Early detection and progression of decay in L-joints and lap-joints in a moderate decay hazard zone

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Abstract

Accelerated test methods are needed to evaluate the initiation and progression of decay in wood exposed aboveground. The relationship between test conditions and initiation of decay, however, is poorly understood. Southern pine and maple L-joints and lap-joints were exposed aboveground in a configuration that encouraged water entrapment at the Valley View Experimental test site near Madison. Wisconsin, (hazard zone 2, moderate decay hazard). Test units were evaluated over 120 weeks for visible signs of decay by the pick test, immunodiagnostics, and fungal isolation. Maple lap-joints began to show evidence of incipient decay afterjust 2 weeks. Ten out of 15 maple lap-joint specimens were positive for the immunodiagnostic wood decay test after 6 weeks. Seventeen out of 21 fungal isolates cultured between 12 weeks and 16 weeks were identified as *Irpex lacteus*, a common white-rot basidiomycete which was isolated from both pine and maple specimens. Maple lap-joints were the first specimens to show visible signs of decay (68 weeks), followed by maple L-joints. pine lap-joints, and pine L-joints. Moisture content of the joint area of shaded specimens was not directly related to rainfall and covering specimens inhibited wetting, thus the onset of decay was delayed. We concluded that under shaded conditions in a moderate decay hazard zone: 1) immunodiagnostic tests were a rapid indicator of incipient decay, 2) decay was detected in lap-joints earlier than L-joints, 3) decay was detected in maple specimens before pine specimens, 4) *Irpex lacteus* was the predominant basidiomycete cultured from test specimens. and 5) moisture accumulation in joints under shaded conditions was not related to measured rainfall.

Aboveground performance of wood has been the subject of numerous studies (Scheffer et al. 1971, Fougerousse 1976, Savory and Carey 1979, Carey et al. 1981, Carey 2002a, Highley 1984, 1993, Carey and Bravery 1986, DeGroot 1992). Aboveground tests are carried out to assess the ability of wood preservation systems to protect wood used in exterior applications such as millwork, decking, and fascia (Nicholas and Crawford 2003). They are also useful for evaluating the natural durability of building components that are intended for applications protected from the environment. For example, millwork joints that are not properly clad experience rapid failure if water becomes trapped between the cladding and the frame. The value of such tests is recognized by standard-setting organizations. such as the American Wood-Preservers' Association (AWPA). The AWPA standard E9-97, *Standard field tests for the evaluation of wood preservatives to be used in non-soil contact,* contains the following statement: "The test employs simulated joinery units (millwork) (L-joints) which are placed out of doors, out of contact with the ground and are thus exposed to the normal environmental and ecological conditions similar to those of exterior joinery" (AWPA 2003a).

A number of test designs have been used in the past, with postrail, cross brace, L-joint, and lap-joint designs used most often (DeGroot and Highley 1995). The relative durability of American woods in aboveground exterior use was reported by Highley (1993) using the cross-brace test design. Studies by Highley and others demonstrated that L-joints were more difficult to protect from decay than cross-brace units (Highley

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1993, Highley et al. 1994). In these studies. untreated pine and maple cross-brace units were severely decayed after 12 years of aboveground exposure at the USDA Forest Service national exposure site near Madison, Wisconsin. Eslyn et al. (1985) published the longevity of several wood species, and associated decay fungi cultured from them, through 12 years of exposure aboveground near Madison, Wisconsin, and in the high-decay hazard climate of southern Mississippi. They first observed visual decay in maple cross-brace units at 4 years and in pine cross-brace units at 6 years. Carey attempted to establish the rate of colonization by decay fungi in Scots pine L-joints and was able to isolate decay fungi from a majority of untreated L-joints after 3 or 4 months, although the isolates were not identified (Carey 2002b). She concluded that the mean life of untreated Scots pine sapwood varied between 8.0 years and 10.7 years, and that the early onset of decay in a particular replicate did not necessarily result in early failure of that replicate (Carey 2002c). Furthermore, direct comparisons could he made between the incidence of colonization and the rate of progress of visible decay.

