Chapter 16

Concepts in the Development of New Accelerated Test Methods for Wood Decay

Darrel D. Nicholas[1] and Douglas Crawford[2]

[1]Department of Forest Products, Mississippi State University, Mississippi State, MS 39762–9820
[2]Forest Products Technologist, Forest Service, Forest Products Laboratory, U.S. Department of Agriculture, Madison, WI 53705–2398

Abstract

Efforts to develop new environmentally friendly wood preservatives are seriously handicapped by the extended time period required to carry out the evaluation needed to establish confidence in the long term performance of new preservative systems. Studies in our laboratory have shown that using strength loss as a measure of the extent of wood decay makes it possible to detect the early stages of decay that results from non-enzymatic reactions. We have developed specialized equipment and techniques that have applications for both above ground and soil contact preservative systems. By coupling these evaluation techniques with a better understanding of moisture control, microbial succession, soil chemistry and soil microbial dynamics, it may be possible to develop improved test methods that can greatly reduce the time required to evaluate wood preservative systems.

Introduction

The development of new or modified wood preservative systems is severely hampered because of inadequate test methods. Although current methods for evaluating the efficacy of wood preservatives provide valid data, the time required to obtain definitive results is too long because reliable decisions on efficacy against wood decay organisms must rely on long-term field test data. In order to reduce the test time, it will be necessary to develop reliable laboratory and field tests that optimize the wood decay process. However, this goal will not be realized until we develop a better understanding of the many variables that influence microbial decay rates, develop improved methods for detecting and quantifying the extent of wood decay and couple these developments with improved designs for test specimens and methods. The factors which affect decay rates are reviewed in this chapter. The variables affecting decay rates are briefly reviewed in this chapter in order to provide the necessary background needed before decay test methodology can be addressed. Following this, detailed suggestions on research needed to develop reliable accelerated wood decay test methods that are critical to the future of the wood preserving industry are presented.

Variables Influencing Microbial Decay Rates

A basic understanding of the many variables associated with microbial decay rates is needed before serious consideration can be given to development of accelerated decay tests. Accordingly, the material presented in this section is presented in order to provide this background.

Water and Oxygen

Water is essential for the growth of wood decay fungi. It serves as a solvent for metabolic processes, for the transport of metabolites, enzymes, and organelles, is a vital skeletal ingredient and is the driving force behind extension growth *(1)*. It is generally accepted that a minimal wood moisture content (MC) at or above the fiber saturation point is needed to support the wood decay processes. At lower levels there is insufficient water to meet metabolic requirements. Furthermore, the MC of wood plays a major role in determining which fungal species colonize wood and the extent to which they utilize this substrate.

The optimal MC for growth of many of the wood decaying basidiomycetes is unknown, but for those that have been studied it ranges between 40 and 80% *(2)*. The reason that optimum decay rates are associated with this MC range is

mainly due to the oxygen requirements for these fungi. At higher MC levels, aeration is reduced and this impacts fungal growth. Rypacek *(3)* studied wood decay in sticks that had MC gradients from one end to the other and found that decay was inhibited when the amount of air in the wood was less than about 10-20% of the void volume. Other studies *(2)* support this conclusion, thus it is generally considered that 20% residual air volume in wood is close to the minimum level needed for basidiomycete wood decay fungi. Since the void volume of wood varies inversely with specific gravity, the MC levels that inhibit fungal growth are considerably higher in lower density wood,

When wood is subjected to exterior exposure it undergoes alternate wetting and drying which can impact the survival and growth rate of fungi inhabiting the wood, but very little information is available on this subject. With the exception of fully saturated wood it does not appear that high MC levels affect the survival of most basidiomycetes *(2).* At MC levels below fiber saturation, prolonged periods of low MC levels appear to have a significant effect on the survival of some fungi. For example, Scheffer and Chidester *(4)* showed that when wood was stored at a MC of 12% seven of eight sapstain fungi present died within 7 months. On the other hand, most of the wood decay fungi such as *Postia placenta, Gloeophyllum trabeum,* and *Gloeophyllum sepiarium* were still alive after 3 years. One exception was *Meruliporia (Poria) incrassata*, a water-conducting fungus that inhabits damp wood structures, which died within 25 days.

In general, soft-rot fungi have a greater tolerance for low oxygen concentration than basidiomycete decay fungi, and as a result they can decay wood that is saturated with water. This explains why these fungi are associated with decay in cooling tower slats and similar products maintained at high MC levels. The influence of low oxygen tolerance by soft-rot fungi is also evident in soils, When soils are maintained at a relatively high MC, basidiomycetes are inhibited and soft-rot fungi become the major wood-degrading microorganisms. This has recently been demonstrated by the junior author when it was shown that only soft-rot fungi were present in a soil bed containing soil with a water holding capacity of 34.1%, determined by AWPA Std. E-10 *(5)* with the soil maintained at a MC of 29% (oven-dry basis) and a corresponding wood MC of approximately 96%. Fungi that were isolated from the soil in this study included species from the genera Fusarium, Penicillium, Phialemonium, Curvularia, Exophiala and Geotrichum. Although all of these genera are common soil-dwellers, many of these species also have soft-rotting capabilities *(6).* This knowledge is useful in conducting soil contact tests by making it possible to screen for either soft-rot or basidiomycete decay based on selection of the appropriate soil MC.

In decay tests that subject wood to soil contact, the MC of the wood and soil are both important because it influences the microbial populations and decay rates. In order to optimize wood decay by basidiomycete fungi the wood MC

should be in the range of approximately 40-80%. However, a linear correlation between the soil and wood MC does not exist. As the soil MC is increased, wood shows a corresponding increase up to a certain level after which the wood MC increases very sharply until it levels off around 100%. The significance of this relationship is discussed in more detail in the section on soil contact decay tests.

