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Soil-Contact Decay Tests Using Small Blocks

A Procedural Analysis

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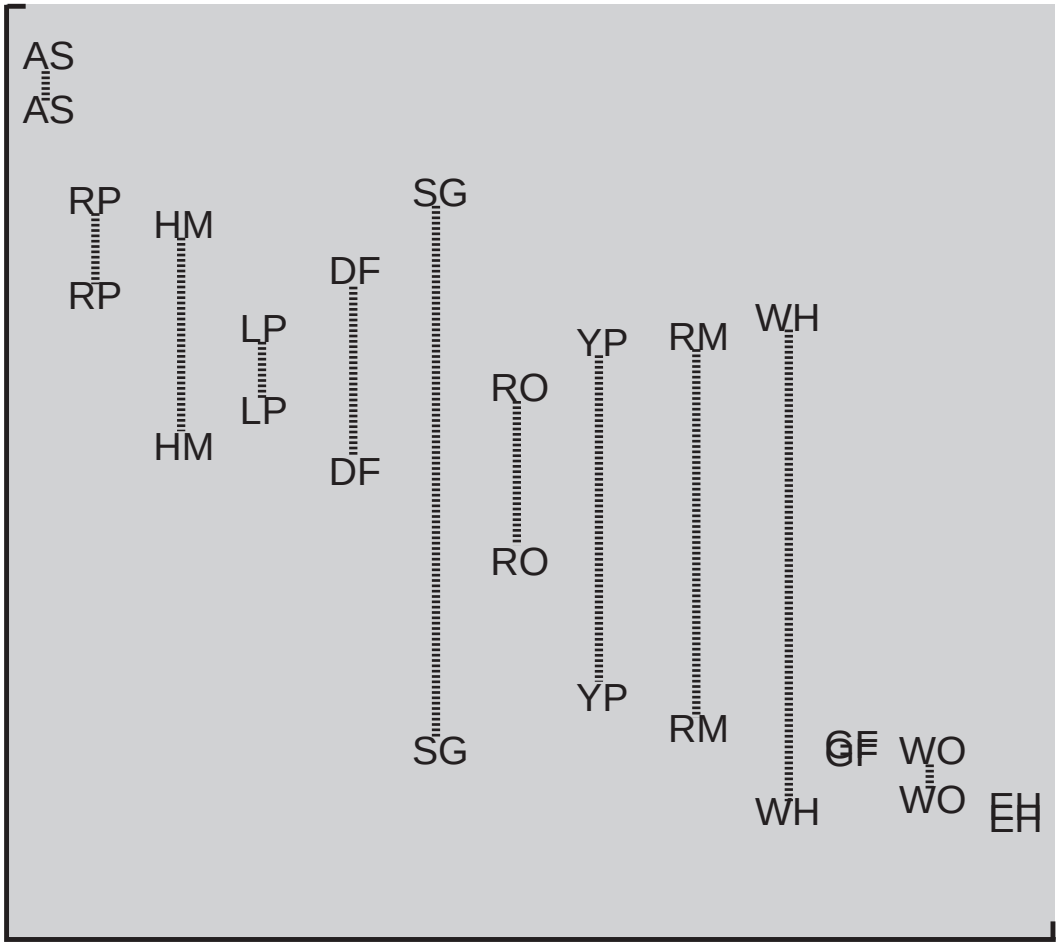

Abstract

Much discussion has been held regarding the merits of laboratory decay tests compared with field tests to evaluate wood preservatives. In this study, procedural aspects of soil-jar decay tests with 1 cm^3 blocks were critically examined. Differences among individual bottles were a major source of variation in this method. The reproducibility and sensitivity of the soil-jar method using small blocks must be further characterized before it can be accepted as a standard protocol for evaluating preservative-treated wood.

Keywords: Decay, preservatives, methods

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Soil-Contact Decay Tests Using Small Blocks

A Procedural Analysis

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Introduction

Although considerable debate exists concerning the merits of using laboratory decay tests compared with field tests to evaluate wood preservatives, the use of small-volume wood blocks in laboratory soil-jar decay studies holds promise as a rapid method to evaluate new preservatives in a variety of wood species (Bravery 1979; Scheffer and others 1987, 1988). The study herein was initiated to (1) characterize the capacity of white- and brown-rot fungi to decay different wood species when using this soil-jar method and (2) examine potential sources of variability within the procedure.