Modifications of standard test set-ups may aim to create a specialized method for a specific application. For example. one modification of the L-joint design incorporated incisions to evaluate difficult to treat western wood species (Morrell et al. 1998). Another modification to the typical lap-joint incorporates thin sticks within the joint for the purpose of repeated mechanical and immunodiagnostic assessments. This modification was made to enhance the rate ofperformance-data collection (Nicholas and Crawford 2003).

Limitations of current aboveground test procedures include the subjective nature of the rating system and lack of accurate methods for detecting and measuring the extent of decay. particularly incipient decay. Both L- and lap-joint tests were designed to effectively trap water and provide conditions favorable for wood decay. The L-joint design favors end-grain penetration of rainwater (Highley 1993). Lap-joints create a relatively large interfacial zone where water can be trapped (DeGroot and Highley 1995). Both are dependent on rainfall. temperature at the chosen test site, and exposure time for a reliable set of performance data. The climate in Madison, Wisconsin is moderately favorable for promotion of decay (decay hazard zone 2). with an absence of decay-supporting conditions during late fall and winter months (Scheffer 1971),

It may he possible to accelerate aboveground tests, even in a climate of moderate decay hazard, by shading the test fence or covering the test units with a tarp to trap condensation and prevent rapid drying. The objective of the this stud) was to determine the amount oftime required to detect incipient decay in shaded L- and lap-joint test units in a moderate decay-hazard zone using traditional and experimental decay detection methods.

Materials and methods

L-joint specimens

L-joint assemblies were prepared from white pine (*Pinus strobus* L.) sapwood and sugar maple sapwod (*Acer sacharum* Marsh.) according to AWPA E9-97 (2003a). Specimens were neither preservative-treated nor end-sealed and had an average initial moisture content (MC) of 8.5 percent. Fifteen specimens of each wood type and test configuration were exposed aboveground near Madison, Wisconsin, under the shelter of a silver maple tree to inhibit drying **(Fig. l).**

Figure 1. – Pine and maple L-joint assemblies.

Figure 2. – Pine and maple lap-joint assemblies

Lap-joint specimens

Lap-joint assemblies were prepared from white pine sapwood and maple according to a modification of AWPA E16-98 (2003b). The standard method specifies an initial waterproof end-seal to prevent water entry and microbial infection. Specimens in this study were neither preservative-treated nor end-sealed and had an average initial MC of 8.5 percent. Fifteen specimens of each wood type were fastened together with stainless steel clips for easy access during multiple inspections. Specimens were then mounted on horizontal aboveground platforms and subjected to shaded exterior exposure **(Fig. 2).** An additional 15 pine specimens were exposed aboveground under the shelter of a tree and were covered with a tarp to prevent drying. The tarp was slit directly over the test specimens to allow wetting with rainwater. Additionally, this group of specimens was watered after every inspection.

Decay detection methods

Five different specimens of each wood type, joint configuration, and test group were evaluated at 2-week intervals from April through October during the first summer, 4-week intervals during the second summer, and once during the third summer. Specimens #1 to =5 were evaluated at 2 weeks, #6 to #10 at 4 weeks, #11 to #15 at 6 weeks and so fort, such that

Table 7. – Lap- and L-joint grading system.

Rating	Condition of joint
0	Sound
1	Trace attack
2	Slight attack
3	Moderate attack
4	Severe attack
5	Failure

during every fourth evaluation, the same five specimens were reevaluated. Color changes, microbial growth, softening (pick test) (Wilcox 1983), moisture. fungal culture, and immunodiagnostics were performed on each specimen evaluated.

Pick test. – An awl was used to pry a splinter, parallel to the grain, away from the wood surface in the joint area of the test assembly. The appearance of the splinter. an indicator of the presence of decay, was used as an aid to grade test specimens.

Visual inspection. – Color changes in the wood. presence ofbleaching and staining, and signs of moisture accumulation in the joint or lap were noted. Joints were rated according to the grading system in **Table 1.** and the average rating of five specimens was recorded.

