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Section 2 Test methodology and assessment

An experimental method to simulate incipient decay of wood by basidiomycete fungi.

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EXPERIMENTAL METHOD TO SIMULATE INCIPIENT DECAY OF WOOD BY BASIDIOMYCETE FUNGI.

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ABSTRACT
At very early stages of decay of wood by basidiomycete fungi, strength loss can be measured from wood before any measurable weight loss. Therefore, strength loss is a more efficient measure of incipient decay than weight loss. However, common standard decay tests (e.g. EN 113 or ASTM D2017) use weight loss as the measure of decay. A method was developed that allowed progressive removal of samples so that all stages of colonisation and decay could be monitored by strength testing, weight loss determination and chemical analysis. Our results indicated that substantial and rapid decay (90% strength loss and 40% weight loss after 12 weeks) of southern pine by brown rot fungi was possible using the method. Our results also demonstrate a direct relationship between strength loss and weight loss and suggest a quantitative relationship between strength loss and chemical composition (hemicellulose sugars) during incipient decay of southern pine by basidiomycete fungi.

Keywords: Incipient decay, test method, strength testing, chemical composition,

INTRODUCTION
At very early stages of biological decay, changes in the chemical composition of wood cause measurable reductions in strength from wood before measurable weight loss occurs. Therefore strength loss is a good indicator of early wood decay.

The study presented in this paper forms part of an ongoing program investigating the relationship between strength and chemical composition. A previous study (Winandy and Morrell, 1993) has shown a close relationship between the degradation of hemicellulose components, such as arabinose and galactose, and wood strength losses. The strength properties and chemical composition of fire-retardant treated wood are also systematically altered by hydrolytic chemicals (Winandy, 1995) with the rate of strength loss and change in chemical composition directly related to temperature (Winandy and Lebow, 1996). The changes in chemical composition appear to be similar in both the biological and chemical systems (Green *et al*, 1991). Understanding the relationship between chemical composition and strength loss may aid in developing a model to determine the strength loss caused by a variety of biological, chemical or thermal agents.

An *in vitro* study of the relationship between chemical composition and strength loss permits better control over the initiation, rate and termination of the biological decay. Decay tests however, normally use weight loss as the decay criteria rather than strength loss. Therefore, the designs of current standard methods are unsuitable for a study of this kind. One objective of this study was to design a suitable standardised methodology for relating chemical composition and strength loss to incipient decay *in vitro*.

In strength testing three point loading systems maximise the stress only directly under the bending head. However, fungal decay may not be uniform throughout the wood specimen and this maximum stress may not coincide with the decayed area. Winandy and Morrell (1993) used a four point bending test rather than the simple three point bending test normally used (ASTM, 1998 a, b, c.) and this was shown to produce a uniform bending force and stress between the supports (Winandy and Morrell, 1993). The four point bending test was part of the design criteria for our standardised method.

A reliable and practical test system was required that was suitable to simulate natural, early uniform decay of wood by brown and white rot fungi using test samples that could be subsequently analysed for strength and chemical composition.

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

METHODS

Preparation of wood specimens

Southern pine (*Pinus spp*) sapwood test specimens (25.3cm x 2.5cm x 0.95cm) and "feeder" strips (21.5cm x 1.9cm x 0.6cm) were prepared, clear of knots, damage or defect and were air dry. A 5cm section was removed from the end of each test specimen for determination of moisture content and estimation of initial dry weight and density.

The specimens were sterilised using steam at 110°C for 1 hour

Test fungi

Three species of fungi were used; 2 brown rot fungi and 1 white rot fungus;

Brown rot fungi

Gloeophyllum trabeum (Pers. Ex Fr) Murr (Mad 617)
Postia placenta (Fr.) M. Larsen and Lombard (Mad 698)

White rot fungus

Trametes (Coriolus) versicolor (L:Fr.) Pilat (Mad 697)

Biological exposure

In the basic system (Les Ferges method in Soltis *et al*, 1992) the 1L of soil was placed into a lidded aluminium pan (33 x 23 x 8.25cm) (Mirro corp. Manitowoc, Wisconsin) so that the bottom of the pan was covered. The media was then formed into a ridge running the length of the long axis. The feeder strip was placed onto the ridge and inoculated with the test fungus. The test sample was placed onto the feeder strip, perpendicular to the long axis of the feeder so that only the middle section of the sample was in contact with the feeder and no part was in contact with the media. For this investigation the basic method was modified as follows:-

Media:- Three different media were used;

- Soil,
- Vermiculite (horticultural grade) (Scotts, Marysville, Ohio, USA)
- Vermiculite/soil mixture.

