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Research on Biodeterioration of Wood, 1987–1992

II. Diagnosis of Decay and In-Place Treatments

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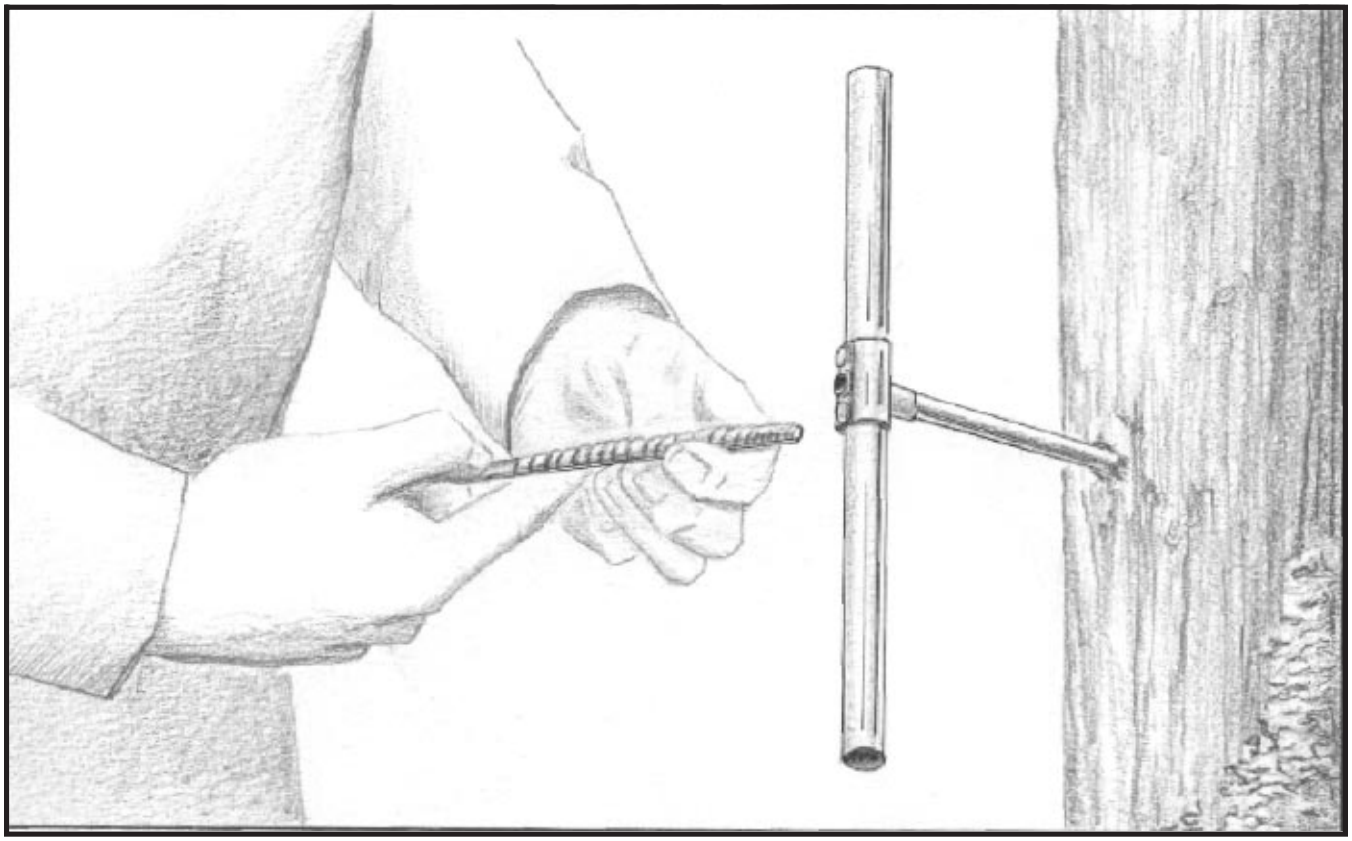

Abstract

Biodeterioration is associated with virtually all types of wood structures, including bridges, mine timbers, utility poles, marine structures, and railroad ties. Scientists in the Biodeterioration of Wood Unit at the Forest Products Laboratory, USDA Forest Service, are exploring new approaches for detecting and preventing wood decay. This report describes progress in developing immunological procedures for detecting decay. The report also describes simple in-place preventive and remedial treatments for protecting wood from biodeterioration by mold, sapstain, and decay fungi.

