

Accelerated detection of brown-rot decay: Comparison of soil block test, chemical analysis, mechanical properties, and immunodetection

C.A. Clausen
S.N. Kartal

Abstract

Early detection of wood decay is critical because decay fungi can cause rapid structural failure. The objective of this study was to compare the sensitivity of different methods purported to detect brown-rot decay in the early stages of development. The immunodiagnostic wood decay (IWD) test, soil block test/cake pan test, mechanical property tests, and chemical analysis were evaluated in southern yellow pine blocks and stakes exposed to *Postia placenta* for 5 weeks. The IWD test was 100 percent positive in blocks after 5 days of incubation; similarly, IWD was 100 percent positive in stakes after 3 days. Weight loss was not accurate for measuring decay in stakes using the cake pan method, but significant weight loss (1.8%) occurred in blocks after 4 weeks of exposure to the fungus in soil block tests. Maximum compressive strength (MCS) and modulus of elasticity (MOE) were reduced 21 and 13 percent, respectively, in blocks exposed to the fungus for 2 weeks. In stakes, MOE and modulus of rupture were reduced 9 percent and 19 percent, respectively, after 4 weeks of exposure. Arabinan, xylan, and rhamnan decreased rapidly in blocks, exceeding 30 percent after 4 weeks of exposure. In stakes, only galactan was decreased beyond 30 percent after 4 weeks. Sample size and shape was a significant factor in successfully detecting early stages of brown-rot decay in laboratory tests. Blocks fostered rapid colonization in soil block tests, and most methods tested were able to detect the fungus sooner in blocks than in stakes. The IWD test was the most rapid method of detecting *P. placenta* (from 3 to 5 days), followed by reduction in MCS in blocks.

Decay caused by brown-rot fungi is the most prevalent and destructive type of wood deterioration because it can cause rapid structural failure. Annual losses of over \$1 billion in the United States result from fungal deterioration of untreated or inadequately treated wood (Scheffer 1973). Losses are difficult to quantify, but it is estimated that 10 percent of the annual timber harvested in the United States is used to replace wood that has deteriorated in service (Zabel and Morrell 1992).

Although incipient brown-rot decay occurs shortly after brown-rot fungi initiate colonization and release enzymes, there is no visible evidence of damage to

the wood. However, chemical changes during initial colonization result in measurable reductions in strength before measurable weight loss (Schmidt et al. 1978, Wilcox 1978, Imamura 1993, Kim et al. 1996). During early decay, slight changes in color or texture occur, but decay is not yet obvious (Zabel and

Morrell 1992, Clausen et al. 2001). By the time 1 percent weight loss is realized, laboratory tests show that losses in toughness range from 6 percent to greater than 50 percent. Strength losses may exceed 50 percent by the time 10 percent weight loss is incurred (Highley 1999).

The authors are, respectively, Research Microbiologist, USDA Forest Service, Forest Products Lab., One Gifford Pinchot Drive, Madison, WI 53705-2398; and Assistant Professor, Department of Forest Biology and Wood Protection Technology, Forestry Faculty, Istanbul Univ., Istanbul, Turkey. The authors would like to acknowledge the technical assistance of Marshall Begel and Lori Schroeder of the Engineering Mechanics Laboratory, Forest Products Lab., Madison, WI. This paper was received for publication in June 2002. Article No. 9509.

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Table 1. — Results of immunodiagnostic wood decay (IWD) test on southern yellow pine blocks and stakes exposed to *P. placenta* over time.^a

Incubation time (day)	Positive detection of decay	
	Blocks	Stakes
	----- (%) -----	
1	0	67
2	0	83
3	0	100
4	67	100
5	100	100
6	100	100

^aEach value represents average of six specimens.

Table 2. — Loss in weight and mechanical properties for southern yellow pine blocks and stakes exposed to *P. placenta* over time.^a

Incubation time (wk.)	Blocks			Stakes		
	Weight loss	MOE reduction	MCS reduction	Weight loss	MOE reduction	MOR reduction
	----- (%) -----					
1	0	3	6	0	5	2
2	3	13	21	0	8	2
3	9	22	37	1	7	8
4	18	34	53	3	9	19
5	26	53	71	7	12	22

^aEach value represents average of six specimens.

The early stages of decay have been the focus of detection research for many years. If a method or combination of methods can detect incipient decay in wood and accurately diagnose the presence of brown-rot fungi, then remedial steps can be taken to arrest fungal growth prior to structural damage.

