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Experimental method to quantify progressive stages of decay of wood by basidiomycete fungi☆

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Abstract

A biological exposure method was developed that allows wood samples to be progressively removed for monitoring colonization and decay by basidiomycete fungi. Monitoring involves strength tests, determination of weight loss, and chemical analysis. To optimize the procedure, several variations of the method were tested using two species of brown-rot fungi (*Gloeophyllum trabeum* and *Oligoporus placentus* (*Postia placenta*)) and one white-rot species (*Trametes versicolor*) against southern pine sapwood. The variations involved type of culture medium and exposure method. All variations enabled substantial and rapid decay. Specimens exposed to brown-rot fungi lost 80–100% strength and 25–40% weight after 12 weeks, the white-rot fungus was less effective, but nevertheless caused 20–40% loss in strength. For both brown- and white-rot fungi, strength loss exceeded weight loss. For brown-rot fungi, there was a direct relationship between strength loss and weight loss, suggesting a quantitative relationship between strength loss and chemical composition (hemicellulose sugars) during incipient decay of southern pine by these fungi. Published by Elsevier Science Ltd.

Keywords: Incipient decay, Test method, Strength testing; Brown-rot decay; White-rot decay; *Gloeophyllum trabeum; Oligoporus placentus; Trametes* (*Coriolus*) *versicolor*

1. Introduction

Changes in the chemical composition of wood at very early stages of biological decay cause measurable reductions in strength before measurable weight loss occurs (Wilcox, 1978; Winandy and Morell, 1993; Kim et al., 1996), suggesting that strength loss is a good indicator of incipient wood decay. The research reported here is part of an ongoing program on the relationship between strength and chemical composition in incipient decay.

Winandy and Morell (1993) showed a close relationship between the degradation of hemicellulose components, such as arabinose and galactose, and wood strength loss. The strength properties and chemical composition of fire-retardant-treated wood are also systematically altered by hydrolytic chemicals (Winandy, 1995), with the rates of strength loss and change in chemical composition directly related to temperature (Winandy and Lebow, 1996). Changes in chemical composition appear to be similar in both the biological and chemical systems (Green III et al., 1991). Understanding the relationship between chemical composition and strength loss may aid in developing a model to predict strength loss caused by a variety of biological, chemical, and thermal agents.

Most standard decay tests, such as EN 113 (British Standards Institution, 1981) or ASTM D2017 (ASTM, 1998a), use weight loss rather than strength loss as the decay criterion. Therefore, the designs of current standard methods are unsuitable for a study of the kind reported here. The objective of this research program is to design a methodology, suitable for later standardization, for relating chemical composition and strength loss to incipient decay in vitro.

This paper presents details of method development and data on the relationship between strength, weight loss, and chemical composition.

2. Methods

The study variables are outlined in Table 1.

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Table 1
Study variables

Fungi	Brown-rot
	Gloeophyllum trabeum
	Oligoporus placentus (*Postia placenta*)
	White-rot
	Trametes versicolor
Culture medium	Soil
	Vermiculite
	Vermiculite/soil
Exposure method	Basic (feeder strip)
	Direct inoculation
Exposure time	6 weeks
	12 weeks
Variations of basic method[a]	Basic
	Basic with overlay
	Basic with support
	Basic with support and overlay
Nutrient amendment	1% malt extract
	0.5% malt extract
	0.1% malt extract
	0% malt extract

[a] Variables for assessing waterlogging potential of vermiculite systems.

2.1 Test specimens

Specimens were made from air-dried southern pine (*Pinus* spp.) sapwood, 9.5 mm radial by 25 mm tangential by 253 mm longitudinal; "feeder" strips were 6 mm by 19 mm by 215 mm. Specimens and feeder strips were clear of knots, damage, or defect. A 50-mm section was removed from the end of each test specimen to determine moisture content and estimate initial dry weight and density. The specimens were sterilized by steam at 110°C for 1 h.