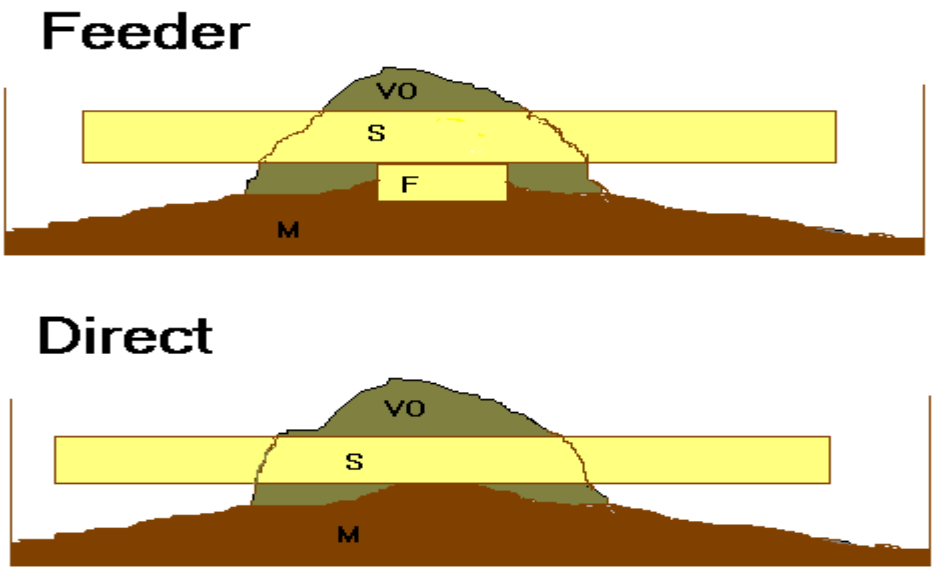

Figure 1 Diagrammatic representation of the feeder strip and direct inoculation methods. (VO = vermiculite overlay, S = specimen, F = feeder strip, M = media)

Preparation of media

The moisture holding capacity (WHC) for each of 6 random samples of each of the media was determined using the method described in ASTM D2017-94 (ASTM 1998d). The pans were then loaded with the media in the following ways: -

Soil:

1L of soil with deionised water added to give a moisture content of 100% of its WHC.

Vermiculite:

1L of vermiculite with 1% aq malt extract solution (10g malt extract, 1litre deionised water) added to give a moisture content of 100% of its WHC

Vermiculite and soil

1L of a 30/70% v/v mixture of soil/vermiculite with deionised water added to give a moisture content of 100% of its WHC.

In each case, the media was shaped into a ridge and feeder strips were added to those trials requiring them.

The pans were then autoclaved at 15 psi and 121°C for 45 minutes.

Fungal inoculation

For each fungus 3 week old liquid cultures (Baileys minimal media pH 4.5 + 0.5% cellobiose (Sigma chemicals St Louis, MO)Highley, 1973)) were combined and then vigorously mixed for 30 seconds using a blender (Waring). The resulting mycelial suspension was applied using 2 methods:-

- Southern pine feeder strips were pre-inoculated with 5ml of the mycelial suspension 7 days before specimen insertion

- The media was directly inoculated with 100ml of the mycelial suspension 7 days before specimen insertion. No feeder strip was used.

Sterile (uninoculated) controls were also used for each media combination.

Specimen insertion
The wood specimens were aseptically inserted into the pans (8 per pan, 2 pans per media/fungus combination) with the middle section resting either on the feeder strip or on the ridge of media, so that neither end touched the surface of the media.