Methods for detecting the earliest stages of fungal decay include tests for mechanical strength (i.e., compression test or measurement of modulus of elasticity [MOE]), electrical conductivity (e.g., moisture meter or shigometer), acoustic detection (e.g., acoustic emission or stress-wave timer), chemical analysis, and laboratory detection (e.g., culturing, microscopy, or serological tests). Culturing and microscopy are currently considered the only definitive methods. Measurements of work to maximum load, toughness, and impact bending are also reported to be sensitive mechanical methods for detecting early decay (Wilcox 1978).

In a study on the effect of hemicellulose degradation on strength properties of wood, Curling et al. (2001) exposed southern yellow pine stakes to *Gloeophyllum trabeum* and analyzed chemical composition, mechanical properties, and representative weight loss. Their

results demonstrated a ratio of strength to weight loss of approximately 40:1. Chemical analysis indicated that early losses in arabinan and galactan were associated with early strength loss. Winandy and Morrell (1993) also demonstrated a relationship between hemicellulose degradation, particularly arabinose and mannose, and strength loss.

One type of serological detection, the immunodiagnostic wood decay (IWD) test, utilizes anti-xylanase antibody to detect minute quantities of fungal xylanase (Clausen and Green III 1996). Because hemicelluloses are rapidly removed by brown-rot fungi during the initial stages of colonization, the presence of endo-1,4- β -xylanase, a hemicellulase, indicates incipient decay (Clausen et al. 1991). The IWD test has been shown to detect brown-rot decay fungi prior to loss in wood weight or strength (Clausen et al. 1991, Clausen and Ferge 1995, Clausen et al. 2001).

The objective of this study was to compare the sensitivity of methods purported to detect brown-rot decay in the early stages of development.

Materials and methods

Steam-sterilized southern yellow pine blocks (10 by 10 by 10 mm) were exposed to *Postia placenta* MAD 698 in a

standard soil block test (ASTM 1998a). Southern yellow pine sapwood (250 mm long by 25 mm tangential by 10 mm radial) were exposed to *P. placenta* MAD 698 in a modified cake pan test (Soltis et al. 1992). For the cake pan test, 1 L of a 1:1 soil and vermiculite mixture was placed in an aluminum cake pan. The surface was covered with rows of southern pine feeders (42 by 29 by 3 mm). The moisture content of the soil/vermiculite mixture was adjusted to 50 percent of the water-holding capacity, and the test apparatus was autoclaved at 103 kPa and 121°C for 45 minutes. When cool, the feeders were inoculated with the fungus by pipetting 100 mL/pan of a macerated 3-week-old liquid culture of *P. placenta* evenly over the feeders. The test apparatus was sealed in a plastic bag to prevent drying and incubated at 27°C and 70 percent relative humidity (RH) for 3 weeks until the feeders were completely covered by fungal growth. Steam-sterilized test specimens were placed on top of the feeders and incubated at 27°C and 70 percent RH. Uninoculated steam-sterilized blocks and stakes served as controls for the block and stake tests.

Six blocks and six stakes were removed from their respective test apparatus after 3, 4, 5, and 6 days. Six uninoculated control blocks and stakes were also tested as follows. Shavings were taken from each stake using a 6.4-mm drill bit. The blocks and shavings were extracted in aqueous 0.1 percent Triton X-100 (Sigma, St. Louis, Missouri), and extracts were tested by the IWD test (Clausen and Green III 1996).

Another six blocks and six stakes were removed from their respective test apparatus weekly for 5 weeks, brushed free of mycelium, and oven dried at 60°C for 48 hours. A matched number of uninoculated blocks and stakes were analyzed weekly. Blocks and stakes were conditioned at 20°C and 65 percent RH prior to testing. Blocks were tested for weight loss, radial compressive strength, and MOE (ASTM 1998a, 1998b). Stakes were tested for weight loss, MOE, and modulus of rupture (MOR) (ASTM 1998a, 1998b). Each specimen was analyzed for carbohydrate content (Davis 1998).