2.2. Test fungi and exposure methods

Three species of fungi were used the brown-rot fungi Gloeophyllum trabeum (Pers. ex Fr.) Murr (Mad 617) and *Oligoporus placentus* (*Postia placenta*) (Mad 698) and the white-rot fungus *Trametes* (*Coriolus*) *versicolor* (L:Fr.) Pilat (Mad 697).

Two exposure methods were used the basic feeder strip method and a direct inoculation method. In the feeder strip method (Soltis et al., 1992), 1 L soil was placed in a lidded aluminum "cake" pan (330 mm by 230 mm by 82.5 mm) so that the bottom of the pan was covered. The medium was then formed into a ridge along the length of the long axis. Next, the feeder strip was placed onto this ridge and inoculated with the test fungus (Fig. 1a). The test sample was placed onto the feeder strip, perpendicular to the long axis of the feeder so that only the center section of the sample was in contact with the feeder and no part was in contact with the medium.

In the direct inoculation method, a part of the specimen was placed in direct contact with the medium (Fig. 1b); no feeder strip was used. The specimen was laid directly on the ridge of the medium so that only its center section was in contact with the medium.

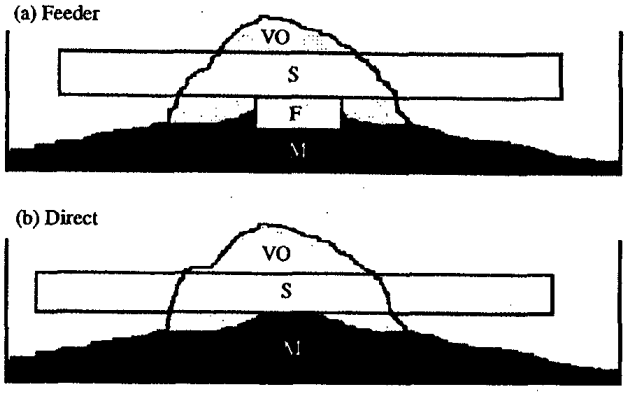

Fig. 1. Diagrammatic representation of basic feeder strip (a) and direct inoculation (b) methods. VO is vermiculite overlay; S, specimen; F, feeder strip; and M, medium.

For this investigation, the basic feeder strip method was modified by using three different media: (1) soil, (2) vermiculite (horticultural grade) (Scotts, Marysville, OH), and (3) vermiculite/soil mixture (70 : 30). The water holding capacity (WHC) of each of six random samples for each medium was determined using the method described in ASTM D2017-94 (ASTM, 1998a). The pans were then loaded with the media as follows:

Soil: 1 L soil with deionized water added to give 100% WHC.
Vermiculite: 1 L vermiculite with 1% aqueous malt extract solution (10 g malt extract (Sigma, St. Louis, MO), 1 L deionized water) to produce 100% WHC.
Vermiculite and soil: 1 L 30%/70% v/v mixture of soil/vermiculite with deionized water added to produce 100% WHC.

In each case, the medium was shaped into a flat ridge, approximately 75 mm wide, and feeder strips were added where required. The pans were then autoclaved at 103.4 kPa and 121°C for 45 min.

Each test fungus was grown for 3 weeks in liquid culture (Bailey's minimal media +0.5% cellobiose) (Sigma) at pH 4.5, 25°C, and 70% relative humidity (Highley, 1973). The mycelia were macerated for 30 s in a blender, and the resulting suspension was applied directly to the feeder strips (5 mL mycelial suspension), or the soil or vermiculite was directly inoculated with 100 mL mycelial suspension 7 days before the specimens were inserted into the pans. Sterile (uninoculated) controls were also prepared for each media combination.

The wood specimens were aseptically inserted into the pans (eight specimens per pan, two pans per exposure combination [medium, fungus, and sampling time]) with the center section resting on either the feeder strip or the ridge of the medium, so that neither end touched the surface of the medium (Fig. 1).