Overlay
An overlay of moist vermiculite was used to maintain a sufficiently high moisture content in the specimens and to confine the majority of fungal activity to the central third of the specimens. The moisture content of the vermiculite was adjusted to 50% of its WHC and sterilized by autoclaving. The overlay was applied, in all trials, to cover the middle third of the specimens (67mm) to a depth of approximately 1 cm.

Exposure period.
The cultures were incubated at 25°C (80°F) and 70% relative humidity for a maximum duration of 12 weeks, with sampling at 6 and 12 weeks.

Assessment of test
At each sampling time the 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 an hour to lower the moisture content of the specimens. The specimens were then allowed to equilibrate to constant weight at 23°C (74°F) and 65% relative humidity prior to mechanical testing. A four point bending test (Winandy and Morrell, 1993) using a load head span of 175mm and a loading rate of 1.25mm/min was used to determine MOR values for the specimens. The MOR value of each specimen was compared to that of unexposed controls to determine the strength loss of each specimen.

Following the mechanical testing two 15mm by full cross section block was then cut from each specimen from within the decay zone near the point of mechanical failure. On eof the blocks was then oven dried. Loss in dry weight was then determined, based on an estimate of original dry weight derived from the dry weight and density of the 5 cm section removed prior to the decay test.

The second block was ground to 30 mesh (547 *m*m). Ground material from each replicate for each fungal-exposure combination was combined and analysed for carbohydrates using high-pressure liquid chromatography (HPLC).

Effect of initial media moisture content
To determine whether waterlogging of the specimens was occurring and inhibiting decay in the vermiculite system, a subsequent study was carried out to determine the optimal initial moisture content of the vermiculite media. Pre-weighed southern pine specimens (50mm x 25mm x 9.5mm) were placed into aluminium pans (33 x 23 x

8.25cm) containing 1 litre of vermiculite adjusted to 50%, 75% or 100% of its WHC (as determined by ASTM D2017-94 (ASTM 1998d)). In the test pans, the vermiculite was inoculated with 5ml of a hyphal suspension of the brown rot fungus, *Gloeophyllum trabeum*. Controls consisted of identically prepared pans that were not inoculated. The length of exposure was 6 weeks, at which point the specimens were removed, cleaned of adhering mycelium, weighed, oven dried and weighed again to determine final moisture content and loss in dry weight.

RESULTS

Weight loss

The weight loss results (Figure 2) show significant decay occurred (over 20% weight loss) with the brown rot fungi under all test conditions after 12 weeks exposure. The direct inoculation method gave higher weight losses than the comparative feeder strip method, although at 12 weeks exposure there were only statistically significant (p0.05) differences between the soil and soil/feeder combinations. In comparing the two brown rot fungi, *G.trabeum* gave statistically significant (p0.05) higher weight losses than *P.placenta* in all but the vermiculite method after 6 weeks of exposure. After 12 weeks exposure however, the only statistically significant difference between the fungi was a higher weight loss caused by the *G.trabeum* in the soil and feeder method.