Temperature

The effects of temperature on the growth rate of fungi have been studied extensively. Most wood decay fungi show optimal growth between 12 to 40°C *(2)*. At the lower and higher extremes of this range growth is either inhibited or sharply retarded. The temperature optimum for fungal growth varies widely within species as well as among species, so it is not possible to select a single temperature that will maximize decay rates. For most common wood-decay fungi studied the optimum temperature for growth, which is correlated to decay rate, appears to be in the range of 20 to 36°C. For the fungi generally used in laboratory decay tests, the optimum temperature for decay ranges from 28 to 36°C.

The fact that some fungi are consistently associated with certain exposures of wood products may be at least partially associated with temperature optima *(2)*. An example is the predominance of *G. trabeum* and G. sepiarium in the exterior wood of various structures which attain fairly high temperatures during the summer months.

Hydrogen Ion Concentration

The pH of wood has an effect on the growth of fungi. In general, wood decay fungi grow best within the pH range of 3 to 6; whereas many bacteria and actinomycetes grow best at a more neutral pH. Brown-rot fungi prefer more acidic conditions than white-rot fungi and have an optimum pH of around 3 *(2)*. In general, the pH of wood decreases during the decay process.

Nutrient Requirements of Fungi

In addition to the carbon compounds supplied by wood, fungi require the nutrients and vitamins development and growth (Table 1). Of these nutrients, nitrogen is unquestionably the most important because substantial amounts are required for the synthesis of proteins and other cell constituents *(7)*. Phosphorous is also very important because a deficiency of this element has

been shown to limit the uptake of nitrogen by fungi *(1).* Wood contains some nitrogen, which originates in the cytoplasm of cambial cells that differentiate and mature into woody plant tissue. The nitrogen is located primarily in the living parenchyma cells *(8).* In trees, the nitrogen content decreases from the cambium to the heartwood and then remains constant.

Table I. Nutrient and vitamin requirements of wood decay fungi *(2,9)*

MajorElements	***MinorElements***	***Vitamins***
Nitrogen	Iron	Thiamine (B1)
Phosphorus	Zinc	Biotin
Potassium	Copper	
Magnesium	Manganese	
Sulfur	Molybdenum	
Calcium		

The total amount of nitrogen present in wood is quite variable within as well as among wood species, ranging from 0.038-0.227% w/w in softwoods and from 0.051-0.106% w/w in hardwoods *(8).* It also appears that the nitrogen content varies within individual growth rings. In this regard, it has been shown that for *Pinus sylvestris* the level of nitrogen in the springwood was 1.5 to 2 times greater than in the summerwood. Other studies with both softwoods and hardwoods suggest that higher levels of nitrogen are common in the springwood compared to summerwood *(7).* Nitrogen levels are extremely low in relation to carbon content, with C:N ratios ranging from approximately 250:1 to 1250:1 *(1).*

A number of studies have demonstrated that the nitrogen level influences the rate of wood decay. For example, Merrill and Cowling *(8)* showed in lab tests that the decay rate by *G. trabeum* and *Trametes versicolor* was twice as great in wood from the outer rings that contained 0.149% nitrogen as compared to that from the inner rings that contained 0.044% nitrogen. Levi and Cowling (IO) obtained similar results for the same fungi with oak wood. In another study, the decay rate by soft-rot fungi could be accelerated 5- to 10-fold by pre-treating wood with 2% casein hydrolysate *(11).* It has also been shown that the addition of nitrogen to agar media, soil, and expanded mica, or use of soils naturally high in nitrogen, resulted in greater weight loss of wood exposed to fungi growing on these media (8). Further substantiation of the importance of nitrogen levels in wood on the degradation by fungi is provided by studies that have shown that when nitrogen is extracted from wood it is less susceptible to decay *(8).*

The type of wood decay that occurs in soil contact appears to be related to the nitrogen content of wood. For example, King *et al. (12)* showed that soft-rot fungi require higher levels of nitrogen than basidiomycetes to decay wood.

Accordingly, they suggested that a nitrogen threshold level of approximately 0.2% w/w is required before decay of wood in soil contact by soft-rot fungi assumes significant proportions. Furthermore, it was demonstrated that the nitrogen levels of wood increase by up to 600%, based on the original pre-burial weight of the wood, during decay by soft-rot fungi in soil contact. This accumulation of nitrogen during the decay process by soft-rot fungi is in sharp contrast to pure culture basidiomycete decay where the nitrogen levels remain relatively constant during the decay process. King *et al. (12)* also found that failure of wood exposed to soils that have high nitrogen levels is invariably caused by soft-rot fungi.

Decay fungi are able to effectively degrade wood despite the relatively low levels of nitrogen in wood. This is somewhat surprising but can be explained by a number of unique mechanisms that wood decay fungi employ to overcome the nitrogen deficiency. Fungi are able to conserve nitrogen by hyphal autolysis and recycling nitrogen *(7).* Wood decay fungi can also parasitize the hyphae of some ascomycetes and Fungi Imperfecti as well as bacterial cells *(10, 13).* This may explain why there is often a close relationship between wood stain fungi, bacteria, and wood destroying basidiomycetes. Wood decay fungi are also able to tranlocate nitrogen and other nutrients from external substrates such as soil *(14).* Some species of bacteria that colonize wood may exhibit nitrogenase activity and are therefore able to fix atmospheric nitrogen *(15,16).* Consequently, invasion of wood by these bacteria would result in elevated nitrogen levels by fixation as well as by the presence of the bacterial cells. When wood is placed in contact with soil, the movement of water into the wood transports nitrogen as well as other nutrients *(17).*

Microbial Colonization of Wood

Because of its cellular structure, wood can be readily colonized by a variety of microorganisms. The overall process is a dynamic one in which the nature of the microenvironment continually changes. A number of studies suggest that the general sequence of wood colonization by microorganisms, either in above-ground or soil contact exposure, is: bacteria ¦ stain-fungi - soft-rot fungi ¦ basidiomycetes *(18-20).* The time required to complete this colonization sequence is variable and depends on a number of factors such as wood species *(21)* and treatment with wood preservatives. Carey *(22)* found that the colonization sequence in untreated wooden joinery was completed in 71 days. Basidiomycetes were not detected in wood treated with either tributyltin oxide or pentachlorophenol until after 221 and 730 days, respectively. However, colonization and detection of visible decay are two different parameters. Our observations indicate that even in high decay hazard climates decay in coniferous sapwood test samples exposed above ground is not evident before six

months of exposure and in some pieces the lag period may extend to 24 months. Obviously, some inhibitory factors are involved since in a pure culture test decay is apparent within a few days.