An overlay of moist vermiculite was used to cover the middle third of the specimen (67 mm) to a depth of approximately 1 cm to maintain elevated moisture content in the specimen and to confine the majority of fungal activity to the central third of the specimen. The moisture content of the vermiculite was adjusted to 50% of its WHC and sterilized by autoclaving prior to applying the overlay.

The cultures were incubated at 25°C and 70% relative humidity for a maximum of 12 weeks, with sampling at 6 and 12 weeks.

2.3. Measurement of strength and weight loss

At each sampling time, eight specimens were removed from the pans, cleaned of any adhering mycelium, and weighed. The specimens were placed in a drying cabinet at 60°C for 1 h to lower the wood moisture content. They were then allowed to equilibrate to constant weight at 23°C and 65% relative humidity prior to mechanical testing.

A four-point bending test (Winandy and Morell, 1993), with the load applied at the third point of a 175-mm span and a loading rate of 1.25 mm/min, was used to determine modulus of rupture (MOR). The four-point bending test was part of the design criteria for our new method. In strength testing, the three-point loading system maximizes the stress directly under the bending head only. However, fungal decay may not be uniform throughout the wood specimen, and this maximum stress may not coincide with the decayed area, introducing undue variability into the strength estimate. Winandy and Morell (1993) described the benefits of a four-point bending test rather than the simple three-point bending test that is normally used (ASTM, 1998b, c) in small-scale testing of mechanical properties. A four-point test produces a uniform bending force and stress between the supports and accurately evaluates strength in the weakest area of the decayed specimen. Specimen MOR values were compared to those of unexposed controls to determine strength loss.

After mechanical testing, two 15-mm by full-cross-section blocks were cut from each specimen from within the decay zone near the point of mechanical failure (Fig. 2). One of the blocks was weighed and oven dried. Weight loss was determined on the basis of an estimate of original dry weight derived from the dry weight and density of the 50-mm section removed prior to the decay test. All weight loss discussed in this paper refers to weight loss of this exposed/decayed portion, not the unexposed ends of each specimen. Thus, both strength and weight loss were used to compare the stage of decay.

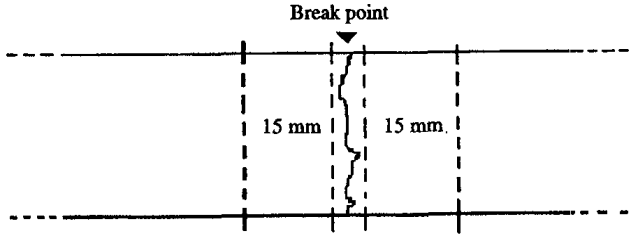

Fig. 2. Cutting scheme for obtaining specimens for weight loss measurement and chemical analysis.

The other block was ground to pass a 30-mesh (547-μm) screen. Ground material from each replicate for each fungal-exposure combination was combined and analyzed for carbohydrates (arabinan, galactan, mannan, and xylan) using high-pressure liquid chromatography (Pettersen and Schwandt, 1991).

2.4. Effect of initial moisture content of medium

The potential for water logging the specimens, which would inhibit decay in the vermiculite system, was assessed by placing preweighed southern pine specimens (50 mm by 25 mm by 9.5 mm) into aluminum pans (33 mm by 23 mm by 8.25 cm) containing 1 L vermiculite adjusted to 50%, 75%, or 100% WHC, as determined by ASTM D2017-94 (ASTM, 1998a). The effects of removing the moist vermiculite overlay (basic method without overlay) and of separating the specimen from the underlying medium using a sterile inert plastic grid to act as a support (basic method with support) were also investigated. Thus, there were four experimental combinations per moisture content, with six replicates per combination:

1. Basic method (without overlay).
2. Basic method with overlay.
3. Basic method with support (without overlay).
4. Basic method with support and overlay.

The vermiculite in the pans was inoculated with 5 mL of a hyphal suspension of *G. trabeum.* Controls consisted of identically prepared uninoculated pans. The specimens were exposed for 6 weeks. They were then removed from the pans, cleaned of adhering mycelium, weighed, oven dried, and reweighed to determine final moisture content and loss in dry weight.