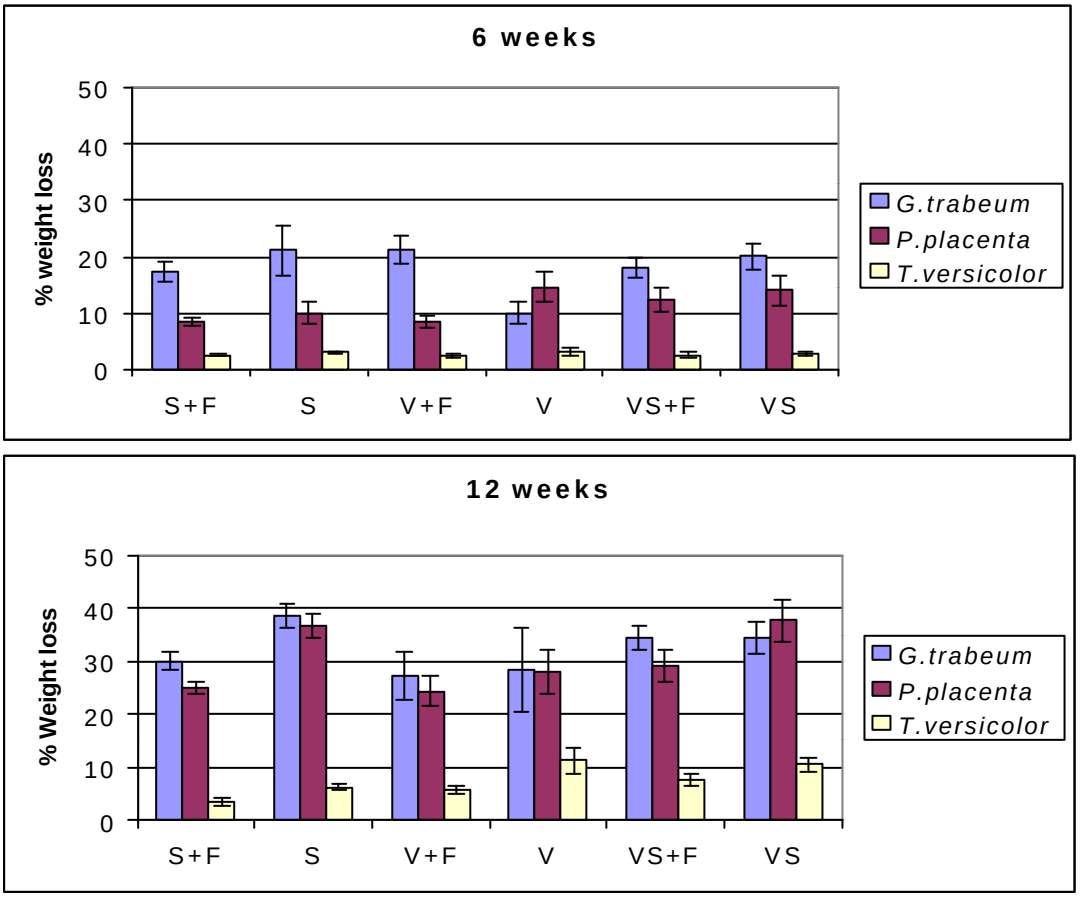

Figure 2 Mean weight loss of samples after exposure to decay fungi for 6 and 12 weeks. (error bars show 95% confidence intervals) (S + F = soil and feeder, S = Soil (no feeder), V +F = vermiculite and feeder, V = vermiculite (no feeder)VS + F = vermiculite/soil and feeder, VS = vermiculite/soil (no feeder))

Generally for the brown rots, weight loss for vermiculite and feeder (V + F) and vermiculite (V) methods was lower than that seen using the other methods. The final moisture content of the specimens in these set ups was also significantly higher (p0.05) than that of specimens in other methods. For example the moisture contents of the specimens exposed to *G.trabeum* under the differing methods are given in table 1.

Table 1. Mean percentage moisture content of specimens exposed to *G.trabeum* for 6 and 12 weeks.

	Soil + feeder	Soil	Vermiculite + feeder	Vermiculite	Vermiculite /soil + feeder	Vermiculite /soil
6 Weeks	30.50	44.11	89.59	119.40	52.56	91.84
12 Weeks	40.80	58.60	82.00	119.51	62.26	100.57

The white rot fungus, *T.versicolor*, showed much lower weight losses than the brown rot fungi. The results do show however, that statistically significant (at p0.05) higher weight losses were seen with the vermiculite method and the vermiculite/soil methods in comparison to the other methods.

Strength loss

Strength losses caused by the brown rot fungi were 40 – 80% after only 6 weeks exposure (Figure 3). *G.trabeum* generally caused higher strength loss, but at 6 weeks the difference was only statistically significant (p0.05) using the soil/feeder, soil and vermiculite/soil methods. At 12 weeks, there were no significant differences (p0.05) between the brown rot fungi. Lower strength losses were seen with the vermiculite/feeder and vermiculite methods in comparison to the other methods for the brown rot fungi.

