Original

Laboratory Evaluation of Chemicals as Wood Prerservatives

(1) Tribromophenol

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Abstract——Three types of tribromophenol (TBP) were tested for their fungicidal effectiveness in the standardized laboratory decay tests. TBP succeeded in protecting treated materials from decay fungi at 1～3 kg/m³ if it was applied to impregnating treatment of wood. Superficial treatment with the chemical was disappointedly less effective, and the qualiative standards required the treatment with 6～8% solutions. When TBP was added to the glue-line of lauan plywoods, the treated plywoods were attacked by white-rot fungus, *Coriolus versicolor* even at 5 kg/m³ contrary to our expectations. However, comparison of the present results with those in bending creep test under low nutrient level for a brown-rot fungus, *Tyromyces palustris* suggested the necessity for the design of the decay tests to examine the effect of treatment, considering commodities and environmental factors relevant to decay hazard.

Key words: 2, 4, 6-tribromophenol, minimum inhibitory concentration, decay test, value of efficiency, glue-line treatment, wood preservative

1. Introduction

With the increased environmental and toxicological concerns about the use of chemicals in the world, a great emphasis has been putting on the search for alternatives to conventional wood preservatives such as chlorinated phenols and organotin compounds.

To look for the promising alternatives, candidate chemicals should be selected on the basis of the following.

(1) Satisfactory preservative effectiveness--fungicidal, insecticidal or termiticidal efficacy should be not less than that of the conventional agents.

(2) Environmentally and toxicologically acceptable--safety considerations strongly support low-toxicity chemicals for any use in the future.

(3) Reasonable price of the product--considering cost efficiency, the lower price is advantageous.

(4) High affinity to other chemicals--as a single chemical can hardly cover the wide range of biocidal spectrum, high affinity of the candidate is desirable when requested to reinforce the effectiveness of formulations by incorporating it with

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other chemical(s).

(5) High stability under various conditions--since stability of the chemical is directly involved in the performance of the treated materials, type of solvent and the derived formulation must be throughly examined.

Among a number of candidates tested so far, 2,4,6-tribromophenol (TBP-OH) and sodium tribromophenate (TBP-Na) have shown a certain level of potential for the practical application to timber preservation fields. When TBP-OH was once subjected to laboratory screening tests to assess its efficacy as a wood preservative at Forest Research Institute in New Zealand, the results revealed that TBP-OH was relatively effective against basidiomycetes, although the chemical could not control soft rot well[1]. In Canada, on the other hand, they demonstrated that TBP-OH was as effective as methylene bisthiocyanate and 2-(thiocyanomethylthio) benzothiazole in controlling molds and sapstain fungi[2].

In the present investigation TBP-OH and its emulsifiable form (TBP-E) and TBP-Na were tested for their fungicidal effectiveness in the laboratory in accordance with standardized decay tests.

2. Materials and Methods

2.1 Chemicals

TBP-OH, TBP-E and TBP-Na were employed in the present investigation. Some properties of TBP-OH are shown in Table 1.

Treating solutions were prepared with methanol for TBP-OH and water for

Table 1. Properties of 2,4,6-tribromophenol*[1]

Melting point	91°C
Density (molten at 100°C)	2.24 g/ml
Appearance	Flake, off white to light tan
Odor	phenolic
Solubility at ambient temperature (g/100 ml)	
Hexane	3.2
Acetone	>100
Benzene	52
Methanol	>100
Carbon tetrachloride	28
Water	0.01
Toxicity	
Acute oral LD_{50}	5,000 mg/kg (rats)*[2]
Acute dermal LD_{50}	>8,000 mg/kg (albino rabbits)

*[1] Supplier's information
*[2] <2,000 mg/kg according to Merck Index (10th edition, 1983)

TBP-E and TBP-Na.

2.2 Agar dilution test

Agar dilution method was firstly taken to compare fungicidal efficacy of three types of tribromophenol. Fundamentally, 0.5～1 g of stock solution of a test chemical was added to medium so that 50 g of chemical-containing medium was prepared. The medium was then shaked well to get an even distribution of the chemical within the medium, and poured into the two sterilized Petri dishes (9 cm in diameter).