The delay in colonization of untreated wood by basidiomycetes may be partially due to antagonism expressed by some of the microorganisms. For example, a number of studies have shown that actinomycetes and species of Eubacteria are highly antagonistic toward wood degrading basidiomycetes *(23).* These bacteria also degrade wood extractives that affect colonization by basidiomycetes. Removal or alteration of these extractives eventually permits rapid colonization by wood decay fungi. However, Hulme and Shields *(24)* suggested that the major factor involved in repression of the basidiomycetes is the rapid depletion of nutrients that are required for rapid growth and successful colonization. Although some of the microorganisms that colonize wood in the early stages are antagonistic to basidiomycetes, others have the opposite effect *(25, 28).* For example, some bacteria develop a synergistic relationship with wood decay fungi *(23).* This synergism stimulates fungal growth and accelerates the rate of woad decay. In this relationship, bacteria are provided with a supply of carbon sources as a result of the extracellular enzyme reactions of the fungus that would not normally be available. Growth of the bacteria depletes concentrations of cellobiose, simple sugars, etc., around the hyphae so that cellulase production is not repressed. In turn, bacteria may supply essential vitamins and other growth promoting substances such as nitrogen to the fungus.

Another aspect of early colonization by actinomycetes, ascomycetes, and Fungi Imperfecti is the ability of some of those microorganisms to detoxify wood preservatives. Merrill and French *(26)* showed that ascomycetes and Fungi Imperfecti detoxified a number of wood preservatives. Carey (18) has shown that a *Phiolophora species* isolated from TBTO-treated wood effectively detoxified this preservative when it was incorporated into agar tests. In another study by King *et al. (27),* it was noted that actinomycetes isolated from treated wood have the ability to biodegrade pesticides in soil. Hence, a number of microorganisms that colonize wood have the potential to detoxify wood preservative chemicals. Such detoxification could then set the stage for wood degradation by basidiomycetes.

Spore Germination

It is generally assumed that fungal spores are the principal infection mode of wood exposed above ground under conditions conducive to decay. Although the presence of spores produced by microorganisms capable of degrading wood are ubiquitous, their germination on wood is problematic *(29, 30).* In order for fungal spores to develop and colonize wood, there must be a sufficient supply of readily assimilable nutrients, a favorable microclimate, including adequate

moisture, and an absence of competitors. Much of the time these conditions do not exist simultaneously so the mere presence of spores does not necessarily ensure that the wood decay process will be initiated. The failure of spores to germinate when they are in contact with wood may be attributed to nutrient and water availability, pH, temperature, wood extractives, and microbial metabolites, etc. *(1).* Unfortunately, only a limited number of studies have been conducted in this area so our understanding of this complex subject is limited.

Morton and French *(31)* studied spore germination with *G. trabeum, G. sepiarium,* and *Fomitopsis roseus* on wood and found that sterilizing wood significantly increased the percentage germination of *G. trabeum* basidiospores, with a greater percentage germinating on wood that was autoclaved dry rather than wet. Furthermore, when wood was subjected to either hot or cold water extraction, the percentage of *F. rosea* spores that germinated was significantly less than that for non-extracted wood. This difference suggests that some water-soluble extractives in Douglas-fir sapwood stimulate germination. In additional experiments, they compared the effect of sodium pentachlorophenate-treated wood on germination of *G. trabeum* basidiospores to the growth of mycelium from the same fungus. From this experiment it was concluded that the basidiospores were about 10-fold more sensitive to the biocide than was the mycelium.

Schmidt and French *(30)* studied the effect of sterilization methods on spore germination of *G. trabeum, Trametes hispida,* and *Poria tenuis* and found that some methods severely retarded germination. They concluded that the best way to minimize variation in spore germination was to briefly dip the wood in boiling water.

One of the problems with spore germination studies is that a given fungus can produce several different types of spores. For example there are basidiospores, chlamydospores, and secondary spores (conidia). These different types of spores may have varying requirements for germination, so experiments with only one type of spore may not apply to the others. A good example of this are the thick-walled resting spores of the white-rot fungus *Ganoderma lucidum* which must pass through a fly larvae gut before they will germinate *(1).*

Test Methodology

Methods of Detecting and Measuring Wood Decay

At the present time, lack of accurate, rapid, non-destructive methods for detecting and quantifying wood decay is a major deterrent to wood preservation research. It will be necessary to develop improved methods for measuring the extent of wood decay in test samples before accelerated test methods can

become a reality. A partial review of current methods and new approaches that may have potential are outlined in this section.

Visual Observation

At present, visual observation is the method normally used to evaluate outdoor exposure wood decay test specimens exposed outdoors. This method is subjective, rather than quantitative, does not detect early decay and consequently (does not provide the information needed for accelerated testing.

Mass Loss

During the decay process, fungi utilize wood as a carbon source and produce CO_2 and water as by-products. As a result, the wood mass is gradually reduced. This can be measured at specific times or progressively by determining the weight of test specimens and comparing this with the samples original weight. This method is widely used in laboratory soil block decay tests and provides reasonably good results. Shortcomings of this method are associated with difficulty in making adjustments for variation in wood moisture content, loss of wood preservatives and inability to make adjustments for the weight gain due to colonization by the fungus.