Procedurally, this soil-jar technique parallels that of the standard ASTM soil-block test (ASTM 1995) in that weight loss is used as a measure of fungal attack in blocks that are incubated under conditions of controlled temperature and humidity for defined periods. The major differences are that the wood blocks are 1 cm^3 rather than 6.9 cm^3 (19- by 19- by 19-mm) in the ASTM technique and the incubation vessels are smaller than those used in the ASTM procedure.

Developmental research (Duncan 1958) that led to the ultimate standardization of a soil-block procedure showed that species of decay fungi respond differently to microenvironmental conditions in the standard 60 ml (8-oz) jars. Most fungi were unaffected by a range of soil moisture levels, but fungi with a relatively high tolerance to a particular preservative were sensitive to variations in soil moisture content. The threshold values for preservatives were judged to be similar for soils having a water-holding capacity within a range of about 20% to 40% if the initial moisture content was about 130% of that capacity.

In a preliminary study, we used the soil-jar method to investigate the effects of both soil moisture content and incubation temperature on the capability of several white-rot fungi to decay wood. Unfortunately, the variability in our results diminished our ability to interpret the potential effects of these two experimental parameters. Subsequently, we conducted a second study at a constant, presumed optimal temperature and with soil at a uniform initial moisture content.

Methods

Both softwood and hardwood species were used in this study (Table 1). Seven decay fungi were used (Table 2), but not in a completely balanced design. *P. merismoides* was used only with softwoods; *X. frustulatus* was used only with hardwoods.

Untreated 1-cm^3 wood blocks were cut from defect-free sapwood of respective tree species. Blocks were ovendried at 54°C, weighed before being placed in vessels containing deionzed water, subjected to a 30-min vacuum at −92 kPa, followed by a 1-h pressure of 0.86 MPa (125 lb/in^2). This process was used to minimize the time normally required for moisture uptake by the block from the soil and feederstrip. Blocks were at 80% to 100% moisture content at the start of the test. The blocks were blotted dry to remove excess moisture, weighed to determine uptake of water, then sterilized by subjecting them to 25μGy (2.5 mrads) ionizing radiation from a cobalt 60 source.

Small glass bottles (60 ml) were filled with 25 g of forest soil that had a minimum moisture holding capacity of 60%. Moisture content of the soil was adjusted to 100% (wt/wt). A small feederstrip of sapwood (3 by 15 by 15 mm long) was placed on the surface of the soil. Feeder strips of western hemlock (*T. heterophylla*) were used with brown-rot fungi.

Table 1—Wood species[a] used in the decay test

Softwoods		Hardwoods	
Common name	Scientific name	Common name	Scientific name
Grand fir	*Abies grandis* (Dougl.) Lindl.	Red maple	*Acer rubrum* L.
Lodgepole pine	*Pinus contorta* Dougl.	Hard maple	*Acer saccharum* Marsh.
Red pine	*Pinus resinosa* Ait.	Sweetgum	*Liquidambar styraciflua* L.
Douglas-fir	*Pseudotsuga menziesii* (Mirb.) Franco	Yellow poplar	*Liriodendron tuliplifera* L.
Western hemlock	*Tsuga heterophylla* (Raf.) Sarg.	Aspen	*Populus tremuloides* Michx.
Eastern hemlock	*Tsuga canadensis* (L.) Carr.	White oak	*Quercus alba* L.
		Red oak	*Quercus rubra* L.

[a]Sapwood was used with all wood species except aspen, which was used without determining heartwood or sapwood.

Table 2—Fungi used in study of the decay capacity of fungi in different wood species

White-rot fungi	Brown-rot fungi
Trametes versicolor (L.:Fr.) Pilát R105	*Postia placenta* (Fr.) M. Larson et Lombard MAD698
Phlebia merismoides (Fr.:Fr.) Fr 514A	*Gloeophyllum trabeum* (Pers.:Fr) Murr. MAD617
Phlebia subserialis (Bourd & Galzn) Donk RLG10693-SP	*Wolfoporia cocos* (FAWolf) Ryv. and Gilbt. FP104264-SP
Xylobolus frustulatus (Pers.:Fr.) P.Karst 106073-R	

Feeder strips of sweetgum (*L. styraciflua*) were used with white-rot fungi. Then, the jars were loosely capped and sterilized (45 min at 121°C). After cooling, the feeder strip was inoculated with a small agar disc cut from the edge of an actively growing test fungus. The jars were incubated at room temperature until the fungus nearly covered the wood surface. Then, sterile test blocks were added transverse face down. After the sterile blocks were inserted, bottles were incubated at 32°C for 12 weeks. The incubators did not allow for humidity control. Humidity was maintained by placing trays of water on the bottom of the incubators and minimizing opening and closing of the incubator doors.

