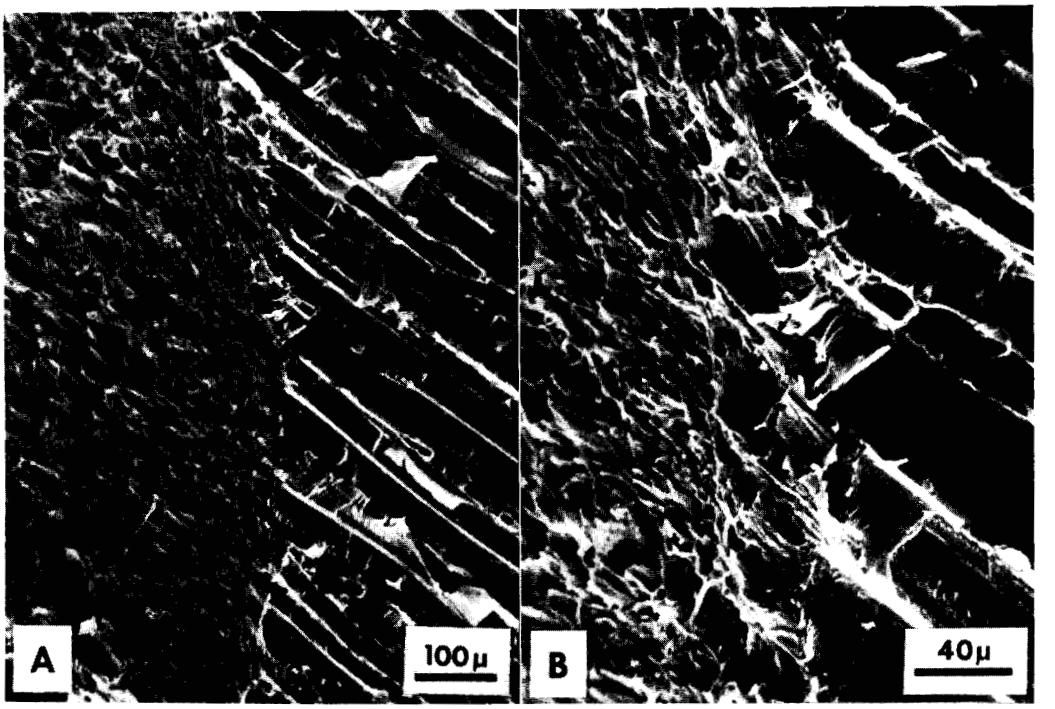
[a] determined on extractive-free samples.

[b] Mg, Cl, Na, Ca, Mn, and I determined after 30 sec irradiation time, 10 min decay, 500 counts. Br determined by 20 min irradiation, 115 hr decay, 2,000 counts. Elements such as Fe and Pb visible on the spectrum, however, no quantitative analyses were determined.

The chemical tabulations (Table 1) on holocellulose and lignin determinations of the sample are indicative of some type of degradation since lignin content is only ⅔ that (30%) of typical softwoods and the holocellulose fraction is also considerably less than normal of extractive-free wood, comprising only 59% of the sample. This is approximately 85% of the normal value of 70% holocellulose content for coniferous woods. Presumably, the lower molecular weight components of the lignin fraction of the wood and of the hemicellulose fraction of the holocellulose component of the wood, were increased during the exposure period and then "lost" in the resultant lignin and holocellulose analyses. It is well known that lower molecular weight "lignins" are lost in the sulfuric acid analyses and the same condition holds for lower molecular weight components of the holocellulose fraction during conventional procedures for estimating the total carbohydrate content of wood.

These observations do not clarify whether such degradation is caused predominantly by borer attack, sea water, exposure, microorganisms, or a combination thereof. Unfortunately, there is a lack of information in the literature to clarify this subject. Studies on feeding habits of marine organisms indicate that, although wood may be the nutrient source, there is evidence (Becker 1959) that symbiosis exists between *Limnoria* and fungi while the studies of Ray (1959) indicate that *Limnoria* feces are lignin-rich. Bacteria also deplete lignin-rich zones of wood.