Fungal culturing

Fungal isolations. – Each joint was surface-sterilized with 95 percent ethanol and drilled with a 6.4-mm drill bit in the lap area of the lap-joint or mortise of the L-joint to collect shavings for testing. Shavings were aseptically placed on Taylor's agar for fungal culturing (Taylor 1971). Taylor's agar is a defined medium which incorporates neomycin sulfate. streptomycin sulfate, and benomyl to suppress growth of bacteria and ascomycetes, so that slower-growing basidiomycete fungi can be isolated. Suspected basidiomycete fungi were isolated by transfer to 2 percent malt extract agar until a pure culture was obtained. Isolates were subsequently transferred onto gallic and tannic acid agar (Lombard and Chamuris 1990) for presumptive identification of white-rot fungi.

Identifications. – Isolates were identified by sequencing the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The DNA was isolated by excising a block of agar approximately 8 by 8 mm from the growing margin of each colony. The agar block was placed in a microcentrifuge tube containing 200 µl of cell lysis buffer (CLB). The CLB contained 1.4 M NaCl, 0.1 M Tris-HCl, 20 mM EDTA, and 2 percent hexadecyltrimethylammonium bromide (CTAB). The block was ground using a plastic pestle, and then an additional 1 mL of CLB was added. Tubes were agitated for 20 seconds and then heated at 65°C for 1 hour. This was followed by centrifugation at 16,000 rcf for 6 minutes, after which 800 µl of supernatant was transferred to a new microcentrifuge tube and 1 mL of −20°C2-propanol added. Tubes were inverted several times and placed at −80°C for 10 minutes. Samples were then centrifuged for 20 minutes at 13,000 rcf at 0°C. Supernatants were discarded and the pellets were washed with 70 percent ethanol. Pellets were air-dried for 5 minutes and then resuspended in 50 µl of water.

The DNA in aqueous solution was cleaned using GeneClean III kits (Qbiogene) with the following modifications. Fifty µl of aqueous DNA solution was combined with 150 µl of NaI solution and 3 µl of glassmilk. Tubes were agitated intermittently for 6 minutes, followed by centrifugation at 16,000 rcf for 8 seconds The supernatant was then discarded and the pellet washed three times using the New Wash solution provided with the kit. After the final wash, pellets were air dried for 15 minutes, and DNA was eluted in 50 µl of water. The DNA solution was then used directly for polymerase chain reaction (PCR).

The fungal-specific primer pair ITS1F and ITS4 (White et al. 1990) was used for PCR. The PCR was performed using 5X Green GoTaq reaction buffer and GoTaq DNA polymerase (Promega Corp., Madison, WI). GoTaq reaction buffer was diluted to a 1x working concentration, and 0.025 units of GoTaq DNA polymerase were added per µl of reaction volume. Each primer had a final concentration of 0.2 µM, and each dNTP (Promega Corp., Madison, WI) had a final concentration of 200 µM. Template DNA (obtained using the GeneClean III kit) was diluted 1:50 in the final reaction volume. Thermocycler conditions were as follows: initial denaturing at 94°C for 2 minutes; 30 cycles of denaturing at 94°C for 40 seconds, annealing at 53°C for 40 seconds, and extension at 72°C for 90 seconds: and a final extension step of 72°C for 5 minutes. The PCR products were cleaned using AmPure magnetic beads (Agencourt) following manufacturer's directions. Sequencing reactions were performed following the Big Dye terminator protocol (ABI Prism) using the ITS4 primer. Sequencing reactions were cleaned using CleanSeq (Agencourt) according to manufacturer's directions. Sequencing products were analyzed at the University of Wisconsin Biotech Center, and final sequences were aligned using Sequencher 4.2 (Gene Codes Corp., Ann Arbor, MI). Sequences were identified using BLAST searches of the GenBank database (NCBI). A match of >98 percent sequence similarity to known isolates was considered a positive identification.

Immunodiagnostic wood decay test. – Samples of wood shavings were obtained by drilling with a 6.4-mm drill bit and collecting sawdust from individual specimens. The drill bit was cleaned with ethanol and flame-sterilized between samples to prevent sample carryover. Fifty-milligram samples were extracted for 2 hours in 2 mL of deionized water containing 0.01 percent Triton X-100 (Sigma-Aldrich, St. Louis, MO) at 25°C. Following extraction, each fluid was tested by immunoassay for the presence of decay fungi (Clausen et al. 1991, Clausen 1994, Clausen and Green 1996). Positive reactions were recorded for each group of specimens.