Because of these problems, the use of mass loss as a method for detecting decay is essentially limited to laboratory soil block tests. When used for this application, the main weakness of this test is its inability to detect the early stages of decay. It is generally conceded that mass losses of less than 2-3% are statistically insignificant because of the inherent variability of this method. Even small mass losses in the early stages of decay are associated with significant strength losses, especially when the wood is colonized by brown-rot fungi *(32)*. For example, it has been shown that the compression strength of wood has been reduced by at least 20% by the time a mass loss of 2-3% has been attained *(33)*.

Mechanical Properties

The mechanical properties of wood are adversely affected by wood decay fungi, so that the progressive measurement of selected strength properties could be used to monitor the wood decay process. This supposition has been verified in a number of studies and appears to have considerable potential *(33)*. Although there are a number of strength properties that can be measured,

compression, bending, and torsion strength are perhaps the most suitable for detecting and measuring decay in laboratory and field tests.

Compression Strength

Recent studies demonstrate that the extent of wood decay can be quantified by measuring the compression strength of wood wafers in the radial direction *(33, 34)*. This method is based on the comparative compression strength at the 5% level of end matched wafers, one of which is exposed to the fungus in a soil block test and the other serving as a non-exposed control. The use of thin wafers (5 mm in the longitudinal direction) and compression strength analysis has made it possible to carry out tests in approximately 6 weeks, compared to approximately 16 weeks necessary for the standard soil block test utilizing mass loss.

Janzen's study *(33)* also indicates that it may be desirable to use modulus of elasticity (MOE) rather than 5% compression strength as a measure of wood decay. Use of MOE results in less variability among samples and appears to provide a better estimate of toxic threshold values.

Bending Stiffness

MOE of wood decreases during the wood decay process *(32)*. As a result, progressive measurement of bending stiffness is a potential method for following the decay process by determining the initial MOE at strain levels below the proportional limit and then making subsequent measurements. Crawford *(35)* used laboratory soft-rot tests to show that there is a good correlation between the reduction of MOE in thin wood sticks and the number of soft-rot cavities. Hence, this is an excellent method for evaluating soft-rot decay test samples. Although detailed studies have not been carried out, it appears that changes in bending stiffness could be used to measure the extent of decay in test specimens that have been attacked by basidiomycetes. This concept has been evaluated in preliminary studies in our laboratory and the results obtained to date are encouraging.

Torsion Strength

The torsional strength of wood is often used to measure the shear strength. As such, it is conceivable that torsional shear might be used to measure the extent of decay in test specimens exposed to wood decay fungi. We have initiated some studies in our lab evaluating the use of torsion strength as a

measure of wood decay. In this process, the sticks (14 mm square by 200 mm long) are torqued at a level below the proportional limit to establish the initial torsion strength. The samples are then exposed to wet, unsterile soil in special tube containers that leave 50 mm of the stick ends exposed to air. These sticks are then removed periodically and re-tested, with reductions in torsion strength serving as a measure of decay. Additional work needs to be done, but this method shows promise for accelerated soil bed tests.

Permeability

Carey *(36)* showed that the permeability of wood exposed above-ground increased during the early stages of decay. This increase is due to the colonization of wood by bacteria and various stain and soft-rot fungi. As a result of this observation it was suggested that measurement of permeability could be used to detect incipient decay. One method of doing this is to measure the uptake of decalin when the samples are dipped in this low viscosity liquid. However, due to the extreme variability of wood permeability, this method has not been very successful. Nevertheless, if better methods of measuring the permeability of wood could be developed then this method might have some potential. In this regard, one possibility is the use of the air pressure method developed by Wallace and Schmidt *(37).*

Immunodiagnosis

In this method, polyclonal and monoclonal antibodies are used to detect the presence of wood decay fungi (See chapter by Clausen). This method is very sensitive and has the potential to detect incipient stages of decay in above-ground test samples. One limitation of this method is the inability to quantify the progressive development of wood decay. As a consequence, it appears necessary to couple this procedure with some other method, such as bending stiffness, to provide adequate evaluation of test samples.

NIR Analysis

In recent years near infrared (NIR) spectroscopy has been used for many applications that involve rapid analysis of various substrates. Preliminary studies with decayed wood indicate that NIR may be useful in detecting and quantifying biodeterioration of wood. By coupling NIR analysis with multivariate regression or principal components analysis, it has been demonstrated that there is a good correlation between the NIR analysis and mass

loss of wood decayed by brown-rot fungi *(38)*. On the basis of additional preliminary studies, there also appears to be a good relationship between NIR analysis and changes in compression strength of brown-rotted wood.

Additional studies will need to be conducted before the potential of this spectroscopic method can be discerned. So far, only wood degraded by brown-rot fungi has been investigated and additional studies with wood decayed by white-rot and soft-rot fungi are needed since these microorganisms are also associated with the wood degradation process. Furthermore, it is not known whether this method can detect the early stages of decay, so additional work is also needed in this area.

The availability of lightweight, portable NIR instruments makes this potential method of decay detection particularly attractive. Furthermore, the fact that these instruments can be fitted with fiber optic probes provides the versatility needed for on-site evaluation of various test sample configurations.

Soil Block Test

In the evaluation of new wood preservatives, the soil block test is typically used to provide initial information on the efficacy against wood decay fungi. Both white- and brown-rot fungi are generally included in this evaluation. The toxic threshold value, i.e., the minimum amount of preservative that inhibits wood decay, is determined in this test by using test specimens that are treated with varying levels of the preservative in question.