The inner wood zone (shipworm area) contains less of the inorganics analyzed than the outer zone (gribble area) with the obvious exception of the "limey burrows." There was no evidence of fungal attack on the sample. The concentration



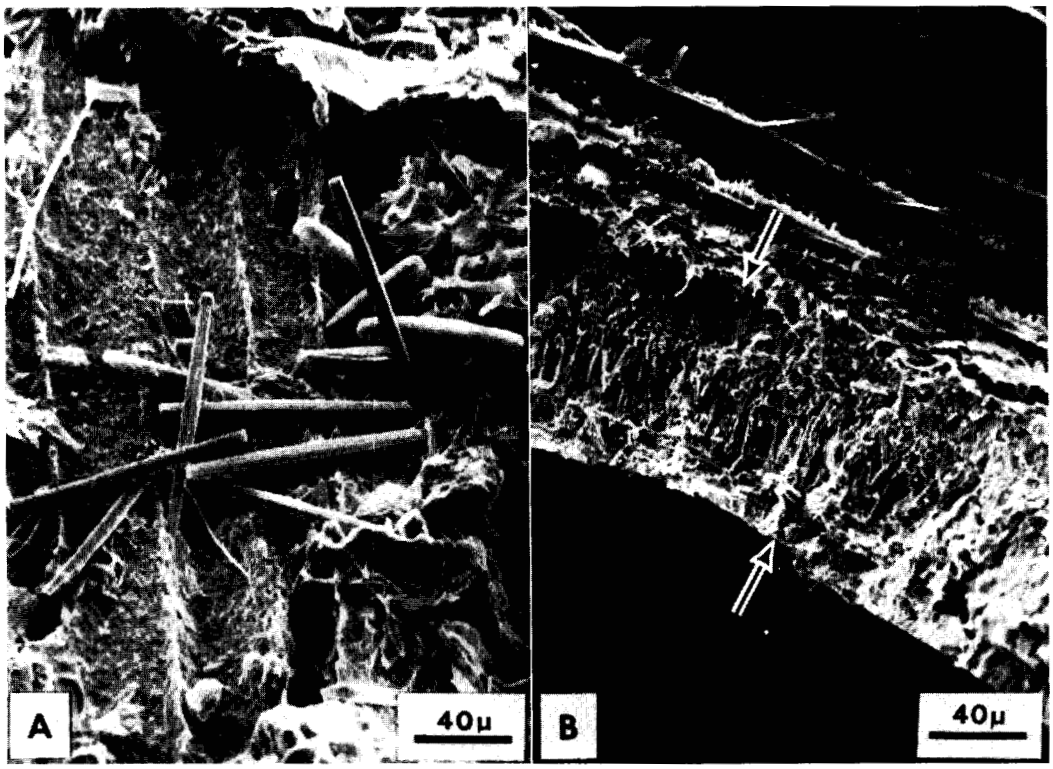
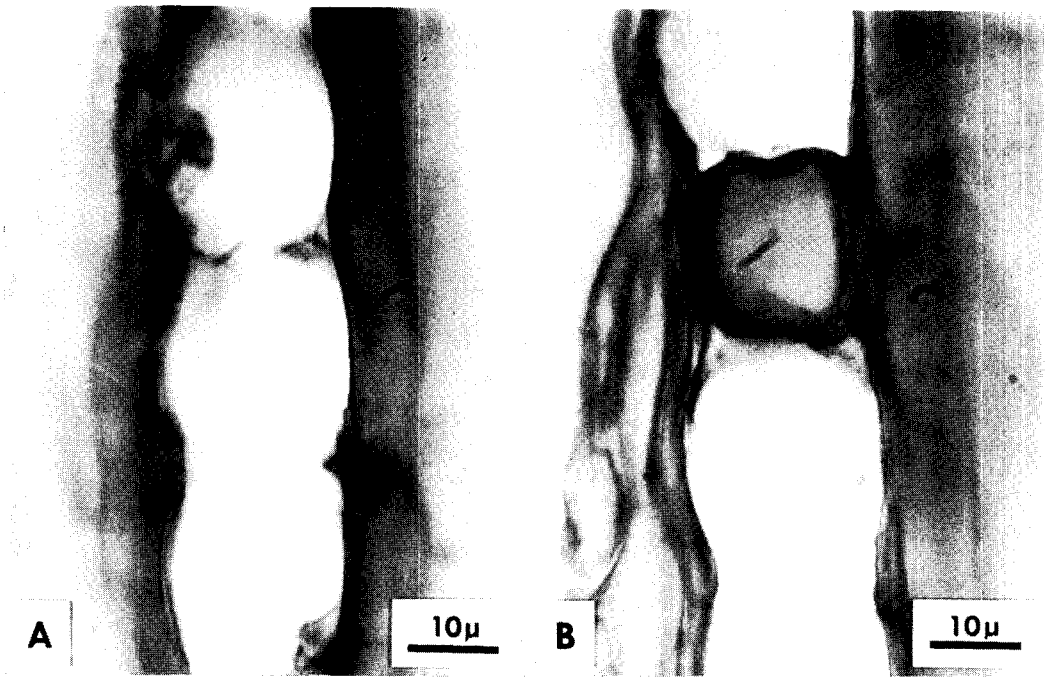