Strength losses caused by *T.versicolor* were considerably less than those caused by the brown rot fungi, with the greatest strength losses being seen in the vermiculite, vermiculite/soil/feeder and vermiculite/soil methods.

Effect of initial media moisture content.

The mean percentage weight losses at the differing moisture conditions are shown in table 2. The weight loss at 100% WHC is statistically lower (p0.05) than the weight losses at both 50% WHC and 75% WHC.

Table 2 Mean weight loss of samples exposed to G.trabeum at different initial media moisture levels.

	Percent of WHC		
	50%	**75%**	**100%**
Mean weight loss	21.50	21.10	8.22
95% confidence interval	6.10	5.00	0.70

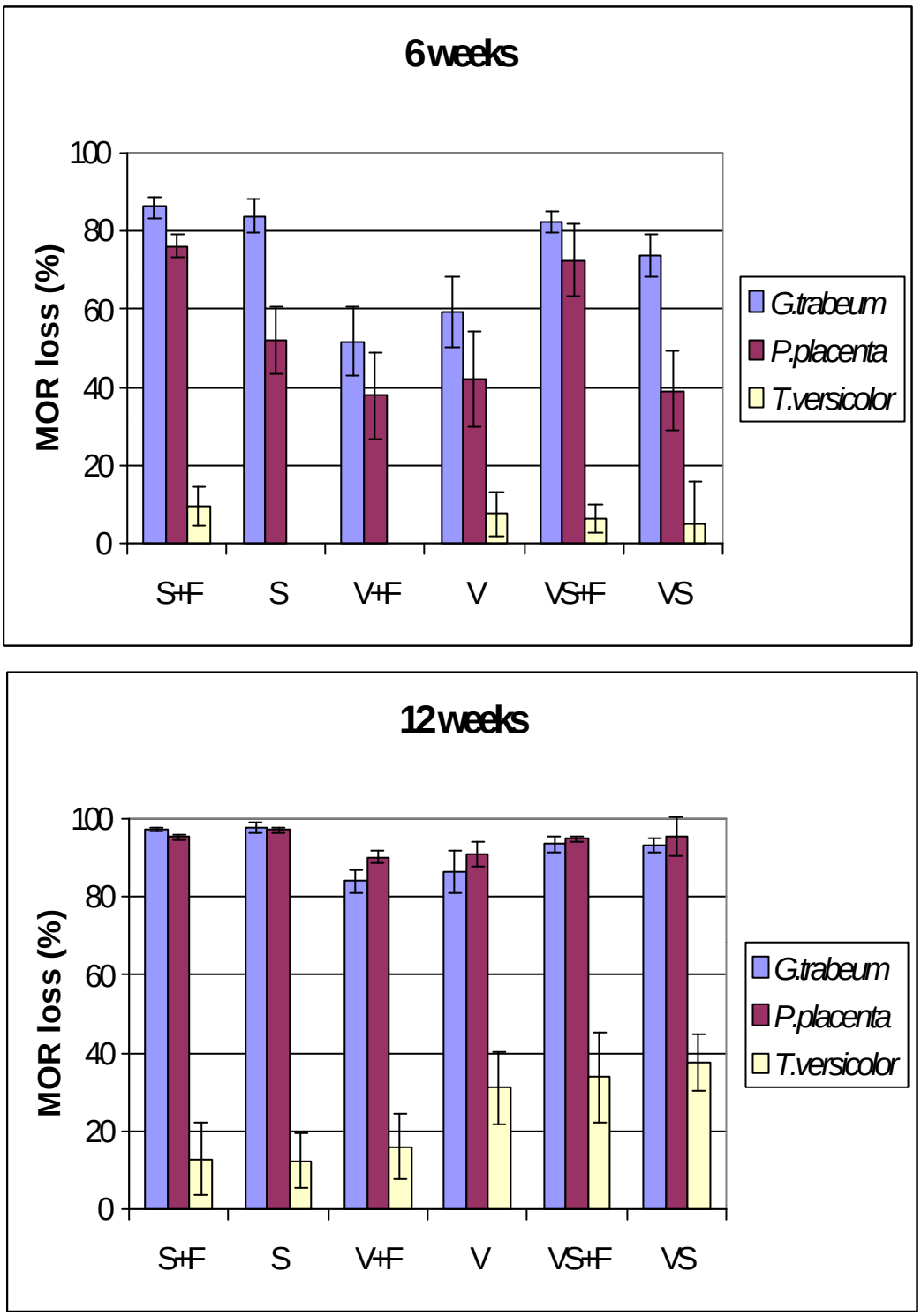

Figure 3 Strength loss of samples after exposure to decay fungi for 6 and 12 weeks. (error bars show 95% confidence intervals) (S + F = soil and feeder, S = Soil, V +F = vermiculite and feeder, V = vermiculite VS + F = vermiculite/soil and feeder, VS = vermiculite/soil)

Strength/weight loss relationship

Strength loss and weight loss data were compared graphically, for each fungus (Figure 4).