The standard soil block test (AWPA E 10) specifies that either 14 mm or 19 mm wood cubes can be used and the presence of decay is determined by mass loss *(5)*. In order to have a valid test the untreated control blocks must attain a minimum of 50% mass loss, which requires about 12 weeks exposure for brown-rot fungi. By using 14 mm cubes the rate of decay is increased and the incubation time can be reduced from 12 to 8 weeks. However, when softwoods are tested against white-rot fungi the exposure time needs to be increased in order to achieve sufficient decay in the untreated blocks. Using this standard method, the total time required to run the test is in the range of three to four months.

Another approach to measuring the extent of decay in the soil block test is to use compression strength, which decreases in proportion to mass loss during wood decay *(39)*. The advantage of using compression strength is that it permits the use of smaller samples without sacrificing accuracy. By using wood samples measuring 5mm thick it is possible to reduce the incubation time to approximately four weeks. Furthermore, the conditioning period required to equilibrate the test samples to a specified moisture content is eliminated *(34)*. As a consequence, the total time required to carry out the test can be reduced to approximately six weeks.

Although the soil block test is fairly rapid, it has a number of limitations. Because it uses a pure culture of a given fungus growing under near ideal conditions, the test is more severe than natural (outdoor exposure) conditions would be, where colonization of the wood is dependent on spore germination and decay fungi must compete against each other as well as other microorganisms. There are also numerous fungi that can decay wood and these can exhibit a wide range of tolerance to different biocides. The virulence of fungi are also dependent on which strain is being used and this can vary with the extent of subculturing, the type of wood, the type of soil, etc. *(40- 42).* Finally, most organic biocides are susceptible to microbial or chemical degradation and this effect is less apparent in the artificial short term lab decay test. As a result of these weaknesses, the soil block test results are best used as an initial screen to compare a given candidate preservative to known preservatives in order to determine whether or not further evaluation in field trials is justified.

When wood is used in exterior above-ground applications, the decay process is primarily initiated by spore germination. This suggests that it might be possible to use spore germination to establish toxic threshold values of candidate preservatives. Morton and French *(31)* carried out a series of experiments to evaluate this concept and concluded that such a test method might be feasible. This concept was subjected to further evaluation by Savory and Cary *(43)* using basidiospores of *G. trabeum.* The toxic threshold values for tributyltin oxide, pentachlorophenol and chromated copper arsenate, determined by inoculation with mycelium and also by spores, were found to be essentially the same. Furthermore, it was found that spores would germinate at some preservative concentrations but failed to colonize the wood. Hence, the concept of using spore germination as a rapid method for establishing toxic threshold values for wood preservatives may not be possible. However, it might be possible to monitor spore germination and further hyphal development for a relatively simple and reasonably rapid test method. Additional research in this area would be desirable.

Above-ground Decay Tests

These tests are carried out to evaluate wood preservative systems that are designed for treated wood used in exterior applications where the product is not subjected to soil contact. Typical applications are millwork, decking, fascia boards, etc. A number of different designs have been used over the years with the most popular being the post-rail, L-joint, and Lap-joint units. In recent years the L-joint and Lap-joint designs have been used for most tests (Figure 1).

A common element in the design of these test units is the inclusion of some type of joint that effectively traps rainwater. Although these units provide a realistic evaluation of the performance of wood preservative treatments, an

extended exposure period, with the time being dependent on the rainfall and temperature at the test site, is needed to develop a reliable prediction of preservative performance. Also, the use of visual observation and probing techniques in the evaluation process makes it difficult to detect incipient decay and provide quantitative data, and the ratings are often dependent on the moisture content of the sample at the time of evaluation.

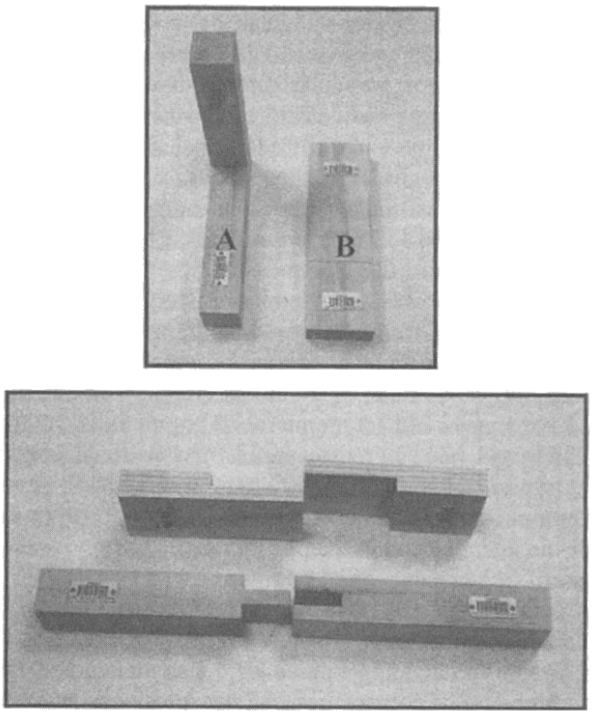

Figure 1. Top and side view illustrations of L-Joint (A) and Lap-Joint (B) above ground test units.

One of the major weaknesses of the current above-ground test unit procedures is the extended time required to evaluate a given preservative. In order to address this problem two concepts need to be examined. These are the development of methods for accelerating the decay process and methods that accurately detect incipient decay and subsequent progressive deterioration of the wood structure.

Accelerating Decay Rates

Optimizing decay rates requires developing a better understanding of the spore germination process as well as providing appropriate nutrient, moisture and temperature levels. Factors influencing spore germination on wood have been discussed in detail in earlier sections of this chapter. From this review, it is apparent that considerably more research is required to permit the development of methods that optimize spore germination on wood. Such information is critical in our quest to develop accelerated test methods.