FIG. 3 (above). Tangential views of wood surface made by gribbles as seen in SEM. Some cellular debris and foreign deposits evident; "limey burrows" caused by shipworms (Fig. 4) absent.

FIG. 4 (below). SEM views of crystal buildup (**A**) and heavy calcium deposits (**B**, arrows) associated with shipworm damage.



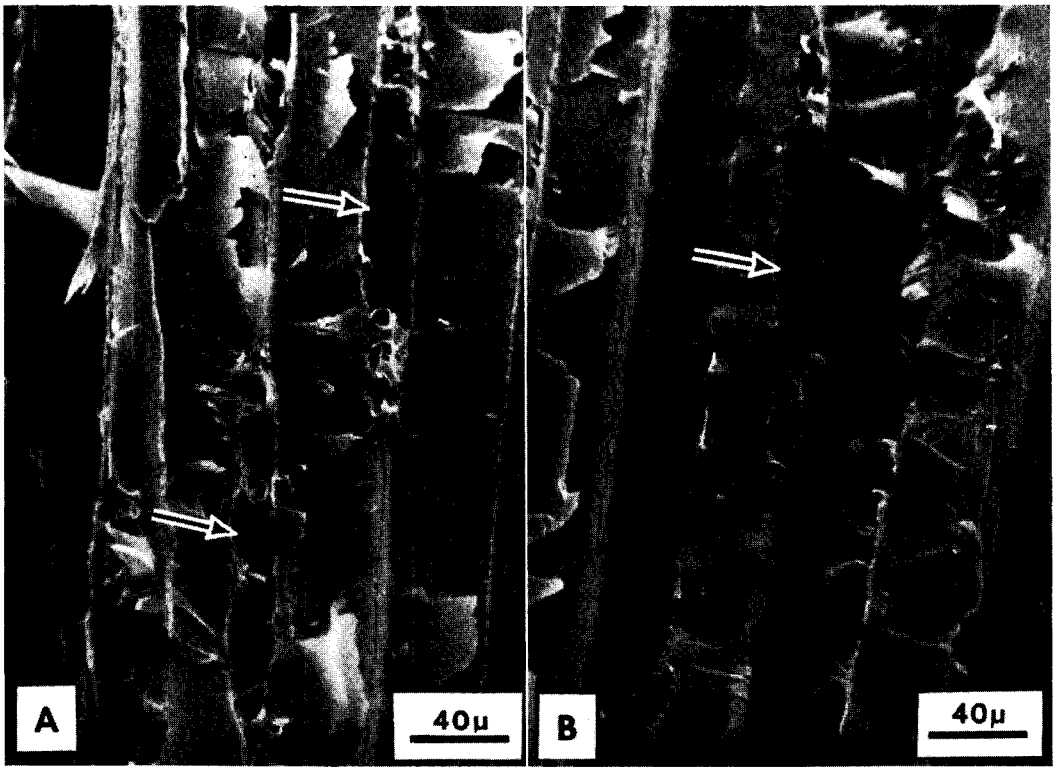


FIG. 5 (above). Tangential views (**A**,**B**) of ray tissue areas; several ray parenchyma cells missing (compare with Fig. 6).

FIG. 6. (below). SEM micrographs of tangential face showing ray tissue loss (arrows) and exposed torn lamella of cell-wall structure (see Table 1) presumably indicative of cell-wall degradation by exposure conditions.