Two blocks of each wood species were incubated in each of two bottles per fungus. The identity of the bottles was followed in this study. This enabled comparison of results between paired bottles per wood species and results for blocks within bottles. The design used for this analysis was a random design, with subsampling and a two-way factorial treatment structure.

Possible changes in wood moisture content during the course of the decay trial were also examined as a source of variation within the experiment. Moisture content of the wood was determined when blocks were removed from the bottles at the conclusion of the incubation.

Results

Data were initially analyzed using a general linear model (SAS 1989). The difference between bottles in rates of decay in blocks of the same wood species was a significant source of variation. Comparisons between paired bottles for each wood species revealed significant differences between bottles, compared to within bottle variation, in percentage weight loss for all species except eastern hemlock, white oak, and Douglas-fir. Results for Douglas-fir were only marginally nonsignificant at the 95% level of probability. Western hemlock and Douglas-fir usually had less weight loss than did most other wood species, regardless of fungus. The susceptibility of white oak to decay varied from midrange to the lower third probability level, depending upon fungus.

When the difference between pairs of bottles and within bottle variation was examined for individual fungi across all wood species, the between bottle difference was significant for *G. trabeum, P. subserialis,* and *T. versicolor*. These fungi were the three most virulent against hardwoods (Tables 3–6)

Table 3—Rank of various decay fungi by percentage weight loss caused within each respective wood species

Fungus	Rank[a] of decay in softwoods					
	Douglas-fir	Eastern hemlock	Western hemlock	Grand fir	Lodgepole pine	Red pine
G. trabeum	1		2	x	1	1
P. merismoides	3	3	x[b]	2	3	
P. subserialis		2	3	3	x	3
P. placenta	2	1	1	1	2	2
T. versicolor			x	x		x
W. cocos			x	x		

Fungus	Rank of decay in hardwoods						
	Aspen	Sweet-gum	Hard maple	Red maple	Yellow poplar	Red oak	White oak
G. trabeum	1	3	2		x	1	x
T. versicolor		x	1	1	2		3
P. merismoides		x			x		
P. subserialis	2	1	3	2	1	x	2
P. placenta	3	2	x	3	3	2	1
W. cocos		x			x	3	x
X. frustulatus		x			x		

[a]Ranked in decreasing order; 1 indicates most decay.
[b]Within each wood species (column), percentage weight loss caused by fungus (indicated by an "x") was less than but not significantly different from maximum weight loss (ranked 1) observed in that wood species.

and were nearly as aggressive in softwoods (Tables 3–5, 7). This finding indicates that the number of replicates per species in this experiment was two (bottles) rather than four (blocks). Therefore, subsequent discussion of results are based upon a statistical analysis of an experiment with two replicates. The mean percentage weight loss of the two blocks in each bottle was used as an individual resultant variable.

When evaluated for all wood species and fungi, final wood moisture content was significantly different between the two bottles per wood species by fungus combination.

Only a few fungi caused a relatively large weight loss. One white-rot fungus, *P. subserialis*, and two brown-rot fungi, *P. placenta* and *G. trabeum*, caused the most decay in nearly all wood species. Exceptions were the maple species, which were most severely degraded by *T. versicolor*, a white-rot fungus. In softwoods, the maximum weight loss was caused by *G. trabeum* or *P. placenta*. *G. trabeum* caused more decay in red oak than did *X. frustulatus*.

The complete array of wood species was challenged with three brown-rot fungi (*G. trabeum, P. placenta,* and *W. cocos*) and two white-rot fungi (*P. subserialis* and *T. versicolor*). Statistically significant differences were not detected between many wood species in susceptibility to those fungi, even though actual differences between means were of some magnitude (Tables 4,5). Parametric and nonparametric analyses of variance (ANOVA), followed by a Tukey multiple comparison test, produced similar results. The absence of a significant difference between species attacked by *G. trabeum* was particularly striking (Fig. 1), even with substantial differences among species in average percentage weight loss. This result was due to the heterogeneity of variance in the experiment. A large variation between bottles was observed with some species but not with others (Fig. 1).