With regard to nutrients, it seems logical that providing appropriate levels of the essential elements for fungal growth will be necessary to accelerate decay test methods. Nitrogen is of particular interest because wood is inherently deficient in this element. Since autolysis of other microorganisms and possibly airborne particles are the only source of additional nitrogen in wood exposed above-ground, serious thought needs to be given to injecting nitrogen compounds into the wood test units. The inclusion of other elements, such as phosphorus which is essential for nitrogen utilization by fungi, also needs to be considered. Fungi also require the vitamins thiamine and biotin which are not present in wood and are probably provided by bacteria that colonize wood prior to basidiomycete attack *(13).* Hence, it would be of interest to see if the addition of vitamins would accelerate the colonization by basidiomycetes. Although the above suggestions may be of value, it should be emphasized that the addition of nutrients could have a major impact on microbial populations and a considerable amount of research will be required to ensure that artificial condition are not created that result in unrealistic test conditions. Great care will need to be taken to ensure that these modifications do not accelerate decay rates in a manner that is unrealistic for the intended end use applications.

As discussed previously, the optimum wood MC for decay by basidiomycetes is in the range of 40-80%. Consequently, developing a system that would maintain wood at these moisture levels should accelerate the decay process as long as appropriate temperatures were also maintained. One approach to maintaining a high MC of non-soil contact test specimens is to place them on concrete blocks that rest on the ground. Further reduction in moisture loss is achieved by covering the test unit with shade cloth, which allows rainwater to enter but reduces evaporation rates. This test method has been used successfully in Hilo, Hawaii to accelerate rates of biodeterioration *(44).*

By giving consideration to the above factors it should be possible to significantly accelerate the wood decay process in above-ground tests. This approach must be rigorously evaluated to ensure that unrealistic artificial conditions are not created so that the results reflect real world conditions. Accordingly, the accelerated method must mimic the results obtained from actual long-term exposure.

An indication of the validity of this approach is provided by our experience with conducting above-ground decay tests in Hilo, Hawaii and Saucier, Mississippi. The Hilo location has ideal temperature and rainfall conditions to support wood decay. In contrast, the Saucier location has considerably less rainfall and lower temperatures in the winter. Our results from testing the same wood treatments at both sites clearly show that the rate of decay is accelerated 2-3 fold at the Hilo test site. Figure 2 shows decay ratings over a 10-year period for dip-treated Ponderosa pine L-joints. Clearly, this represents a significant acceleration. Nevertheless, it may be possible to further accelerate the decay rate by incorporating appropriate nutrients in the test specimens as long as this can be done without creating unrealistic decay conditions that do not apply to real world applications.

In addition to accelerating decay rates, improved methods for detecting and measuring the extent of decay are needed. Methods that appear to have the best potential for above ground tests are based on mechanical tests, NR analyses and immunodiagnosis. A conceptual test unit for using a combination of immunodiagnosis and mechanical test is shown in Figure 3. This test utilizes a typical lap-joint with thin sticks incorporated in the joint. The immunoassay will be used to detect the onset of colonization by decay fungi by periodically removing small samples near the ends of the sticks. In addition, the sticks will be removed periodically and subjected to a bending test to measure the MOE, which decreases as decay develops.

If additional research confirms that both brown- and white-rot decay can be detected and quantified by NIR analyses, it would be a valuable tool for use in above ground decay tests. Portable NIR units are available and it may be possible to measure decay in lap-joints as well or other test units by using small fiber optic probes.

Soil Contact Decay Tests

Treated wood products are often used in applications that involve soil contact. This results in a very severe exposure condition for a number of reasons. Soil contains large populations of microorganisms, some of which can biodegrade wood. A number of these microorganisms also have the ability to biodegrade organic wood preservatives while others can modify inorganic preservatives and make them more susceptible to leaching. Soil contains numerous chemical components that provide essential nutrients required by the fungi and also have an impact of the rate of depletion of wood preservatives. The leaching of wood preservatives is generally more severe when the wood is

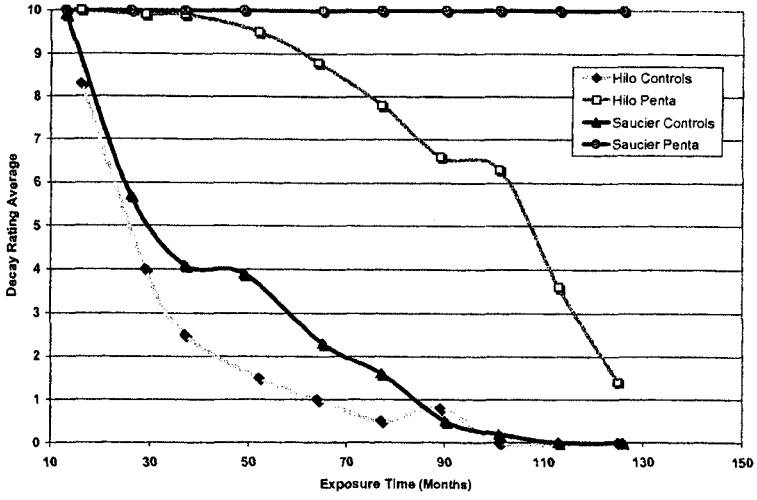

Figure 2. Comparative decay rating for ponderosa pine L-joints after exposure at Hilo, Hawaii and Saucier, Mississippi. The test samples were dip treated with 5% Penta in mineral spirits and the controls were dip treated with escorez resin (5%) in mineral spirits. A 10 rating denotes no decay and a 0 denotes failure.

exposed to water that contains soil than to water alone. Soil also serves as a reservoir for water, which helps maintain wood MC levels in the range that favors wood decay.