Research on how fungi decay wood and how this information will lead to better control methods is described in a companion report, Research on Biodeterioration of Wood, 1987–1992. I. Decay Mechanisms and Biocontrol (FPL–RP–529).

Timber has been estimated to increase in value nearly 24 times between the stump and delivery of the finished product. Thus, research that improves the service-life of wood has potential for high financial return.

Keywords: Wood decay, in-place treatment, decay detection, stain, mold

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II. Diagnosis of Decay and In-Place Treatments

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Introduction

Wood is liable to biological degradation when exposed to dampness, which can encourage the development of fungal growth or insect attack. This waste of valuable timber can be avoided by proper application of wood preservatives prior to installation. Maximum protection against wood decay is provided by pressure impregnation of the wood with preservative. However, in-place or supplemental surface treatments can reduce the incidence of decay in (1) newly installed untreated wood, (2) untreated wood exposed by weather checks or mechanical damage of poorly pressure-treated wood, and (3) untreated wood exposed when pressure-treated wood members are cut or drilled. The surface type of treatment is effective when applied before decay is established. Surface chemical treatments, applied by dipping or spraying, also protect freshly cut timber from discoloration by mold and sapstain fungi.

Until the late 1970s, pentachlorophenol or its sodium salt was essentially the only chemical used to protect green wood from stain and mold organisms and to protect wood exposed above ground from decay; the chemical was applied without pressure—by brush, dip, or spray treatment. Environmental concerns have eliminated the use of pentachlorophenol for this purpose, giving rise to an urgent need for alternative chemicals.

Economical and effective improvements for *in situ* treatments depend on the ability to locate interior decay (particularly early decay) within the structure. Other than culturing and microscopic observation, there is no reliable method for determining early decay in structures. Timber inspectors untrained in microbiological techniques or without access to microbiological laboratories need some kind of diagnostic method. Immunodiagnostic methods, such as used in agriculture to detect plant diseases, may be used to detect early infection of wood by decay fungi.

If decay is detected in a structural member before significant strength reduction occurs, then remedial treatments can be used to arrest the decay. Internal decay in wood is difficult to eradicate by application of conventional wood preservatives because of their shallow depth of penetration. High vapor pressure fungicides (fumigants), which have successfully stopped internal decay in Douglas-fir poles, are promising for remedial application. However, additional information is needed about the efficacy of fumigants in other species, about alternative, safer chemicals, and about performance of the treatment under different exposure conditions, such as in horizontal timbers exposed above ground.

The purpose of the research described in this report is to improve the performance of wood by protecting it against fungi that discolor or decay the wood during processing or in service, primarily above ground. To accomplish this objective, we developed (1) methods for diagnosing fungal attack in the early stages and (2) simple preventive and remedial means for controlling both stain and decay fungi.

Research Accomplishments

Immunodiagnostic Detection of Early Decay

Detection of decay in wood structures, particularly in the early stages, has long been a major problem to those responsible for appraising and maintaining structures. Sounding of wood, visual inspection of borings from wood, radiography, sonics, and various mechanical probing devices are useful for detecting advanced decay. However, early stages of decay are often difficult to detect with these methods. Application of chemical indicators to core samples has shown some promise for detection of early decay, but results have often been unclear or have been difficult to interpret. An electronic-type detector, the Shigometer, has been used to detect early internal decay in trees and utility poles. However, this instrument gives unreliable results when the moisture content of wood is between 38 and 45 percent (Morris and Dickenson 1984).