The weight losses caused by white-rot fungi (Table 5) in hardwoods and softwoods were not significantly different, even though a considerable range in weight loss occurred among the wood species. Hardwoods and softwoods were intermixed in their susceptibility to brown-rot fungi. Hardwoods are considered to be more susceptible to white-rot fungi, but this distinction can disappear when wood is in soil contact.

Table 4—Weight loss of wood species as a result of decay by brown-rot fungi

Fungi	Species	Mean weight loss (%)	Significance[a]
G. trabeum			
	Aspen	73.9	A
	Red pine	60.3	A
	Hard pine	53.6	A
	Lodgepole pine	50.7	A
	Douglas-fir	50.7	A
	Sweetgum	42.5	A
	Red oak	42.1	A
	Yellow poplar	38.5	A
	Red maple	37.2	A
	Western hemlock	34.9	A
	Grand fir	20.1	A
	White oak	17.7	A
	Eastern hemlock	15.9	A
P. placenta			
	Yellow poplar	55.1	A
	Red pine	51.7	A B
	Douglas-fir	50.1	A B
	Red maple	45.9	A B C
	Sweetgum	43.8	A B C
	Lodgepole pine	43.6	A B C
	Eastern hemlock	43.3	A B C
	Hard maple	42.1	A B C
	White oak	41.5	B C
	Aspen	41.1	B C
	Western hemlock	40.5	B C
	Red oak	33.7	C D
	Grand fir	26.4	D
W. cocos			
	Yellow poplar	40.7	A
	Aspen	35.9	A B
	Sweetgum	22.2	A B
	Hard maple	16.7	A B
	Red maple	16.2	A B
	Grand fir	14.9	A B
	Eastern hemlock	13.4	A B
	Lodgepole pine	10.2	A B
	Douglas-fir	8.4	A B
	White oak	7.7	B
	Red pine	6.1	B
	Western hemlock	3.5	B
	Red oak	3.5	B

[a]Means with same letter are not significantly different ($\alpha = 0.05$).

Table 5—Weight loss of wood species as a result of decay by white-rot fungi

Fungi	Species	Mean weight loss (%)	Significance[a]
P. merismoides			
	Lodgepole pine	29.1	A
	Grand fir	23.3	A B
	Eastern hemlock	18.3	A B
	Douglas-fir	14.8	A B C
	Red pine	7.5	B C
	Western hemlock	-1.6	C
P. subserialis			
	Aspen	71.4	A
	Yellow poplar	67.7	A B
	Sweetgum	57.1	A B C
	Red maple	54.1	A B C
	Hard maple	51.2	A B C
	White oak	29.5	A B C
	Red pine	28.8	A B C
	Western hemlock	25.2	A B C
	Grand fir	22.9	A B C
	Lodgepole pine	21.5	A B C
	Eastern hemlock	20.2	B C
	Red oak	18.9	B C
	Douglas-fir	13.6	C
T. versicolor			
	Red maple	82.8	A
	Hard maple	71.8	A
	Yellow poplar	59.5	A B
	Aspen	40.3	A B C
	Sweetgum	38.1	A B C
	White oak	18.0	B C
	Red pine	15.9	B C
	Red oak	13.6	B C
	Lodgepole pine	12.4	B C
	Douglas-fir	9.8	C
	Grand fir	6.2	C
	Eastern hemlock	4.6	C
	Western hemlock	1.1	C

[a]Means with same letter are not significantly different ($\alpha = 0.05$).

As shown in Tables 4 and 5, aspen, hard maple, red maple, sweetgum, and yellow-poplar were the species most susceptible to decay. No significant difference was detected among fungi in their ability to decay sweetgum and yellow-poplar. Even though significant differences were detected among fungi in their capacity to decay aspen and the two maple species, both white- and brown-rot fungi were included within the group that produced the most decay within each respective wood species (Table 6).

Douglas-fir, eastern hemlock, grand fir, red oak, and white oak were the least susceptible to decay. Mean percentage weight loss caused by fungal species was not significantly different for grand fir. *G. trabeum* caused the most decay in red oak and the pines. With hardwoods (Table 6) and softwoods (Table 7), the relative difference between mean percentage weight loss caused by the most virulent or the two most virulent fungi and the remaining fungi was often quite large.

The initial uptake of water was uniform within individual wood species, but varied among species. At the conclusion of the test, the mean wood moisture content was not limiting for decay.