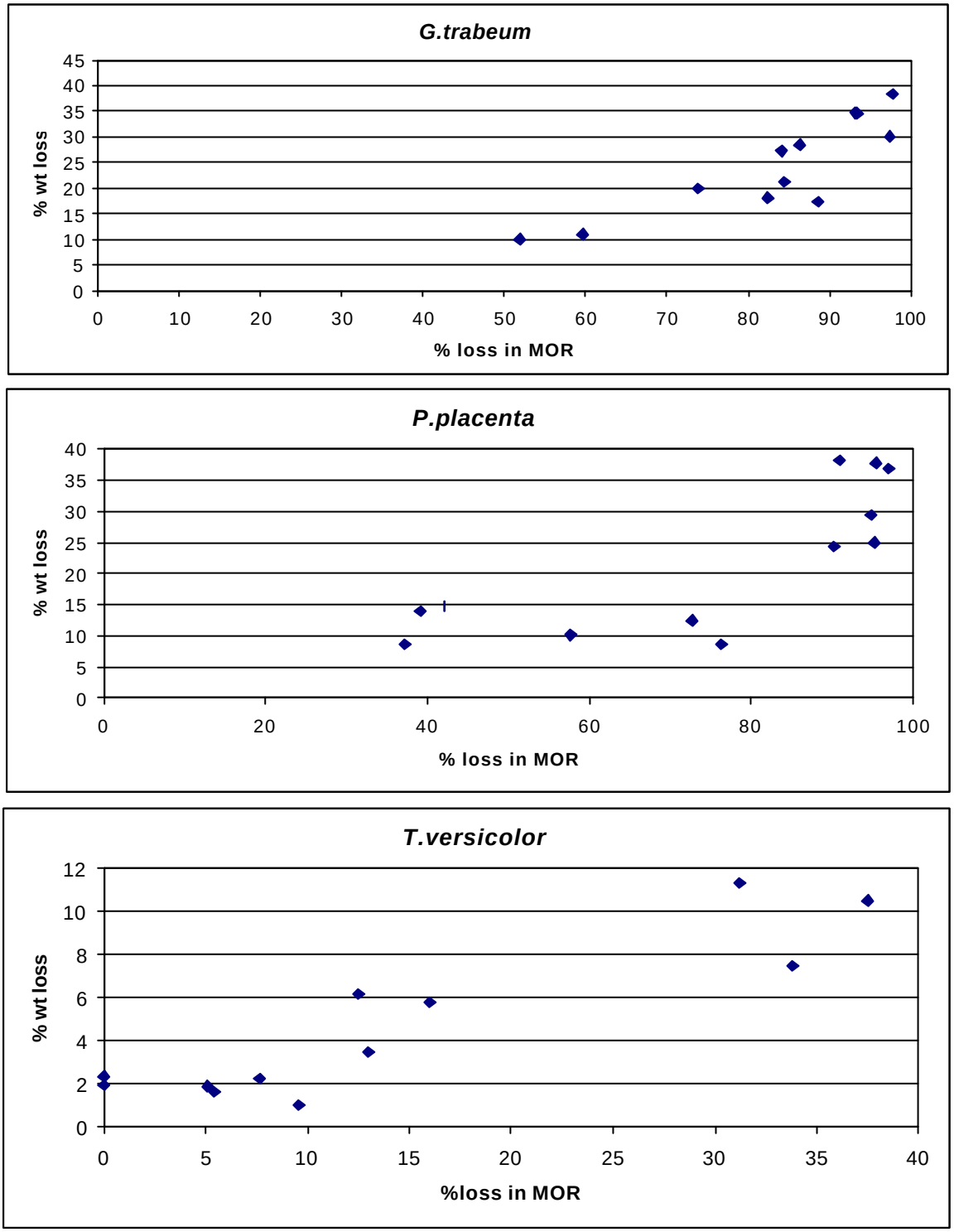

Figure 4. Relationship between weight loss and strength loss

Chemical analysis

The percentage occurrence of the hemicellulose sugars arabinan, galactan, xylan and mannan is compared graphically to loss in MOR in Figure 5. The data for the brown rot fungi shows that the levels of arabinan decrease with increasing loss of MOR until at 60 – 70% loss in MOR the levels reached approximately half of their original value. In contrast, levels of xylan and mannan appeared to remain stable until 30-40% MOR loss was achieved at which point the levels began to decrease. In the case of the white rot fungus, *T.versicolor*, the levels of all components remained stable, however it should be noted that the loss in MOR with this fungus was considerably lower than that seen with the brown rot fungi.

DISCUSSION

The *in vitro* test methods were shown to be effective for enabling substantial and rapid decay of southern pine by brown rot basidiomycete fungi. That the white rot fungus did not cause substantial levels of decay is most likely due to the wood species used, i.e. a softwood. If a hardwood species were used, instead of the softwood, substantially higher weight and strength losses could be expected with white rots, using this method.

In terms of effectiveness of the method, using direct inoculation rather than feeder strips showed some slight advantage, although this advantage was not statistically significant. Other factors favour direct inoculation however. Direct inoculation is simpler (fewer steps) and may be a more accurate simulation of natural colonisation in ground contact. The vermiculite systems appeared to be less effective