Immunological methods have been successful in detecting early fungal infection in agricultural crops and thus may have promise in detecting fungi in wood. The goal of our research has been to develop an immunodiagnostic procedure capable of detecting incipient brown-rot decay before significant structural damage has occurred. The ideal detection system should meet several criteria: (1) specificity without cross-reactivity, (2) speed, (3) ability to adapt as a field test for use by nontechnical inspectors, (4) low cost, (5) usability with small sample size, and (6) usability with multiple samples with automated readings.

Agglutination assays are considered to be the most rapid immunodiagnostic procedure (Wiedbrauk and others 1989). Immuno-dot blot assay and enzyme-linked immunosorbent assay (ELISA) are also rapid immuno-diagnostic procedures that combine sensitivity with ease of visualizing results (Wiedbrauk and others 1989). We compared ELISA, immuno-dot blot, and particle agglutination assays for the early detection of six brown-rot fungi on southern yellow pine (*Pinus* sp.) and correlated these methods with wood-block weight loss.

Polyclonal rabbit antibodies were produced with antigen derived from liquid culture filtrates from six common brown-rot fungi: *Postia placenta*, MAD-698; *Gloeophyllum trabeum*, MAD-617; *Antrodia carbonica*, MAD-141; *Neolentinus lepideus (= Lentinus lepideus)*, MAD-534; *Serpula incrassata*, MAD-563; and *Coniophora puteana*, MAD-515. Pooled culture filtrates were fractionated to give a molecular weight range of 10–100 kDa prior to injection. Murine monoclonal antibodies (mAbs) were produced with antigen derived from extracted wood blocks decayed with *P. placenta*. The extract was partially purified by gel chromatography prior to injection. Monoclonal antibodies were used to isolate hemicellulases of *P. placenta* with affinity chromatography.

The results from this work demonstrated that immunodiagnostic procedures are capable of detecting the presence of brown-rot organisms early in decay before weight loss has occurred (Clausen and others 1991, 1993) (Fig. 1). The different methods often gave different results.

Overall, agglutination assays for wood-decay antigens lacked the sensitivity of the other assays. Agglutination of coated latex beads showed increased sensitivity and reproducibility compared to coated charcoal particles. Strong cross-reactivity was seen with *C. coerulescens* in the charcoal agglutination test, but none of the non-brown-rot organisms cross-reacted in the latex particle assay. All non-brown-rot test organisms were incubated for 4 weeks; therefore, less cross-reactivity would have occurred during incipient colonization of *C. coerulescens*. Latex particles that were uniform and provided greater surface area for attachment of antibody were superior to charcoal. Despite 45 percent weight loss in some samples, only moderate agglutination reaction was observed for all of the test samples. Interpretation of results for any agglutination assay is subjective; experience is required to evaluate reactions and to avoid misinterpretation of slightly positive data. This assay would be a good presumptive field test if performed by qualified personnel.

The immuno-dot blot detected most brown-rot decay antigens, giving strong reactions in instances where weak or no agglutination occurred. *Postia placenta* gave no immuno-dot blot reaction but did agglutinate latex particles moderately. Non-brown-rot organisms did not react in the test. The immuno-dot blot procedure, requiring more time and skill, would provide an effective preliminary assay for qualitative determination of wood decay by brown-rot fungi.

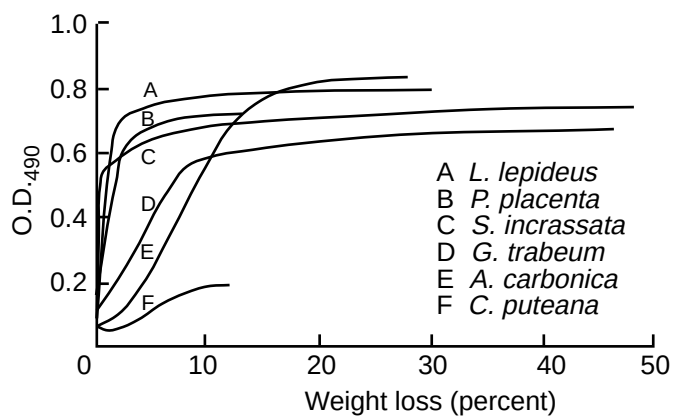

Figure 1—Weight loss of southern yellow pine wood blocks decayed by six brown-rot fungi compared to ELISA binding results of extracts prepared from the same decayed wood blocks. (A) *L. lepideus*, (B) *P. placenta*, (C) *S. incrassata*, (D) *G. trabeum*, (E) *A. carbonica*, (F) *C. puteana*. Data represent the average of three replications.