Results

Tables 1 and 2 show the results of the IWD test and tests for mechanical prop-

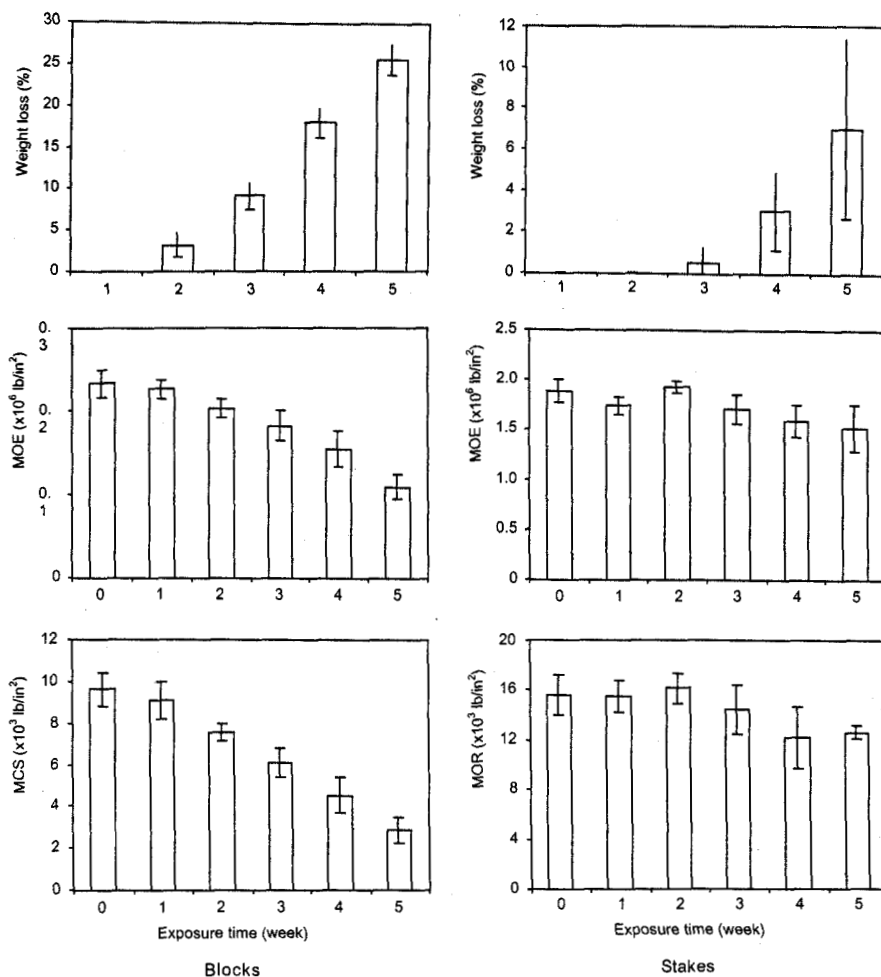


Figure 1. — Weight loss, MOE, MCS, and MOR of southern yellow pine blocks and stakes exposed to *P. placenta* for 5 weeks compared to that of uninoculated controls. (Bars represent standard deviation; $n = 6$).

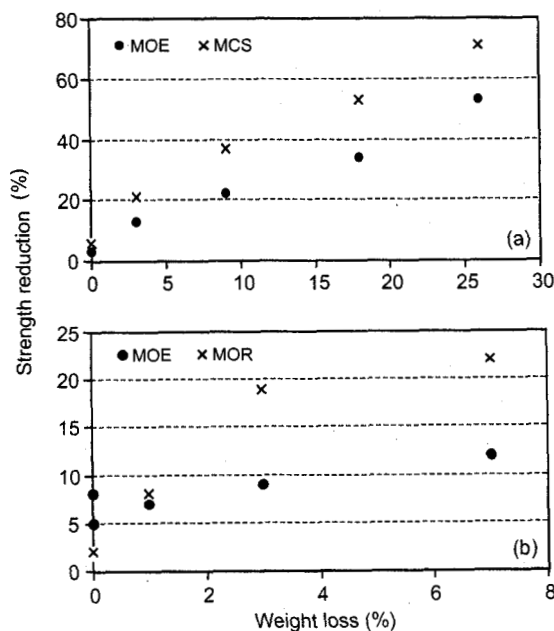


Figure 2. — Relationship between weight loss and strength reduction for blocks (a) and stakes (b) exposed to *P. placenta* for 5 weeks.

erties for blocks and stakes after various periods of exposure to *P. placenta*. Stakes tested 100 percent positive by IWD after 3 days of exposure to the fungus, whereas blocks tested 100 percent positive after 5 days. The data on mechanical properties show the total percentage of reduction compared to the same properties in uninoculated controls. MOE of blocks was reduced 13 percent after 2 weeks of incubation, but MOE of stakes was reduced only 12 percent after 5 weeks of incubation. Of the mechanical properties tested, maximum compressive strength (MCS) showed the most rapid change. In blocks, MCS decreased 21 percent after 2 weeks. The MOR of stakes decreased 19 percent after 4 weeks of incubation.