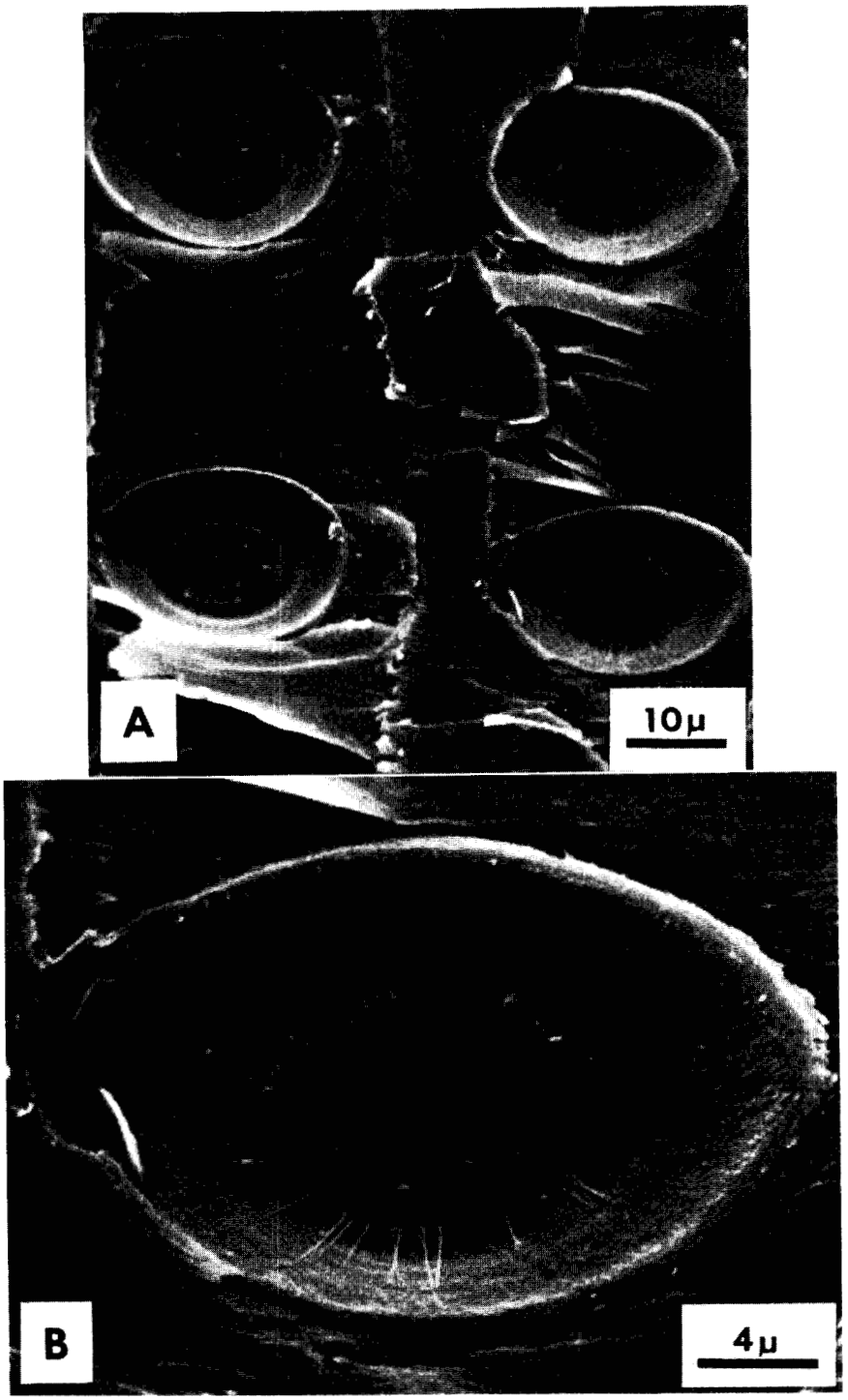


FIG. 7. Representative views (**A**) of bordered pit structures showing minimal loss of anatomical features. Pit structure enlarged in (**B**) showing microfibril structure intact.

(with exception of "limey burrows") of inorganics in the outer wood (gribble zone) may be attributed to greater sea water accessibility (wood permeability) on the outer wood surface in this zone; however, this is an assumption at present.

The use of light and SEM techniques in this study fills a void in published English texts dealing with marine borer damage to wood at the cellular level and points out that microscopy is a valuable support tool in such studies by indicating preferential loss of ray parenchyma cells in the sample studied—such action possibly due to microorganism (bacteria) presence and thus resultant increased water permeability of the sample (through ray destruction) thereby contributing to an increase of marine borer population and/or penetration.

REFERENCES

BECKER, G. 1959. Biological investigations on marine borers in Berlin-Dahlem. Pages 62–83 *in* D. L. Ray, ed. Marine boring and fouling organisms. University of Washington Press. Seattle, WA.

BROWNING, B. L. 1967. Methods of wood chemistry. v. 2. Chapter 34, Determination of lignin. John Wiley and Sons, Inc., New York. Pp. 785–791.