The ELISA assay was the most reliable quantitative test method and was very sensitive, picking up significant signals at 0 to 13.4 percent weight loss for five of the six test fungi. Interestingly, *P. placenta* was easily detected by ELISA and not detected at all by immuno-dot blot, even at 11.7 percent weight loss. Also, the organism that gave the strongest dot blot reaction, *C. puteana*, was the only organism that did not react in ELISA, even at 12.2 percent weight loss. No cross-reactivity was observed with the non-brown-rot organisms tested. The mAb followed binding patterns similar to polyclonal antibody for *P. placenta*. More importantly, the mAb followed the polyclonal binding patterns of the other brown-rot fungi tested, but did not react with the non-brown-rot isolates. This suggests a common antigenic epitope among the brown-rot fungi that is not shared with other groups of fungi; that is, white-rot, sapstain, or mold fungi.

In previous studies utilizing ELISA to detect *P. placenta*, the wood substrate had an inhibitory effect (Jellison and Goodell 1989). Our studies exhibited a high nonspecific background in ELISA in tests of wood extracts. Antigen from liquid culture was chosen in hope of alleviating the ELISA background problem, as well as determining the ability of liquid-culture-derived antisera to react to the same organisms grown on their natural substrate (wood). The nonspecific background was subsequently eliminated by preadsorption of the antisera to sawdust (Palfreyman and others 1988).

Even though the assays did not correlate with each other, they did compliment each other. ELISA was the most reliable quantitative method and agglutination the fastest presumptive test. Two of these assays, ELISA and latex beads, most effectively detected incipient decay of common brown-rot fungi at very low weight losses. The sensitivity, speed, and small sample size necessary to obtain data at very low weight losses may result in a practical application of these assays to forest products diagnostic procedures.

In-Place Preservative Treatment To Control Decay

The purpose of in-place or supplemental treatments is to prevent or arrest decay in existing structures. Such treatments can provide a safe, effective, and economical method for extending the service-life of wood. In-place treatments have seldom been used, partly because decay had not been detected until it had become visible or a member had weakened or failed to some degree. Early detection of decay by frequent and thorough inspections eliminates this problem. In-place treatments have been applied to some products, but their full potential has not been realized.

Two types of in-place treatment are commonly used: surface treatment and fumigants. Surface treatments are used to prevent infection of exposed wood, and fumigants are used to treat internal decay.

Surface Treatment

Although pressure treating provides maximum protection against decay according to American Wood-Preservers' Association standards, in-place surface treatments can reduce the incidence of decay in certain situations, as described in the introduction. The main structures that may need additional in-place treatment are utility poles, railroad ties, wharves, bridges, millwork of buildings, decking, and fencing. This type of treatment is effective when applied before decay is established. In-place surface treatment can be used for maintaining structures because of its ease of application and effectiveness as a toxic barrier to new infection. However, the shallow penetration of surface treatments limits their effectiveness for established internal decay; in these cases, fumigants are much more effective in arresting decay. Such treatments are not intended to substitute for pressure treatment of new wood.

In-place preservative treatments consist of various liquid or heavier grease-type preservative compounds. The effectiveness of surface treatment depends on the thoroughness of application, wood species, and moisture content of the wood at the time of treatment. Wet wood absorbs less preservative than does dry wood during brush or spray treatments.