Constituents of the test medium were:

Peptone ……………… 5 g
Malt extract…………10 g
Glucose ………………25 g
KH_2PO_4……………… 3 g
$MgSO_4 \cdot 7H_2O$ ……… 2 g
Agar …………………20 g
Distilled water…1,000 ml

The medium was also used to get inoculum. Either of *Coriolus versicolor* (Linn. ex Fr.) Quél. or *Tyromyces palustris* (Berk. et Curt.) Murr. was inoculated onto the medium in a Petri dish and incubated at 26±2°C for 6～10 days. Fully-grown mycelial mat of the test fungi was punched out with a 6 mm-diameter cork borer, and the agar disc with its top covered with a test fungus was aseptically placed on the center of the medium containing a test chemical. Three replicates were prepared for each test concentration.

The assembled dishes were incubated at 26±2°C to measure the speed of fungal growth in diameter and to determine the rate of growth inhibition until the full growth was observed for untreated controls.

The rate of growth inhibition was calculated from the following equation:

Rate of growth inhibition $= [(D_1 - D_2)/(D_1 - 6)] \times 100$ where, D_1: mean diameter of fungal growth (mm) on the untreated medium, D_2: mean diameter of fungal growth on the treated medium (mm).

2.3 JIS (Japanese Industrial Standard) decay test

Decay test was conducted acoording to JIS A 9302. Sapwood blocks with a size of 20(T) ×20(R) ×10(L) mm were prepared from *Cryptomeria japonica* D. Don, and vacuum/soak impregnated with each treating solution to obtain 24 replicates. After drying the blocks under the ambient conditions, a half of the treated blocks was subjected to weathering cycles so that 6 each of weathered and unweathered blocks were assingned to a decay fungus. Weathering procedure consisted of wet and dry cycles. Blocks were exposed to running water for 1 hr and oven dried for 22~23 hr at 60±2°C and the cycle repeated 9 times.

All the blocks were oven dried and sterilized with ethylene oxide gas prior to decay test. The blocks of the same treatment were then exposed to the monoculture mycelium of *C versicolor* or *T palustris,* and incubated for 90 days at 26±2°C. After incubation, the blocks were cleaned off adherent mycelia and oven dried again to determine percentage weight losses. To facilitate comparison of efficacy of each treatment, value of efficiency was calculated from the following equation:

Value of efficiency= $[(W_1-W_2)/W_1]\times 100$ where, W_1: mean percentage weight loss of untreated controls and W_2: mean percentage weight loss of treated blocks. Details should be referred to the standard.

2.4 JWPA (Japan Wood Preserving Association) decay test

Decay test was carried out according to JWPA Standard-I.

Sapwood blocks with a size of 20 (R) ×5 (T) ×40 (L) mm were prepared from *Fagus crenata* Blume, *C japonica* and *Pinus densiflora* Sieb. et Zucc. Eighteen blocks each of the 3 timber species were brush-treated with test chemicals at a rate of 110±10 g/m^2 after sealing the end grain of the blocks with epoxy resin. After drying the blocks under the ambient conditions for about 3 weeks, 9 of each treatment and timber species were subjected to weathering cycles. Blocks were immersed in non-running water for 30 sec, kept in a dessicator with water at the bottom for 4 hr at 26±2°C, then transferred into an oven for 20 hr at 40±2°C and the cycle repeated 9 times. Treating concentrations are shown in Table 4 together with the results of the JWPA decay test.

All treated and untreated blocks were oven dried at 60±2°C, weighed and sterilized with ethylene oxide gas. Three blocks of the same treatment and timber species were inserted into 2 teflon frames so that 5×40 mm sides faced nearest to the medium when they were aseptically placed in a round glass jar which contained a medium of 250 g quartz sand plus 80~85 ml nutrient solution with growing mycelia of a decay fungus on it.

Blocks of *F. crenata*, *C. japonica* and *P. densiflora* were respectively exposed to the monoculture mycelium of *C. versicolor*, *T. palustris* and *Serpula lacrymans* (Wulf. ex Fr.) Schroet., and incubated for 8 weeks at 26±2°C, except for *S. lacrymans* which was incubated at 20±2°C. After incubation, all blocks were cleaned and dried again in an oven, and reweighed to calculate percentage weight losses. Details should be referred to the standard.