Moisture. – Moisture readings were taken at approximately 5-mm depth with a Delmhorst RDX-1 moisture meter (Delmhorst Instrument Co., Towaco, NJ) in the joint of five L-joint and five lap-joint specimens for each type of wood and set of conditions during each inspection. Average moisture readings were recorded for each group of specimens. Rainfall data were collected at the experimental test site with an RM Young Model 52.202 heat-tipping bucket rain gauge and Paroscientific MET3A weather station that was interfaced with a National Instruments (NI) data collection system using NI LabView software.

Results and discussion

Visual ratings combined with pick-test results were averaged for the five specimens during each inspection **(Fig. 3,** Table 2). Wilcox (1983) described three distinct stages of decay determined b? the pick test. Yon-decayed wood is dense. difficult to penetrate. and results in a fibrous or splintering

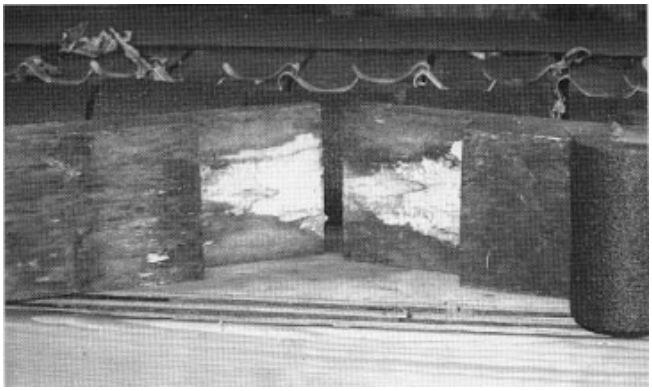

Figure 3. – Bleaching of maple lap-joint by white-rot after 72 weeks.

Table 2. – Average decay ratings summary of test units exposed under shade through 120 weeks.

	Specimen type and conditions				
	Pine			Maple	
Time	Lap-joint	Covered lap-joint	L-joint	Lap-joint	L-joint
(wks)					
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	1	0
6	1	0	0	1	1
8	1	0	1	0.6	1
10	1	1.4	1	1	1
12	1.4	0	1	0.8	1
14	1.4	0.4	1	1.4	1
16	1	1	1	1	1.2
18	1	1	1	1	1
22	1	0	1	1	1
26	1	1	1	1	1
52	1	1	1	1	1
56	1	1	1	1	1
60	1	1	1	1	1
64	1	1	1	1.4	1
68	1	1.2	1	2	2
72	1	1	2	2	2
120	0	0	0	2.5	1

break. In a fibrous break, splinters are long and separate from the wood surface far from the tool. A splintering break results in numerous splinters directly over the tool. A pick test on non-decayed wood will give an audible sound that one would expect to hear when wood breaks. A pick test on decayed wood will result in a brash or brittle failure across the grain with few, if any, splinters. The sound will not be as loud. The pick test can subjectively differentiate between sound and decayed wood in weathered specimens that might otherwise be mistaken as decayed under comparable conditions. Both maple lap- and L-joints were the first specimens to show visible signs of decay at the 68-week inspection (Fig. 3) with an average rating of 2. Differences in rating averages were a result of subjectivity between multiple inspectors and interpretation of "discoloration" (AWPA 2003b).

All of the tests were conducted in the shade of a tree in an effort to delay drying of condensation and rainwater. The test

Table 3. – IWD and culture result summary for white pine lap-joint test units exposed under shade.

	IWD		
Time	Number positive	Cumulative number of positives	Fungal culture
(wks)			
2	0	0	
4	1	1	
6	1	2	
8	0	2	
10	1	3	
12	0	3	
14	0	3	
16	1	4	(4) *I. lacteus*[a]
18	0	4	
22	0	4	
26	0	4	
52	0	4	
56	1	5	
60	2	7	
64	2	9	
68	2	11	
72	0	11	
120	2	13	

[a]Number of isolates in parentheses

design included one set of pine lap-joints covered with a poly tarp to further trap moisture. Slits were made in the tarp directly over test specimens to allow rain water to reach specimens. During the initial inspections. the MC of these specimens was unusually low compared to the adjacent uncovered specimens. Following each inspection thereafter. this group of specimens was sprinkled with 4 L of water prior to reinstallation of the tarp. This also ensured that this group of specimens was repeatedly wetted at regular intervals during the summer months. Despite regular repeated wetting. few differences were seen in the rating of this group of specimens and the uncovered adjacent group of pine lap-joints.