More than 30 years ago, the Forest Products Laboratory (FPL) Biodeterioration of Wood Unit established tests of the efficacy of dip treatment of simulated building joints and platforms exposed in low-to-moderate decay hazard situations (that is, above ground) at sites in Gulfport, Mississippi, and Madison, Wisconsin. This work demonstrated the merit of surface application of preservatives to wood used above ground, whether by brushing, spraying, or a short period of soaking, which could be done at the construction site or in a routine maintenance program (Highley 1980, Highley and Scheffer 1993, Scheffer and Eslyn l978, 1982). Dismantlement of the samples at the Mississippi site showed that penta dips still afforded some protection after 20 years (Scheffer and Eslyn 1982). In the less severe decay climate of Wisconsin, such treatments have provided excellent protection of post-rail units for at least 34 years (Highley and Scheffer 1993). Surprisingly, painting has been an overriding factor that has controlled decay in the post-rail units. There has been no evidence that type of oil carrier or incorporation of a water repellent improves effectiveness of treatment. Three- or 15-min dips in penta have been equally effective. Penta-grease applied to unit ends only has been effective.

The need for similar information on new, environmentally safe chemicals led to establishment of new tests involving many chemicals and substantial numbers of different, commercially important woods. These tests are also being conducted above ground in Mississippi and Wisconsin. This work and, in fact, most of the studies on in-place treatments, have been financed in part by the U.S. Navy.

Highley (1990) compared the efficacy of several fungicides applied as brush treatments as alternatives to chlorinated phenols for protection of wood above ground. Cross-brace and L-joint test units, constructed of southern pine, Douglas-fir, maple, or red oak, were treated just before assembly and exposed outdoors from 5 to 12 years at two sites. After 12 years at the Wisconsin site, untreated pine and maple cross-brace units were severely decayed, red oak had only limited decay, and Douglas-fir had no decay. After 12 years at the Mississippi site, untreated pine, maple, and red oak cross-brace units were severely decayed, and Douglas-fir had no decay. The hardwood species were more difficult to protect from decay than were the softwood species. No treatment completely prevented decay in maple cross-brace units exposed for 9 to 12 years in Mississippi. Most treatments only slightly reduced the development of decay in red oak cross-brace units exposed in Mississippi; many treatments protected pine cross-brace units. The L-joint units were exposed for only 5 and 6 years at the Mississippi site, but they were more difficult to protect from decay than were the cross-brace units. Several alternative treatments were as effective or more effective than the chlorinated phenols in preventing decay in both types of test units.

The Biodeterioration of Wood Unit has conducted studies to reduce the incidence of decay in waterfront structures. Most waterfront structures are built of wood because of its low initial cost and permanence when properly treated. However, premature failures caused by decay in above-water portions can occur even though the wood has been pressure treated with preservatives (Highley and Scheffer 1989). Decks and large horizontal structural members (curbs, wales) are particularly prone to decay, often as a result of seasoning checks that expose untreated heartwood. Also, fender piles are frequently damaged by interior decay resulting from infection of untreated heartwood exposed when the pile top is cut to height. The problem is particularly severe with species such as Douglas-fir that contain large portions of heartwood, which are difficult to pressure treat.

In 1969, a study was initiated to determine the potential of (1) in-place treatment of deck planks with fungicides for preventing decay in untreated wood or pressure-treated wood with checks that penetrate the zone of preservative treatment and (2) cap and fungicidal treatments for protecting the cut tops of newly installed piling from decay. The results of this work showed that remarkably long service-life can be obtained by simple in-place treatments of decking and cut tops of piling (Fig. 2). With Douglas-fir, annual copious brushing of the upper surface of decking with various penta formulations and with fluor-chrome-arsenate-phenol (FCAP) in years 2 through 9 after construction protected the decking from decay for 20 years. Two supplementary treatments applied after construction in years 2 and 6 were less effective than the annual supplementary treatments. Nevertheless, supplementary treatment with sodium penta and water repellent, penta in mineral spirits plus water repellent, and