2.5. Effect of nutrient amendment

The effect of malt extract solution on decay was assessed by placing preweighed southern pine specimens (50 mm by 25 mm by 9.5 mm) into aluminum pans (33 mm by 23 mm by 8.25 cm) containing 1 L vermiculite adjusted to 75% WHC (as determined by ASTM D2017-94 (ASTM, 1998a)), using 1%, 0.5%, 0.1%, and 0% malt extract solution, with six replicates per concentration. The vermiculite was inoculated with 5 mL of a hyphal suspension of *G. trabeum* and incubated for 6 weeks. The specimens were removed, cleaned of adhering mycelium, weighed, oven dried, and reweighed to determine final moisture content and loss in dry weight.

2.6. Statistical methods

Differences in strength and weight loss values; between experimental methods for each fungal species were tested for significance using single factor analysis of variance (ANOVA) comparisons. The data derived form the ANOVA table were used to determine the least significant difference. This value was used to identify significant differences between methods. All comparisons were made at a statistical level (p) of 0.05.

3. Results

3.1. Weight loss

Specimens exposed to brown-rot fungi lost considerable (over 20%) weight under all test conditions after 12 weeks of exposure (Fig. 3). The direct inoculation method resulted in higher weight loss than did the basic feeder strip method. However, at 12 weeks of exposure, significant ($p \leqslant 0.05$) differences were observed for only the soil and soil/basic method. G. trabeum produced significantly ($p \leqslant 0.05$) higher weight loss than did 0. placentus in all media except vermiculite after 6 weeks of exposure. After 12 weeks of exposure, however, the only statistically significant ($p \leqslant 0.05$) difference between the fungi was higher weight loss caused by *G. trabeum* in the soil/basic method.

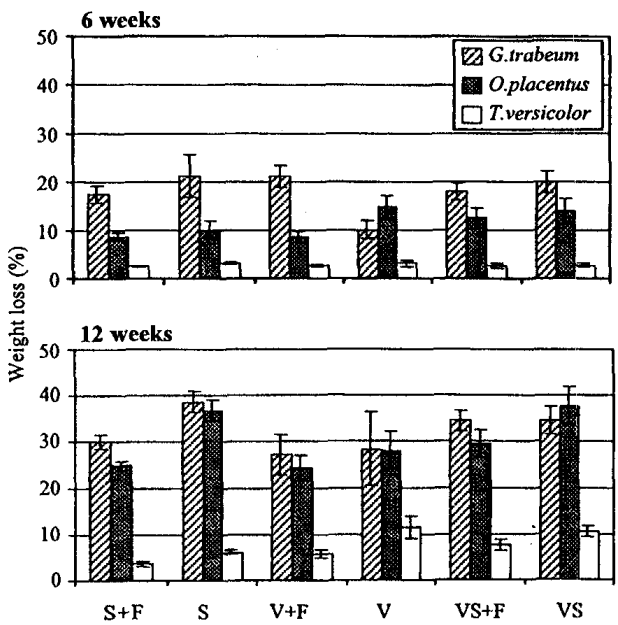

Fig. 3. Weight loss of southern pine specimens after exposure to decay fungi for 6 or 12 weeks. Error bars show 95% confidence intervals. S+F designates soil and basic feeder strip method, S, soil and direct inoculation method; V+F, vermiculite and feeder strip; V, vermiculite and direct inoculation; VS+F, vermiculite/soil and feeder strip; VS, vermiculitc:/soil and direct inoculation.

Generally, specimens exposed to brown-rot fungi on vermiculite by both the basic and the direct inoculation methods experienced lower weight loss than did specimens exposed to brown-rot fungi on other media. Final moisture content of specimens on vermiculite was also significantly higher ($p \leqslant 0.05$) than that of the other specimens (Table 2).

Exposure to *T. versicolor* resulted in much lower weight loss than did exposure to the brown-rot fungi (Fig. 3). However, in regard to *T. versicolor*, significantly ($p \leqslant 0.05$) higher weight loss occurred for specimens on vermiculite and vermiculite/soil.