Figure 1 summarizes weight loss, MOE, MCS, and MOR for blocks and stakes exposed to *P. placenta* for 5 weeks. Significant weight loss (>10%) was measured in blocks after 4 weeks. Weight loss proved to be an inaccurate method of measuring decay in stakes exposed to *P. placenta* in the modified cake pan method, compared to the standard ASTM soil block method (ASTM 1998a). The size, shape, and greater volume of stakes slowed the progression of fungal colonization. Standard deviation was also greater at each test interval for stakes compared with blocks. Figure 2 shows the relationship between strength reduction and weight loss in blocks and stakes. In blocks, the reduction in MCS occurred sooner and more rapidly than the reduction in MOE (Fig. 2a). By the time 9 percent weight loss had occurred, MCS was reduced 37 percent. In stakes tested by the cake pan method, MOR was reduced 19 percent by the time 3 percent weight loss had occurred.

Table 3 summarizes the results of carbohydrate analyses for blocks and stakes. Figure 3 shows the percentage of reduction of each carbohydrate during the 5-week exposure to *P. placenta*. For blocks, losses in arabinan, rhamnan, and xylan exceeded 30 percent after 4 weeks of exposure when weight loss was 18 percent, MOE was reduced 34 percent, and MCS was reduced 53 percent. Analysis of stakes revealed a 30 percent loss in galactan after 4 weeks, when no significant weight loss had occurred, MOE was reduced 9 percent, and MOR was reduced 19 percent.

Table 3. — Carbohydrate analysis of blocks and stakes exposed to *P. placenta* for 5 weeks.^a

Specimens	Time	Carbohydrate composition					
		Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan
	(wk.)	----- (%) -----					
Blocks	0	1.01	1.37	0.07	42.82	5.88	11.32
		(0.07)	(0.12)	(0.01)	(0.87)	(0.81)	(0.80)
	1	0.87	1.40	0.07	42.56	5.05	12.15
		(0.14)	(0.02)	(0.00)	(0.88)	(0.98)	(1.08)
	2	0.78	1.10	0.06	42.46	5.60	10.32
		(0.08)	(0.15)	(0.01)	(1.05)	(0.90)	(0.91)
	3	0.68	1.02	0.05	42.23	4.82	9.65
		(0.08)	(0.16)	(0.00)	(0.94)	(0.93)	(0.92)
	4	0.55	1.07	0.04	41.89	3.91	10.73
		(0.07)	(0.13)	(0.01)	(0.92)	(0.40)	(0.16)
5	0.61	0.86	0.04	39.13	4.36	7.87	
	(0.06)	(0.02)	(0.00)	(0.53)	(0.63)	(0.24)	
Stakes	0	1.02	2.63	0.09	41.97	6.44	9.84
		(0.03)	(0.07)	(0.00)	(1.12)	(0.19)	(0.27)
	1	0.93	2.20	0.09	42.80	6.39	10.43
		(0.03)	(0.40)	(0.01)	(1.21)	(0.35)	(0.57)
	2	0.94	2.13	0.09	42.07	6.61	10.53
		(0.01)	(0.07)	(0.01)	(0.23)	(0.04)	(0.20)
3	0.86	2.05	0.08	42.23	6.21	10.62	
	(0.01)	(0.04)	(0.01)	(0.50)	(0.04)	(0.19)	
4	0.82	1.69	0.07	41.25	6.13	9.84	
	(0.01)	(0.27)	(0.00)	(0.87)	(0.43)	(0.81)	
5	0.77	2.04	0.07	40.48	5.93	8.98	
	(0.03)	(0.19)	(0.01)	(1.79)	(0.56)	(0.19)	

^aEach value represents an average of six specimens; numbers in parentheses are standard deviations.