Tables 3 through **5** show the number of positive immunodiagnostic wood decay (IWD) tests during each inspection and the cumulative number of positives for the lap-joint test groups (n = 15). Even though only five specimens were tested during each rotating inspection. specimens showing a positive IWD reaction were retested during the next inspection to confirm the reaction. Thereafter, these specimens were not retested. Confirmed positives are not included in the cumulative number ofpositives in **Tables 3** through **7.** One of the shaded white pine specimens was positive by week 4, but overall, both shaded and covered pine specimens hadcumulative positive IWD reactions in 47 percent and 26 percent of the specimens at week 60, respectively. Maple lap-joints (Table 5) had a positive reaction in three specimens after 2 weeks, and 10 out of 15 positive by 6 weeks. Nearly all maple specimens were positive for IWD by the end of the first summer (16 weeks).

IWD test results for L-joint specimens **(Table 6)** showed that pine specimens had two positive results by week 4, but overall cumulative results showed a steady increase in positives over time. Greater than 50 percent positive results for

Table 4. – IWD and culture result summary for covered white pine lap-joint test units exposed under shade.[a]

Time	IWD: Number positive	IWD: Cumulative number of positives	Fungal culture
(wks)			
2	0	0	
4	0	0	(1) *I. lacteus*[b]
6	0	0	
8	--	0	
10	0	0	
12	--	0	
14	1	1	(1) *I. lacteus*
16	2	3	
18	0	3	
22	1	4	
26	0	4	
52	0	4	
56	0	4	
60	0	4	
64	4	8	
68	3	11	
72	1	12	
120	0	12	

[a]Specimens covered with a slitted tarp.
[b]Number of isolates in parentheses.

Table 5. – IWD and culture result summary for maple lap-joint test units exposed under shade.

Time	IWD: Number positive	IWD: Cumulative number of positives	Fungal culture
(wks)			
2	3	3	
4	3	6	
6	4	10	
8	0	10	
10	0	10	
12	1	11	(1) *Schizophyllum commune*
14	1	12	(4) *I. lacteus*, (1) *T. versicolor* (1) unknown Basidio[a]
16	2	14	
18	0	14	
22	0	14	
26	0	14	
52	0	14	
56	0	14	
60	0	14	
64	0	14	
68	0	14	
72	0	14	
120	0	14	

[a]Number of isolates in parentheses

pine specimens were seen after 14 weeks. Similar to the lap-joint findings, four IWD tests were positive for the maple specimens at the 2-week inspection and over 50 percent ofthe specimens were positive by 10 weeks **(Table 7).**

Table 6. – IWD and culture result summary for white pine L-joint test units exposed under shade.

Time	IWD: Number positive	IWD: Cumulative number of positives	Fungal culture
(wks)			
2	0	0	
4	2	2	
6	1	3	(1) *I. lacteus*
8	1	4	(1) *I. lacteus*
10	2	6	
12	0	6	(1) *I. lacteus*
14	2	8	
16	0	8	(2) *I. lacteus*[a]
18	0	8	
22	1	9	
26	1	10	
52	0	10	
56	1	11	
60	0	11	
64	4	15	
68	0	15	
72	0	15	
120	0	15	

[a]Number of isolates in parentheses.

Table 7. – IWD and culture result summary for maple L-joint test units exposed under shade.

Time	IWD: Number positive	IWD: Cumulative number of positives	Fungal culture
(wks)			
2	4	4	
4	0	4	
6	1	5	
8	0	5	
10	4	9	
12	3	12	
14	0	12	(1) *Sistotrema brinkannii.* (1) *I. lacteus*
16	0	12	(4) *I. lacteus*[a]
18	0	12	
22	0	I2	
26	1	13	
52	0	13	
56	1	14	
60	0	14	
64	0	14	
68	1	15	
72	0	15	
120	0	15	

[a]Number of isolates in parentheses.