In testing wood preservatives for soil contact applications, treated test specimens of various sizes ranging from ¾ inch square by 18 inches long to full size pole stubs are inserted to one-half their length into pre-drilled holes in the ground at selected test sites. These test specimens are inspected periodically and rated for the extent of decay and insect attack. These are generally long-term tests that require a minimum of five years of exposure of smaller test specimens in locations that have relatively high decay hazard conditions. Although the small test specimens decay faster, they do not provide information on potential preservative distribution problems and may exacerbate preservative depletion rates.

One method for accelerating the decay rate when wood is subjected to soil contact is to control the soil moisture content and temperature conditions so that they are optimum for wood decay fungi. This can be done either by selecting test sites that have warm/wet climates or by the use of soil beds that are artificially watered and housed in buildings that have temperature and humidity

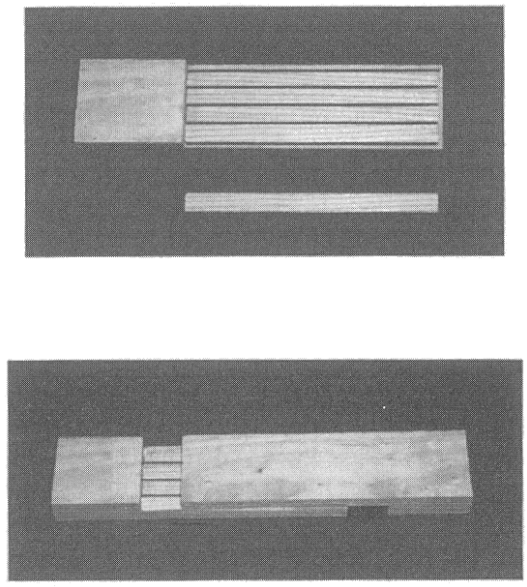

Figure 3. The modified Lap-joint designed to incorporate bending strength and immunoassay to detect and measure decay.

controls. This concept was originally developed by Gersonde and Becker *(45)* and further refined by others *(44,46-48).* On the basis of these studies and additional work by Archer *(49),* a soil bed test procedure was developed (5). The use of this accelerated method to predict long-term performance of treated wood exposed to soil has not been widely accepted and long term field tests are still required before one can have confidence in the performance of new wood preservative systems. Some concerns about this method are discussed in recent publications *(49, 50).* The basic problems with this concept are associated with difficulty in maintaining consistent moisture levels and failure to recognize the role that soil physical and chemical properties play in wood preservative depletion rates and microorganism populations. Another major problem is the lack of accurate methods for detecting and quantifying the extent of decay in test specimens.

Moisture Control

Most soil beds are operated on the basis of controlling moisture at some specified MC in relation to the water holding capacity of the soil. Accurate

control of soil MC is extremely difficult and wide variations generally exist in the soil beds, which are often overlooked. Nevertheless, even if adequate soil MC control is achieved this approach is often flawed because it fails to take into consideration the soil/wood MC relationships. Wood MC is critical because basidiomycete fungi are most active when the wood MC (oven dry basis) is in the range of 40 to 80%. Recent studies in our laboratory on the MC of wood in contact with soil shows that the relationship between soil MC and wood MC is not linear (Figure 4). From this graph it is apparent that there is a sharp rise in wood MC when the soil MC reaches about 22%. This steep rise then levels off when the soil MC reaches about 25%. The soil used in this study had a water holding capacity of 34.6%. We have observed similar relationships for other soils, but the inflection point in the curve occurs at widely different soil moisture contents. Hence, it is important that soil/wood MC relationships be developed for wood samples used in soil bed tests. Although the MC of some areas of the stakes - at ground line and slightly above – may be in the desired range the soil MC is still important because it can influence the type and number of microorganism present.

Physical and Chemical Properties of Soils

Often little consideration has been given to the potential impact of soil types, chemical composition and soil collection methods on the results obtained in soil beds despite their potential impact on biocide depletion, microbial populations and fungal virulence. Biocide depletion rates are an important factor in soil bed tests because of the relatively small test specimens and consistently high MC levels. A recent study has shown that wood preservative depletion rates vary widely in different types of soils *(51)*, but the specific components or properties of the soil responsible for leaching differences were not identified. Additional research is needed in this area. With regard to microorganism populations, a study by Nilsson and Daniel *(52)* has shown that some soils favor brown-rot fungi and others favor white-rot fungi and garden compost favors soft-rot fungi. The reason for these differences is not clear, but is undoubtedly related to the soil chemical and physical properties. As discussed previously, several elements and vitamins are critical for the growth and development of fungi. Since the relative amounts and types of these compounds in soil undoubtedly have an influence on the rates of wood decay, analysis of soil and addition of deficient elements would help assure that optimum decay rates are achieved in soil bed tests.

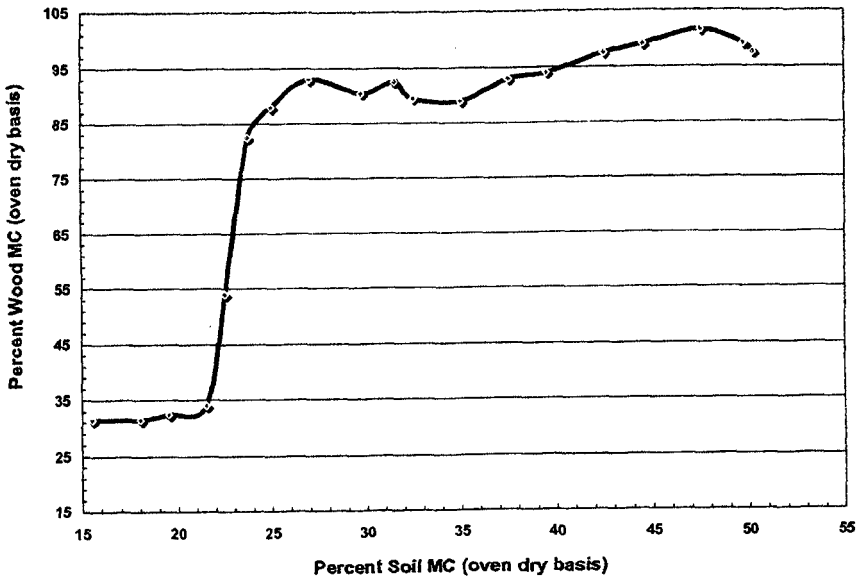

Figure 4. Relationship between soil MC and the EMC of southern yellow pine sapwood sticks buried in the soil. The water holding capacity of the soil determined using AWPA standard E10 (5), was 34.6%.