FINDLAY, W. P. K. 1953. Dry rot and other timber troubles. Hutchinson's scientific and technical publications. London. 267 pp.

HUNT, G. M., AND G. A. GARRATT. 1957. Wood preservation. McGraw-Hill, 3rd edition, New York. Pp. 62–68.

HOCHMAN, H. 1973. Degradation and protection of wood from marine organisms. Pages 247–275 *in* D. D. Nicholas, ed. Wood deterioration and its prevention by preservative treatments. v. 1. Syracuse University Press. Syracuse, N.Y.

HUMASON, G. L. 1962. Animal tissue techniques. Chapter 7, Nitrocellulose method. W. H. Freeman and Co. Pp. 70–79.

PANSHIN, A., C. DEZEEUW, AND H. BROWN. 1964. Textbook of wood technology. Chapter 10, Wood deterioration and stains; natural durability of wood. McGraw-Hill, 2nd edition, New York. Pp. 378–382.

RAY, D. L. 1959. Nutritional physiology of limnoria. Pages 46–61 *in* D. L. Ray, ed. Marine boring and fouling organisms. University of Washington Press, Seattle, WA.

ST. GEORGE, R. A. 1950. Marine borers. Pages 66–69 *in* F. C. Craighead, ed. Insect enemies of eastern forests. USDA Misc. Pub. 657.

WILCOX, W. W. 1970. Anatomical changes in wood cell walls attacked by fungi and bacteria. Botan. Rev. 36(1):1–28.

WISE, L. E., M. MURPHY, AND A. A. D'ADDICCO. 1946. Chlorite holocellulose—its fractionation and bearing on summative wood analyses and on studies on the hemicelluloses. Paper Trade J. 122(2):11–19.