Table 6—Weight loss caused by six decay fungi in three hardwoods

Hardwoods	Fungi	Mean weight loss (%)	Signi-ficance[a]
Aspen			
	G. trabeum	73.9	A
	P. subserials	71.4	A
	P. placenta	41.1	B
	T. versicolor	40.3	B
	W. cocos	35.9	B
	X. frustulatus	31.9	B
Hard maple			
	T. versicolor	71.8	A
	G. trabeum	53.6	A
	P. subserials	51.2	A
	P. subserials	42.1	A B
	W. cocos	16.7	B C
	X. frustulatus	10.0	C
Red maple			
	T. versicolor	82.8	A
	P. subserials	54.1	A B
	P. placenta	45.9	A B
	G. trabeum	37.2	B
	W. cocos	16.2	B
	X. frustulatus	11.9	B

[a]Means with the same letter are not significantly different ($\alpha = 0.05$).

Table 7—Weight loss caused by six decay fungi in three softwood species

Softwoods	Fungi	Mean weight loss (%)	Signi-ficance[a]
Douglas fir			
	G. trabeum	50.5	A
	P. placenta	50.1	A
	P. merismoides	14.8	B
	P. subserials	13.6	B
	T. versicolor	9.8	B
	W. cocos	8.4	B
Eastern hemlock			
	P. placenta	43.3	A
	P. subserials	20.2	B
	P. merismoides	18.3	B C
	G. trabeum	15.9	B C
	W. cocos	13.4	B C
	T.versicolor	9.8	C
Lodgepole pine			
	G. trabeum	50.7	A
	P. placenta	43.6	A B
	P. merismoides	29.1	A B
	P. subserials	21.5	A B
	T. versicolor	12.4	B
	W. cocos	10.2	B

[a]Means with the same letter are not significantly different ($\alpha = 0.05$).

There was a significant correlation between percentage weight loss in individual wood species and final moisture content of wood blocks at time of removal. The most pronounced correlation between these two parameters within an individual wood species was demonstrated by red oak (Fig. 2). A similar, but less well-defined, correlation occurred for red maple. The comparison of moisture content and weight loss data for aspen fell into two clusters. One cluster of data indicates 30% to 40% weight loss was associated with final moisture content of about 50% to 90%. Two other observations of weight losses of about 70% had wood moisture content levels greater than 200%. The relationship between percentage weight loss and final wood moisture content was less apparent for the other wood species.

There was no overall correlation between percentage weight loss as a result of individual fungi and final wood moisture content (Fig. 3). Only with *G. trabeum* was there a suggestion of a correlation between final wood moisture content and percentage weight loss, particularly at weight loses greater than 30%. Final wood moisture content levels were grouped rather tightly across all wood species for *W. cocos*, but not for the other fungi.

Discussion

Although the practice of incubating two experimental wood blocks in one vessel is not unique to this study (AWPA 1994), the fundamental consequence of potential loss of independent observations must be recognized. Furthermore, it would seem that verification of independence of replicates should be a criterion for acceptance of any comparative data that utilizes this procedure. Definition of the number of replicates needed to add precision to this type of test also seems in order. In this study, we often observed substantial differences of magnitude between mean values for various experimental combinations, without detecting a statistically significant difference.

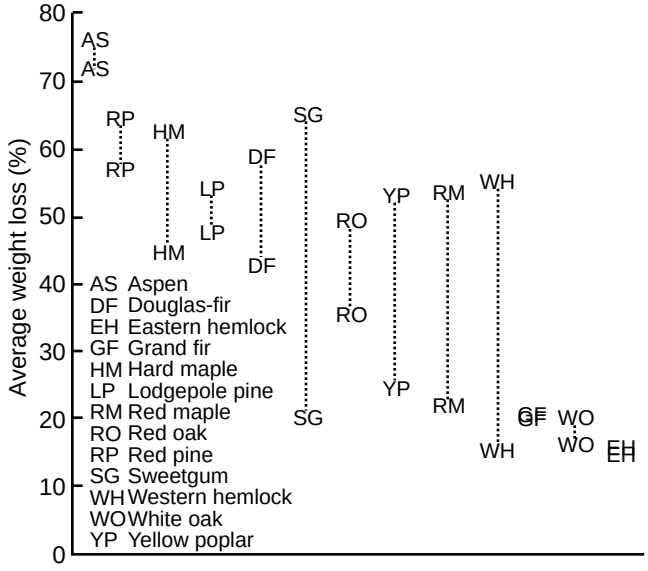

Figure 1—Variation between paired bottles in average percentage weight loss (two blocks of one wood per bottle) caused by *G. trabeum*. Range between paired bottles is shown as dashed line. Percentage weight loss between wood species is not significantly different.