Oxygen level in soil is also an important factor that has not been given much attention. In the typical soil beds, which have stakes inserted vertically, optimum oxygen levels are undoubtedly available at the ground line areas much the same as they are in field stakes. In the soil bed designs, such as the tube test described below in this paper, the oxygen levels at the interior portion of the stakes may or may not have optimum oxygen levels. Research is needed in this area to determine whether or not aeration of the soil beds would enhance decay rates.

Microbial Populations in Soil

Soils contain large populations of microorganisms–fungi, bacteria, nematodes, protozoa and microarthropods. The types and relative numbers of these microorganisms is highly dependent on the composition, moisture, oxygen levels, and temperature of the soil. These factors need to be considered to optimize wood decay rates in the soil, but very little research has been carried

out in this area. Nevertheless, the potential of this approach is illustrated by a study recently carried out in the laboratory of the senior author. In this study, a soil obtained from a forested area was fortified with composted wood that added additional nitrogen, phosphorus and organic matter (Table II). This soil and non-amended soil were used in a soil bed test with untreated pine sapwood stakes. Mer 8 months of exposure, the amount of decay in the stakes in the amended soil was considerably greater (Table II). These two soil samples were also analyzed for bacteria and fungal populations. It is apparent from Table III that the addition of compost had a significant effect on microbial growth, particularly on the basidiomycete populations. Although limited in scope, this study illustrates the promise of this approach to accelerating decay rates in soil bed tests.

Table II. Chemical Analysis of Soils Used in a Soil Tube Test and Decay Ratings for Untreated Pine Sticks After Eight Months Exposure

Soil Composition	*Total Organic Carbon (µg/g)*	*Total Organic Nitrogen (µg/g)*	*Total Organic Phosphorus (µg/g)*	*Decay Rating*[a]
Soil	27104	988	44	9.8
Soil + Compost (25% w/w)	91543	3603	2585	7.5

[a]A rating of 10 denotes sound and 0 denotes failure.

Table III. Microbial Analysis of Soils Used in a Soil Tube Test

Soil Composition	*Active Bacterial Biomass*	*Total Bacterial Biomass*	*Active Fungal Biomass*	*Total Fungal Biomass*	*Average Hyphael Diameter*[a]
Soil + Compost	2.8	216	3.7	287	3

[a]Hyphael diameter of 3 denotes a community dominated by basidiomycetes.

New Soil Bed Design

Accurate control of soil and wood moisture contents is critical in soil bed tests. Many soil bed facilities use large containers, such as bathtubs or large concrete bins. In general, the larger the container the more difficult it is to control soil and wood moisture levels. As a result, we have been investigating

the use of small PVC tubes as soil containers (Figure 5). In this configuration the test specimens (14 x 14 x 250 mm long sticks) are inserted into holes in the side of the tube so that only the center portion is in contact with the soil. The soil MC is controlled by weighing the tubes periodically and adding water through a perforated tube extending along the bottom of the tube. The wood MC is controlled by inserting thin wood sticks into the soil from the top opening in the tube, and then removing them periodically and determining the MC by weight.

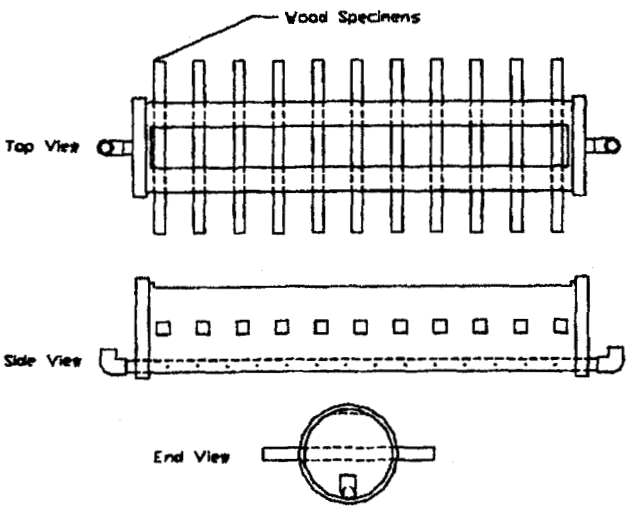

Figure 5. Schematic of soil tube test apparatus.

CONCLUSIONS

There is a need to develop accelerated test methods for evaluating the efficacy of wood preservatives. To achieve this goal it will be necessary to develop improved methods for detecting and quantifying the extent of wood decay. It will also be necessary to develop a better understanding of the many variables that influence microbial wood decay rates. In this regard, we need more knowledge on the process of microbial colonization of wood and the interaction of the various types of microorganisms involved in the process. In addition, our knowledge of the role that essential nutrients play in the decay process and their effect on the efficacy of wood preservatives is limited and needs to be addressed. Once we develop a better understanding of these relationships it should be possible to develop vastly improved wood decay test methods. However, it needs to be emphasized that there is a danger in developing accelerated test methods because the results may not provide a realistic evaluation of commercial treated wood products. Consequently, it is

essential that comparative field tests with known preservatives be included in the development process.

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Barry Goodell, Editor
University of Maine

Darrel D. Nicholas, Editor
Mississippi State University

Tor P. Schultz, Editor
Mississippi State University

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