3.2. Strength loss

Brown-rot fungi caused from 40% to 80% loss in strength after only 6 weeks of exposure (Fig. 4). *G. trabeum* generally caused higher strength loss than did *O. placentus*, but at 6 weeks the differences were statistically significant ($p \leqslant 0.05$) for only the soil/basic, soil/direct inoculation, and vermiculite/soil/direct inoculation methods. At 12 weeks, there were no significant ($p \leqslant 0.05$) differences between the brown-rot fungi. Lower strength losses occurred with the vermiculite/basic and vermiculite/direct inoculation methods in comparison with the other methods.

Since the white-rot fungus *T. versicolor* prefers hardwoods to softwoods, it is not surprising that strength loss caused by this species was considerably lower (30–40%) than that caused by the brown-rot fungi. The greatest strength loss with *T. versicolor* occurred in the vermiculite/direct inoculation, vermiculite/soil/basic, and vermiculite/soil/direct inoculation methods.

The relationship of strength to weight loss is shown in Fig. 5.

3.3. Effects of culture moisture content and nutrient amendment

For the basic and basic + overlay systems at 100% WHC, weight loss was statistically ($p \leqslant 0.05$) lower than was weight loss for the supported systems at 100% WHC and for all systems at both 50% and 75% WHC (Fig. 6). There were no statistically significant differences between the systems at 50% and 75% WHC.

The 1% malt extract concentration resulted in the greatest weight loss (Fig. 7). Weight loss decreased with a decrease in malt extract concentration, although the differences in the amount of weight lost were not statistically significant ($p \leqslant 0.05$).

3.4. Chemical analysis

The chemical analysis data for arabinan, galactan, xylan, and mannan content in wood decayed by brown-rot fungi were averaged to determine general trends in comparison to loss in MOR (Fig. 8). As loss in MOR progressed, the

Table 2
Mean moisture content of specimens exposed to *G. trabeum* for 6 and 12 weeks

	Moisture content (%) of specimens by medium and exposure method[a]					
Exposure time (weeks)	Soil + basic	Soil + direct	Vermiculite + basic	Vermiculite +direct	Vermiculite/soil + basic	Vemiculite/soil + direct
6	30.5 ± 1.3	44.1 ± 4.5	89.6 ± 15.1	119.4 ± 7.4	52.6 ± 7.1	91.8 ± 10.3
12	40.8 ± 1.6	58.6 ± 3.7	82.0 ± 9.4	119.5 ± 6.4	62.3 ± 7.5	100.6 ± 7.8

[a]Basic designates the feeder strip method, direct, the direct inoculation method.

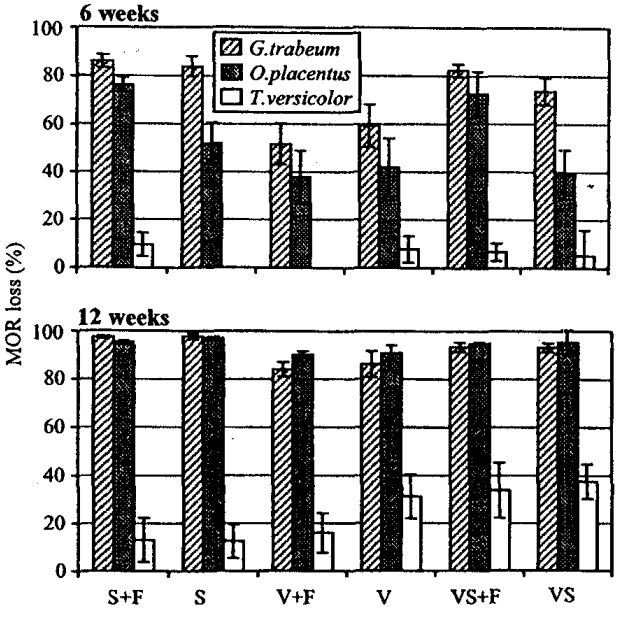

Fig. 4. Strength loss of specimens after exposure to decay fungi for 6 or 12 weeks. Error bars show 95% confidence intervals. See Fig. 3 legend for definitions.