2.5 Decay test for tribromophenol-treated plywood

Specimens with a size of 30×30×12 mm were prepared from the 5-ply (thickness of veneers: 1.7, 3, 3, 3, 3, and 1.7 mm) manufacture-made lauan (*Shorea* sp.) plywoods treated with TBP-OH by incorporating it into the glue-line of melamine urea formaldehyde resin at rates of 1, 3 and 5 kg/m^3.

Decay test was carried out basically by the method of JWPA Standard-III. Details should be referred to the standard.

3. Results and Discussion

3.1 Agar dilution test

Results are shown in Figs. 1 and 2. As shown in Fig. 1, any conspicuous difference was not noticed among the types of tribromophenol in controlling the growth of *T. palustris*. Those were satisfactorily effective at 15~25 ppm. The concentration range which succeeded in inhibiting the fungal growth indicated that the chemical was as effective as organotin compounds, and superior to 2-(4-thiazolyl) benzimidazole (unpublished data, Tsunoda).

Higher concentrations were requested to control the growth of *C. versicolor* as can be seen in Fig. 2. TBP-OH was proven to be more effective than others. Against the white rot fungus, the present agar dilution test demonstrated that tribromophenol was not so effective as tributyltin oxide which could control the fungal growth at 20 ppm (unpublished data, Tsunoda).

Based on the results shown in Figs. 1 and 2, minimum inhibitory concentrations of tribromophenol were determined and given in Table 2. The results indicated that tribromophenol, regardless of the types of chemical, was worthy of further consideration as a wood prerservative.

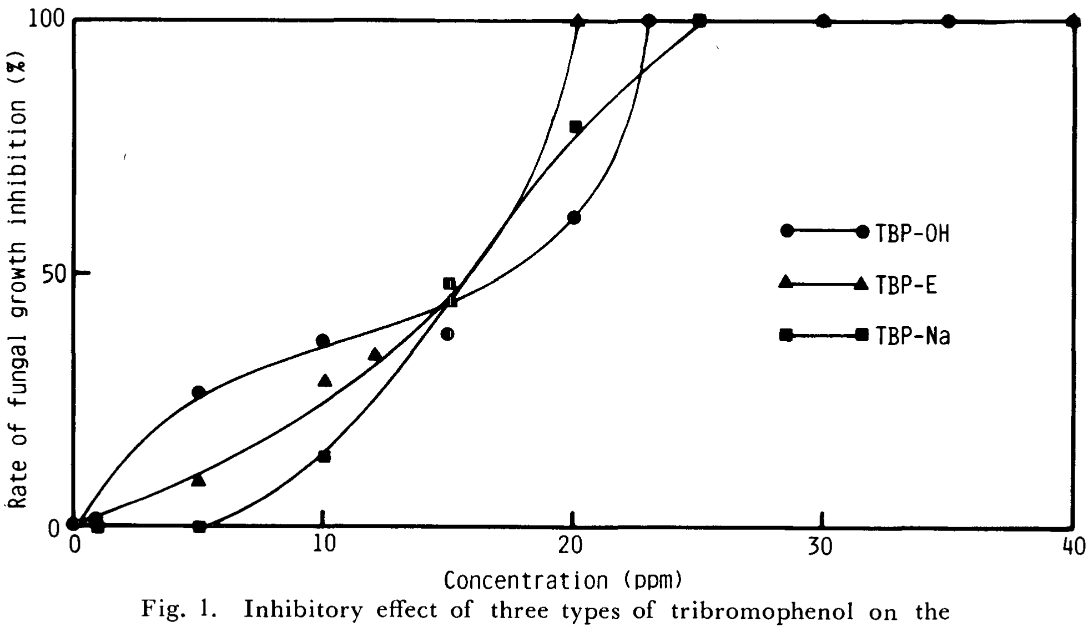

Fig. 1. Inhibitory effect of three types of tribromophenol on the growth of *Tyromyces palustris* in agar dilution test