Discussion and conclusions

The IWD test clearly detected *P. placenta* just 3 days after inoculation. This result mirrors observations from a number of studies in which IWD was able to detect the presence of decay fungi prior to weight loss (Clausen et al. 1991, Clausen and Ferge 1995, Clausen et al. 2001) and strength loss (Clausen et al. 2001). The sampling method may account for differences seen in IWD test results from blocks and stakes. Enzymes were extracted from intact blocks; for stakes, shavings were used. The in-

creased surface area of the shavings may have increased the amount of enzyme extracted for detection. The dimensions of the stakes (250 by 25 by 10 mm) used in this study, though necessary for the standardized MOR test, hindered early detection of *P. placenta* by all methods except the IWD test. Size, shape, and volume of test specimen, as well as configuration of a test apparatus, are important factors in rapid initiation of fungal colonization. Ideally, test methods for early brown-rot decay should detect initial changes in colonized wood or fungal metabolites, and accelerated testing

therefore relies on rapid fungal colonization.

MCS showed the earliest and most rapid decline of mechanical properties tested in the 19-mm-square blocks, followed by MOE (Table 2). Significant weight loss (>10%) did not occur until blocks had been incubated for 4 weeks. By 3 weeks, a 4:1 ratio in strength loss to weight loss was observed. Smith and Graham (1983) showed that compressive strength losses were highly correlated to and evident before weight loss in Douglas-fir decayed by *P. placenta*.

For stakes, MOR showed the most rapid decline of mechanical properties tested. Kim et al. (1994) demonstrated that significant strength loss could be detected in full-sized lumber after 5 weeks of exposure to *Gloeophyllum trabeum*. In our study, stakes showed a 6:1 ratio of strength loss to weight loss after 4 weeks of incubation. Nevertheless, weight loss was not greater than 7 percent, and standard deviations in weight loss values were high after 5 weeks of incubation. We conclude that the cake pan method used in this study is not conducive to accurate or early weight loss measurements of stakes.

Carbohydrate losses were inconsistent for decayed blocks and stakes. Carbohydrate analysis of blocks showed significant losses (>30%) in arabinan, rhamnan, and xylan after 4 weeks of incubation, while analysis of stakes showed a similar loss for only galactan after this incubation period. Coinciding with high losses in arabinan, rhamnan, and xylan, blocks also showed significant weight loss (18%), 34 percent reduction in MOE, and 53 percent reduction in MCS. On the other hand, a 36 percent reduction in galactan for stakes coincided with no significant weight loss (3%), 9 percent reduction in MOE, and 19 percent reduction in MOR. We conclude that carbohydrate analysis is not an accurate or reliable method for detecting incipient decay by the decay methods used in this study.

This study illustrates that the sensitivity of methods for detecting incipient decay varies greatly, and therefore methods must be used in combination to evaluate in-service wood for decay. A positive IWD does not necessarily indicate a potential strength reduction if conditions for sustained decay do not exist for the particular wood member being sampled. Rather, it can serve as an indicator

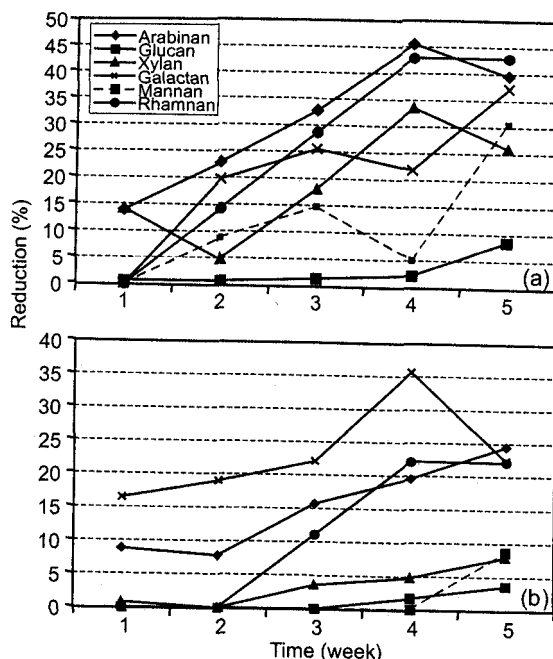


Figure 3. — Total percentage of reduction in carbohydrates in blocks (a) and stakes (b) exposed to *P. placenta* for 5 weeks, compared to carbohydrate reduction in uninoculated controls.

for 1) additional testing with other methods for early detection; 2) potential losses if steps are not taken to protect in-service wood from further exposure to moisture and decay fungi; or 3) areas where remedial treatments to arrest decay are appropriate

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