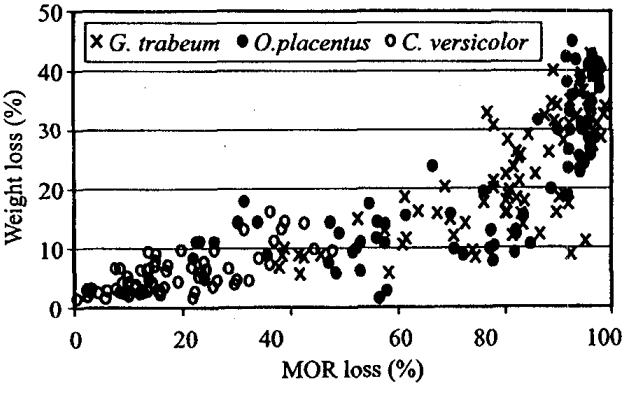

Fig. 5. Relationship between weight loss and strength loss for specimens exposed to decay fungi for 6 or 12 weeks.

hemicellulose was clearly degraded, with some indications (inconsistent due to variation in the data) that degradation of arabinan and galactan began at an earlier stage than did degradation of xylan and mannan.

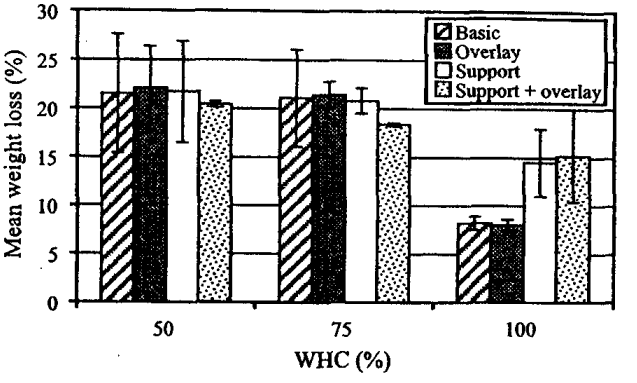

Fig. 6. Mean weight loss of specimens after 6 weeks exposure to *G. trabeum* under different initial culture moisture content levels. Error bars show 95% confidence intervals.

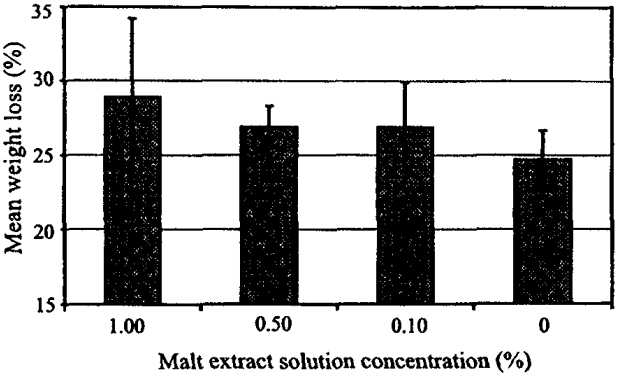

Fig. 7. Mean weight loss of samples after 6 weeks exposure to *G. trabeum* at different concentrations of added malt extract. Error bars show 95% confidence intervals.

4. Discussion

The in vitro test methods developed in this study produced substantial and rapid decay of southern pine by brown-rot basidiomycete fungi. The inability of the white-rot fungus to produce substantial levels of decay was most likely due to the use of a softwood species. Hardwoods contain higher levels of syringyl-type lignin as opposed to the guaicyl lignin found in softwoods. Guaicyl lignin is more resistant to degradation by white-rot fungi than is syringyl (Highley, 1982). Consequently, the use of a hardwood species