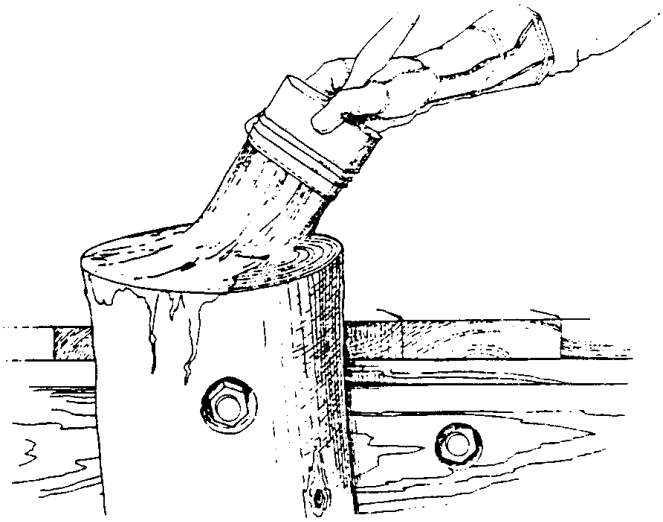

Figure 2—Application of fungicide to cut top of newly installed piling.

FCAP provided protection against decay in the Douglas-fir decking for 17 years. Addition of a water repellent to sodium penta improved protection of Douglas-fir decking somewhat. Scheffer and Eslyn (1978) also found that a water repellent added to penta improved protection moderately. Thus, retreatment of Douglas-fir decking about every 5 years appears to be a conservative schedule for long service-life. With southern pine, the penta treatments did not protect decking from decay, but annual flooding in years 2 through 9 with FCAP provided protection for 20 years. The longevity results from the better treatments for decking found in our study are similar to those found by Scheffer and Eslyn (1978) for penta-dipped floor panels.

The results of these studies lend encouragement to in-place treatment of wood items other than marina decking, especially porches and decks, as well as all platforms that are above ground.

For pile tops, all fungicidal treatments in combination with an intact cap offered excellent protection against decay for at least 20 years (Highley 1983, Highley and Scheffer 1991). Bituminous compounds proved best as capping materials because of their low cost and ease of application and repair. Because pile caps on working piers are often damaged or pulled off by hawsers, the pile top should be treated by flooding with a preservative before capping. A waterborne preservative, such as ammonium bifluoride or FCAP, is apparently the most effective. These chemicals remain inactive as long as the cap remains sound, but they are activated in the presence of moisture and move into checks. The preservative can also provide protection even when the pile cap remains intact. By penetrating the wood, the preservative can eliminate shallow decay below the surface caused by delay in covering the pile top.

Grease-type preservatives troweled onto the cut top of piling (pile cutoff) are also effective in preventing decay. The piling need not be capped. However, such preservatives would not be desirable in many situations because the slick piling top could result in accidents from slippage. The ease of application and cleanliness of waterborne preservatives make them preferable to oil-type preservatives.

Fumigant Treatment

Despite pressure treatment with a preservative, large timbers such as curbs, chocks, and wales can develop internal decay through deep checks that penetrate the treated shell; decay can also enter through cutoff ends. The problem is most serious with shallow-treated species such as Douglas-fir. Preservatives applied by ordinary flooding from a brush or spray penetrate the wood only slightly and so cannot stop decay. Fortunately, a type of preservative has been developed that can move through and permeate wood that cannot be conventionally treated. The chemicals, which migrate as gas, are called fumigants, and offer a widely useful means for eliminating deep-seated decay. Fumigants are applied in liquid or solid form in predrilled holes; they then volatilize into toxic gas that moves through the wood, eliminating decay fungi and insects (Fig. 3).

Several studies have demonstrated that fumigants can effectively eradicate important wood-decay fungi from within large, horizontally oriented southern pine, Douglas-fir, red oak, and white oak timbers (Eslyn and Highley 1985; Highley 1986, 1991; Highley and Eslyn 1981, 1989a, 1989b). These studies have also provided insight into how far fumigants move horizontally through timbers and how long they remain effective. Fumigants were less effective in untreated pine timbers than in treated Douglas-fir timbers. However, fumigant movement and persistence in southern pine was enhanced by wrapping the timbers in polyethylene after fumigation. Fumigants were also less effective in white oak than in red oak. Wrapping enhanced fumigant movement in both species, but persistence was only improved in red oak.