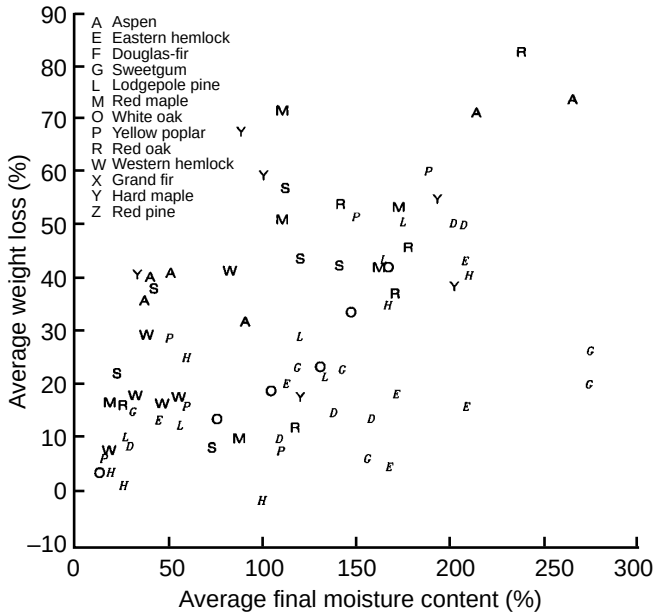

Figure 2—Relationship, by wood species, between average final percentage wood moisture content and average percentage weight loss in wood blocks. Each entry represents an individual fungus species in wood (identified by a letter).

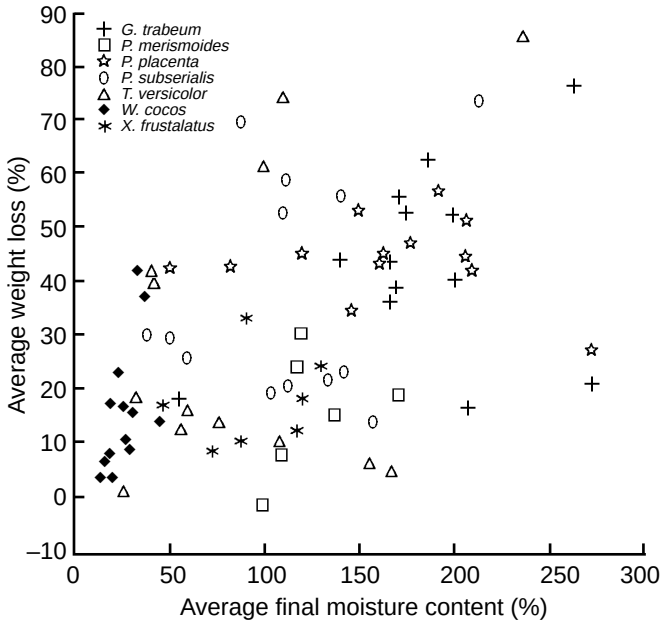

Figure 3—Relationship, by fungus, between average final percentage wood moisture content and average percentage weight loss in blocks. Each entry represents a separate wood species challenged by fungi (identified by a symbol).

Because of the magnitude of differences in apparent capacity of fungi to decay either specific wood species or selected groups of woods, it seems that efficiency with this type of procedure could be gained by minimizing the number of assay fungi and maximizing the number of independent replications. This would require careful selection of fungi on the basis of presumed tolerance to the preservatives being assayed and the capacity to decay the wood species that are included in the test.

The final wood moisture content did not appear to be a limiting factor for the weight loss of the wood. Some correlations between high levels of weight loss and final moisture content were expected, recognizing that as decay progresses, the reference base for ovendry weight determination decreases.

Conclusions

The soil-jar procedure using small wood blocks requires only small amounts of wood material and soil. In this study, the procedure was prone to a large variability within results. One important source of variation was the difference among bottles. Before this procedure can be accepted as a standard protocol for evaluating preservative-treated woods, the source of procedural variability, reproducibility, and sensitivity must be defined.

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