The fungal culture identifications are noted on **Tables 3** through **7.** The most common basidiomycete isolated from both pine and maple test specimens was *Irpex lacteus* (Fr.) Fr. *Irpex lacteus* is a ubiquitous white-rot fungus that can tolerate

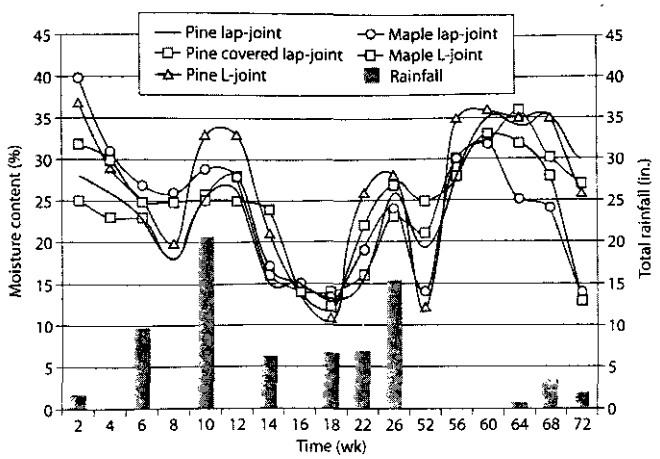

Figure 4. – Average MC in joint area of test specimens and corresponding cumulative rainfall (bars).

a wide range of environmental conditions. In the United States, this species has been documented in 42 of the 50 states and has been recorded from more than 100 different woody plants, including angiosperms and gymnosperms (Gilbertson and Ryvarden 1986, Ginns and Lefebvre 1993). *Irpex lacteus* accounted for 17 of the 21 fungal isolations (81%), indicating that this fungus dominates the early stages of colonization and decay under the conditions experienced during this study. In addition to *I. lacteus,* other decay fungi isolated were *Trametes versicolor* (L.) Lloyd, *Sistotrema brinkmannii* (Bres.) J. Erikss., *Schizophyllum* commune Fr., and one unknown basidiomycete related to *Oligoporus* (= *Postia*) species. During the 120-week test period, the majority of isolations were made between weeks 12 and 16. Eslyn et al. (1985) also isolated predominantly white-rot fungi, including *I. lacteus,* from both hardwood and softwood test units exposed aboveground near Madison, Wisconsin. Only one unidentified brown-rot fungus was isolated. Although the brown-rot fungus *Gloeophyllum* trabeum is often associated with decay in aboveground building components, this species was not obtained by Eslyn et al. (1985). nor did we isolate it. Because wood decay is a complex process involving a succession of different fungi over time, isolations from early stages of decay may not indicate the type of decay to ultimately occur in wood.

Figure 4 shows trends of average MC in the test-unit joints at each inspection and corresponding cumulative rainfall. Apparently, heavy shade coverage by tree foliage inadvertently blocked some rainwater from reaching the specimens. It also appeared that MC of the specimen joint was dependent on trapped condensation and not rainfall, at least for this test setup since moisture measurements did not correspond to measured rainfall. There were lengthy periods oftime from weeks 12 to 22 when the average MC remained below 20 percent while corresponding rainfall was greater than 6 inches per month. Coincidentally, during that same time frame, a majority of the fungal isolations were made. Conversely, average MC was greater than 20 percent during periods of time (weeks 52 to 60) with no significant rainfall. It is possible that carbohydrate was being converted to CO_2 and water during this period of incipient decay. Despite unusually low rainfall during the second summer season (from weeks 52 through 72) cumulative positive IWD results continued to increase, but no additional decay fungi were isolated during that time frame.

Conclusions

Under conditions of moisture entrapment, visual signs of decay occurred first in maple lap-joints. followed by maple L-joints, pine lap-joints, and pine L-joints. Pine lap-joints covered with a tarp were drier than adjacent uncovered specimens, resulting in decreased progression of decay. *I. lacteus,* the primary fungal isolate, was cultured from both pine and maple specimens. IWD test results were an excellent predictor of incipient decay, but they did not correlate with visual ratings, which are indicative of later stages of decay or MC readings.

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