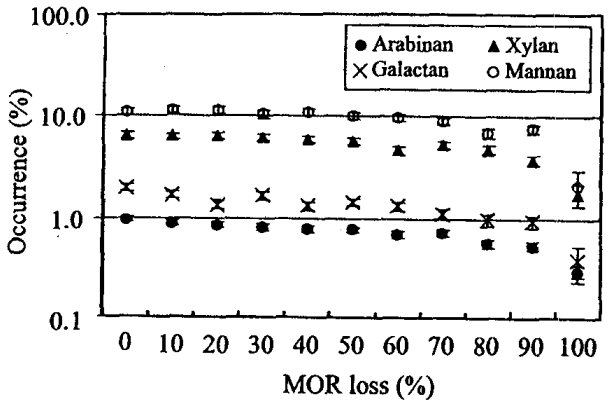

Fig. 8. Changes in hemicellulose content compared to loss in strength of specimens. resulting from brown-rot decay (mean data with 95% confidence interval error bars).

with the white-rot fungus would be expected to result in substantially greater weight and strength losses compared with the use of a softwood species (Schultz and Nicholas, 1997).

The direct inoculation method was slightly more effective than the basic feeder strip method, although the difference between these methods was not statistically significant. Nonetheless, other factors favor direct inoculation. Direct inoculation is simpler (fewer steps) than using feeder strips, and it may be a more accurate simulation of natural colonization in ground contact.

Waterlogging of the specimens exposed to fungi using the vermiculite systems reduced the levels of decay observed. However, reduction of the initial moisture content of the vermiculite from 100% to 75% WHC alleviated this problem. This action may remove the apparent decrease in decay potential of vermiculite as the culture medium in comparison to that of soil and vermiculite/soil. However, for *T. versicolor*, the greatest strength and weight losses were seen in the vermiculite systems, suggesting that the white-rot fungus favors the higher moisture level. This theory is supported by previous studies (Highley and Scheffer, 1970; Lea, 1982; Lea and Bravery, 1986). Therefore, if *T. versicolor* is used against a hardwood species, it can be inferred that the greatest levels of decay would be seen with vermiculite. Accordingly, it may be possible to modify the method to favor either brown- or white-rot fungi by changing the moisture content of the medium. Reducing the level of moisture used in the vermiculite system would likely make it as effective as the soil system. Vermiculite is also a more defined medium than is soil, and its use should improve reproducibility and remove questions on soil type and source. The method provides more information, i.e., strength data, than do other commonly used fungal test methods, e.g., BS 113 (British Standards Institution, 1981) or ASTM D2017 (ASTM, 1998a). The method also compared favorably, in terms of control of conditions and reproducibility, to a previous fungal/strength test method (Winandy and Morell, 1993). Thus, our method of choice for further studies is direct inoculation of vermiculite media at 75% WHC.

The results showed a direct relationship between strength and weight loss for both brown- and white-rot fungi (Fig. 5). Strength loss was a considerably more sensitive measure of incipient decay than was weight loss. For example, strength loss of 30% (*T. versicolor*) to 60% (*G. trabeum*) occurred at 10% weight loss.

The chemical analysis suggests a relationship between hemicellulose composition and the strength properties of wood. Degradation of arabinan and galactan appeared to increase as MOR loss increased, whereas mannan and xylan appeared to be degraded only in the later stages of the decay process, after approximately 60% MOR loss (Fig. 8). This supports previous work (Winandy and Morell, 1993) and provides the basis of the ongoing study on the relationship between strength properties and chemical composition.

5. Conclusions

In all cases, the "cake pan" exposure technique proved to be a useful tool. The use of feeder strips was shown to be unnecessary—direct inoculation of the substrate produced similar or greater levels of decay in the specimens. Although waterlogging of specimens exposed using the vermiculite system was a concern, the problem was circumvented by reducing the initial moisture content of the vermiculite. Vermiculite offers practical and experimental advantages over soil and is therefore our selected method for further studies. Optimizing parameters of our methodology may enable later standardization of this technique as a quantitative measurement, more sensitive than weight loss, of basidiomycete decay of untreated wood. Further studies are required to determine the extent of the relationship between strength loss, weight loss, and hemicellulose composition.

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