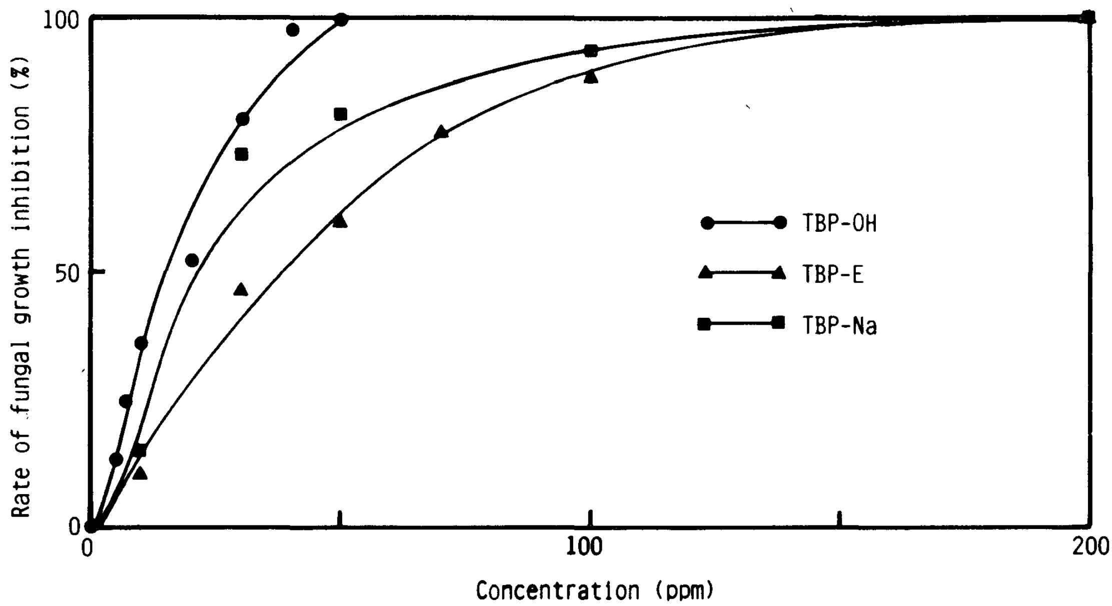

Fig. 2. Inhibitory effect of three types of tribromophenol on the growth of *Coriolus versicolor* in agar dilution test

Table 2. Minimum inhibitory concentrations of three types of tribromophenol

Chemical	Minimum inhibitory concentration (ppm)	
	Tyromyces palustris	*Coriolus versicolor*
TBH-OH	20—25	40— 50
TBP-E	15—20	100—200
TBP-Na	20—25	100—200

3.2 Fungicidal effectiveness in JIS decay test

Summarized data are shown in Table 3. In accordance with the qualitative standards prescribed in JIS A 9201, grade A wood preservatives require values of efficiency of at least 90 and 80 respectively against *T. palustris* and *C. versicolor*, regardless of weathering.

TBP-OH was effective against both decay fungi at a retention of 1.0 kg/m^3 when the treated blocks were not weathered before the decay test. After weathering, however, the chemical could not inhibit the decay by *T. palustris* at the lowest retention of 1.0 kg/m^3, while the retention was high enough to control *C. versicolor*. At higher retentions, decay was fully inhibited.

TBP-Na produced the similar fungicidal performance in the JIS decay test. The chemical was highly effective against both *T. palustris* and *C. versicolor* at a retention of 3.0 kg/m^3 even after weathering.

Comparing the results in the JIS decay test with those in the agar dilution test, it is interesting to note that when applied to timber, tribromophenol was

Table 3. Fungicidal effectiveness of TBP-OH and TBP-Na (value of efficiency determined in JIS decay test)

Chemical	Retention		*Tyromyces palustris* Weathering		*Coriolus versicolor* Weathering	
	kg/m³	mg/g	No	Yes	No	Yes
TBP-OH	1.0	2.2	96	36	100	94
	3.0	6.6	100	100	100	100
	5.0	11.1	100	100	100	100
TBP-Na	0.9	2.0	98	70	99	99
	2.8	6.1	100	100	99	99
	4.6	10.1	100	100	98	99

equally effective against both decay fungi, while *C. versicolor* was twice or more tolerant than *T. palustris* in the agar dilution test. Therefore, it seems unnecessary to evaluate candidate chemicals by agar dilution method as an initial step, although the technique is quick and simple.

3.3 Fungicidal effectiveness in JWPA decay test

Results are summarized in Table 4 and Figs. 3 and 4. Values of efficiency were calculated in the same manner described in 2.3 for easy comparison of fungicidal performance of the chemicals. The qualitative standards (JWPA Standard-VII) require that a wood preservative which is considered to provide good protection should have values of efficiency higher than 80 in any case.