Of the many fumigants evaluated, chloropicrin moved the farthest from point of application and persisted in all timbers for the longest test period. Generally, metham sodium performed second best, followed by Basamid. Although methylisothiocyanate (MIT) is an excellent fumigant, it performed poorly in the pellet form used in our studies because it probably was lost from the pellets prior to treatment.

Antistain Treatment

Mold and sapstain fungi can rapidly discolor freshly sawn lumber before it is dried. Such discoloration can greatly reduce the value of the wood and make it unacceptable for certain uses. These fungi can also discolor unfinished, stained, or painted wood in-service, as long as its moisture content is above 20 percent. Mold and sapstain fungi have traditionally been controlled in the United States by the application of pentachlorophenol or its salt as a dip or brush treatment. The restriction of this compound, as a result of environmental concerns, has created a demand for new antistain chemicals. These compounds must exhibit broad-spectrum fungitoxicity, longevity of efficacy, environmental safety, low cost, solubility in water, and fixation to wood.

Many different compounds have been screened for their ability to inhibit mold and sapstain fungi. Chlorothalonil, a compound used to control many plant diseases in the United States, has been added to commercial preparations of captafol, folpet, and thiophanate methyl to increase mold and stain control in New Zealand. Chlorothalonil has also been reported to compare favorably with pentachlorophenol in preventing decay in laboratory soil-block tests and fungus cellar tests. We looked at this compound in combination with other fungicides in the laboratory (Micales and Highley 1989) and in field tests on hardwood and softwood species. Some chlorothalonil formulations were as effective or more effective than presently used antistain chemicals.

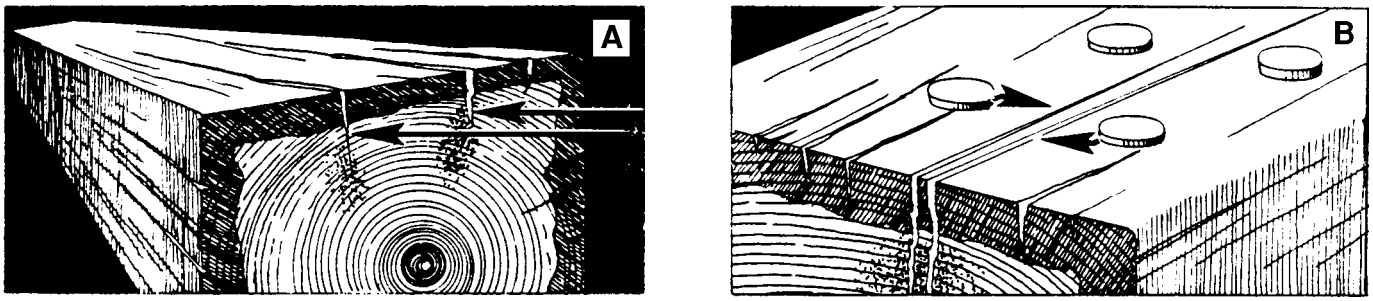

Figure 3—Deep seasoning checks can penetrate the shell of large pressure-treated exterior timbers: (A) internal decay caused by water trapped in checks, (B) plugged holes in timbers. Fumigant applied through holes stopped internal decay. Note that treating holes are located on both sides of deep checks.

Research Needs

Evaluation of Wood Degradation

Areas for research include immunodiagnostic detection of early decay, improved carpogenesis and basidiosporogenesis of brown- and white-rot fungi, and annual loss in wood products resulting from biological degradation.