than the other methods. This was shown to be due to waterlogging of the specimens inhibiting the decay to some degree. Reduction of the initial moisture content of the vermiculite from 100% of WHC to 75% of WHC was shown to alleviate this problem. This action would also remove the apparent decrease in decay potential of vermiculite in comparison to soil and vermiculite/soil as the test media. However, the white rot fungus appeared to favour the higher moisture. Its is therefore apparent that the test can be modified for both brown and white rot fungi by changing the moisture content of the media.Vermiculite is also a more defined media than soil and its use should improve replication of results in subsequent tests and remove questions on soil type and source. The method of choice for further studies is therefore vermiculite media (at 75% of WHC) using direct inoculation.

Comparison of the strength loss and weight loss data (Figure 4) demonstrates that a direct relationship exists between strength loss and weight loss, in both the brown and white rot fungi tested. Strength loss was also shown to be a considerably more sensitive measure of early decay than weight loss with, for example, strength losses of 30% (*T.versicolor*) to 60% (*G.trabeum*) measured at 10% weight loss.

The data on the chemical composition suggest that there is a relationship between the hemicellulose composition and the strength properties of the wood. The arabinan (a side chain element of hemicellulose) appears to be degraded first by *G.trabeum* (Figure 5). With *P.placenta* the decrease in arabinan is accompanied by a similar decrease in galactan (also a side chain element of hemicellulose)