- **Immunodiagnostic detection of early decay**
  Transferring immunocytochemical technological advances from the areas of agriculture and human and animal health is crucial for developing a marketable field test for detecting wood-decay fungi before advanced decay compromises the structural integrity of in-service wood. Double-antibody "sandwich" (DAS) ELISA utilizes monoclonal and polyclonal antibodies. Polyclonal antibodies increase sensitivity by increasing binding on the bottom of the sandwich, whereas monoclonal antibodies increase specificity for the desired antigen on the top of the sandwich. The advent of over-the-counter pregnancy test kits sparked a surge in single-use immunodiagnostic test kits. Two styles of the commonly marketed tests combine nitrocellulose, a stable high-capacity carrier, and latex beads, a lower-capacity carrier with low background binding (Porstmann and Kiessig 1992; Kemeny 1992). These tests are referred to as particle-capture ELISAs (Bangs 1990), and they perfectly meet the criteria of a field test for incipient brown-rot decay. We need to utilize this technology to increase sensitivity and simplicity for developing a marketable diagnostic kit for detecting incipient brown rot.

- **Carpogenesis and basidiosporogenesis of brown- and white-rot fungi**
  Better and faster laboratory techniques are needed for evaluating the durability of wood when used above ground. Basidiospores are the primary source of infection of such wood. However, mycelium inoculum is usually used in laboratory tests because basidiospores are difficult to obtain. Inhibition of basidiospore spore germination can involve different targets than does inhibition of growth (hyphal extension). Spore germination should be easier to inhibit than hyphal growth. If methods can be developed to provide an immediate source of basidiospore inoculum, they can be used to test wood durability. Results of such tests will provide a more accurate reflection of durability to be expected in wood above ground in the field.

- **Annual loss in wood products resulting from biological degradation**
  Data on annual loss of wood are essential for obtaining public and governmental support for wood deterioration and protection research, in amounts appropriate to the magnitude of the problem. Current figures on wood product losses resulting from the action of micro-organisms, insects, and marine animals are outdated. In addition, most of these data appear to be based upon an insufficient and/or unknown data base. To set research priorities and to obtain funding for this research, we need up-to-date and reliable data on losses encountered in different wood products as a result of attack by various biological agents. To compile such data will require information from economists and those involved in maintenance and repair of wood structures.

New Fungicides With Low Mammalian Toxicity

Research topics include control of mold and sapstain, evaluation of treatments for aboveground wood components, studies of decay rate, remedial treatments for decaying wood, and modification of structures to exclude moisture.

- **Mold and sapstain control**
  Chlorophenates, which were previously used to protect wood from mold and sapstain fungi, have been found to contain dioxins and consequently have been abandoned as antistain treatments. Many alternatives to chlorophenates are now commercially available, but none is completely satisfactory. The more environmentally acceptable materials tend to lack broad-spectrum fungitoxicity and longevity of efficacy. This problem is best overcome by using a combination of fungicides that collectively give a broad spectrum of fungitoxicity. Chemicals such as chlorothalonil, which have low mammalian toxicity and are not classified as hazardous waste, need further testing. Emphasis should be given to protection of hardwood species, which are often used for items such as furniture in which discoloration is not tolerated.

- **Treatment for aboveground wood components and rate of decay studies**
  We need to test new commercial formulations—with emphasis on environmental safety and low mammalian toxicity—under different climatic conditions and subject to varying microbial species to determine the least toxic treatment necessary to protect wood under given use conditions. Rate of decay studies are usually carried out in the laboratory using small pieces of wood. Decay studies need to be conducted, particularly above ground, on construction-sized timbers in different hazard climates.

- **Remedial treatments for decaying wood members**
  We have found that fumigants, such as chloropicrin, are very effective in eradicating decay fungi in large softwood and hardwood timbers. However, other fumigants that are less toxic and more easily applied than those previously investigated need to be evaluated for this purpose. In addition, further testing is needed for diffusable preservatives such as fluorides and borates.

- **Modification of structures to exclude moisture**
  Physical exclusion of moisture from wood will prevent decay. Often, simple structural modifications can accomplish this, such as extension of roof overhang and use of vapor barrier, flashing, sealants, and water repellents. New water-repellent treatments need to be tested on wood species with varying durability, which are exposed under varying climatic conditions.

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