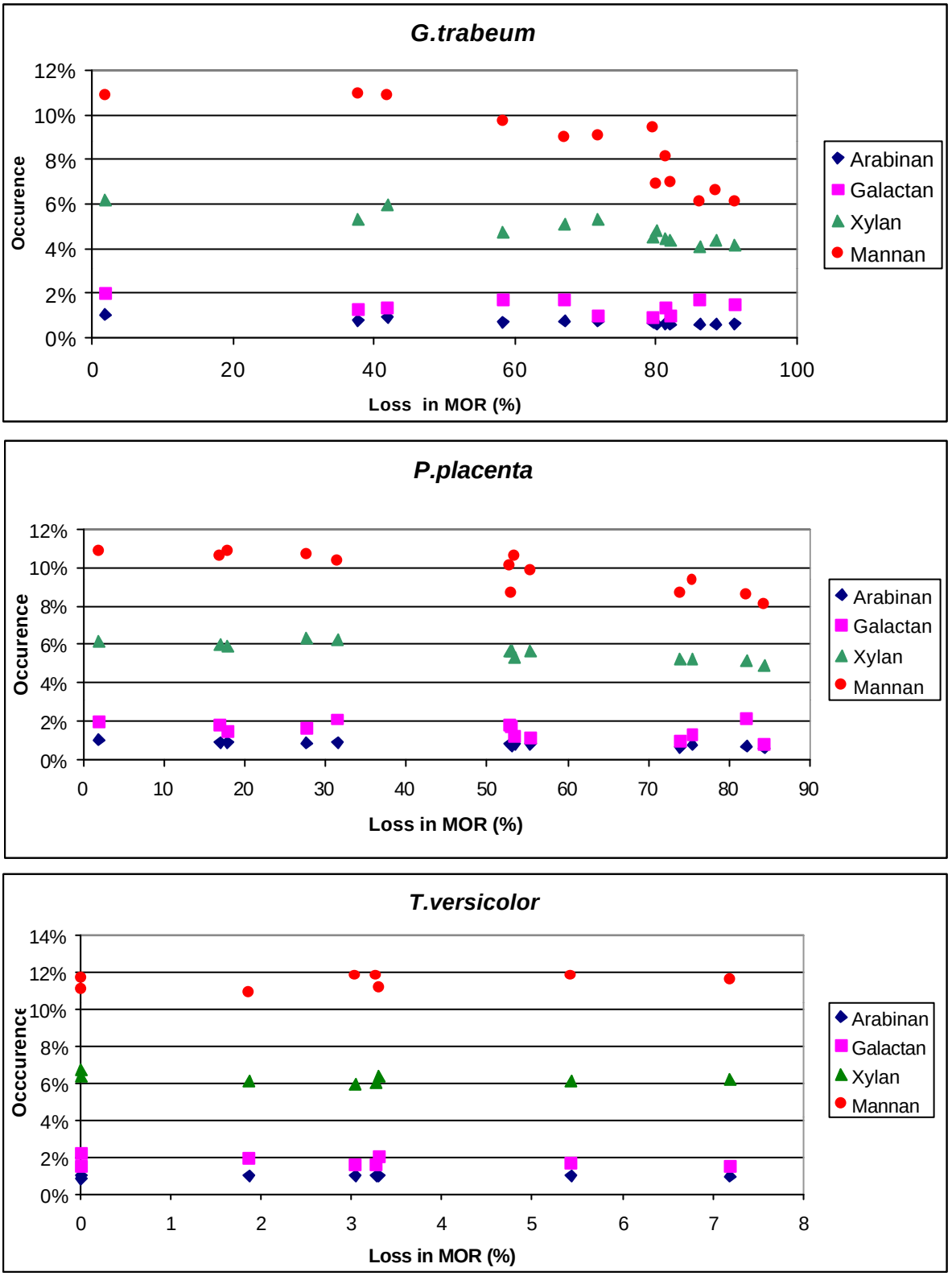

Figure 5 Comparison of hemicellulose composition and loss in MOR.

Mannan and possibly xylan ("back-bone" elements of hemicellulose) appear to be degraded later in the decay process e.g. after approximately 40% MOR loss (Figure 5). This supports previous work (Winandy and Morrell, 1993)

The technique detailed in this paper provides a useful investigation tool for further studies. Optimising parameters of our methodology will enable standardisation of this technique as a quantitative measurement for incipient decay.

CONCLUSIONS

From these results a number of conclusions can be drawn:

- Strength loss is a more sensitive measure of incipient decay than weight loss.
- A direct relationship exists between strength and weight loss.
- A relationship exists between strength loss and hemicellulose composition.
- The "Cake-pan method" using vermiculite media and direct inoculation is an effective system for investigating basidiomycete decay of untreated wood using strength as the decay criteria.

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