Contrary to our expectations, TBP-OH did not perform well when superficially applied to timber, although it could be supposed to protect wood as well as tributyltin oxide or other wood preservatives on the basis of the results in the agar dilution test. Fungicidal effectiveness of TBP-OH was proven to be inferior to metallic naphthenates, 2-(thiocyanomethythio) benzothiazole and organoiodine compounds[3]. The chemical could not prevent decay by the two test fungi at treating concentrations lower than 4%. Against *T. palustris*, TBP-OH protected timber from decay at the concentrations of 6~8%, and it controlled *C. versicolor* at 4~6%. In contrast, a satisfactory protection was exceptionally accomplished against *S. lacrymans* at lower treating concentrations as shown in Table 4.

The present results in both JIS and JWPA decay tests suggest that mycotoxic effectiveness of chemicals is variably affected by test methods. Consequently, it might be necessary to reexamine the efficacy of the candidate chemicals actually when the promising data are produced in any single test.

Only TBP-OH was tested here because water-soluble forms were generally considered to leach out readily at weathering and would fail in protecting timber

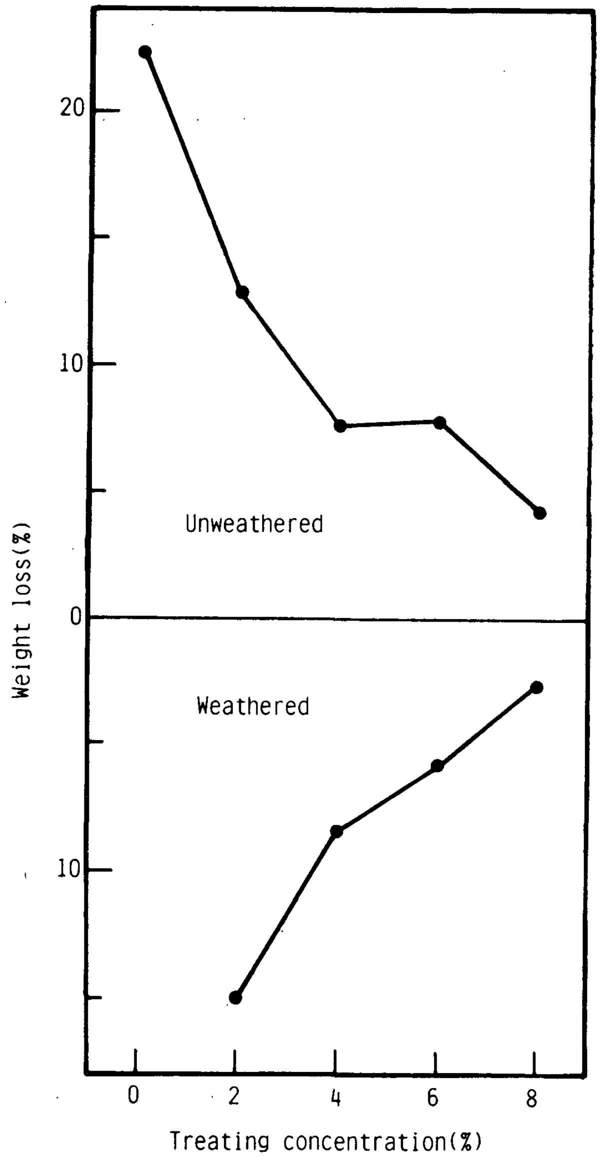

Fig. 3. Fungicidal performance of *Fagus crenata* sapwood brush-treated with TBP-OH against *Coriolus versicolor*

from decay. Weathering cycles, however, did not cause such unfavorable effect on the timber treated with TBP-E[3]. In consequence, the standardized weathering cycles of JWPA should be reconsidered if emulsifiable formulations easily become unstable under certain actual conditions.

3.4 Performance of tribromophenol-treated plywood against decay fungi

As *T palustris* did not attack untreated plywood well due to the timber species of plywoods, *Pycnoporus coccineus* (Fr.) Bond. et Sing. was employed instead. Mean percentage weight losses are shown in Table 5 together with the range of percentage weight losses.

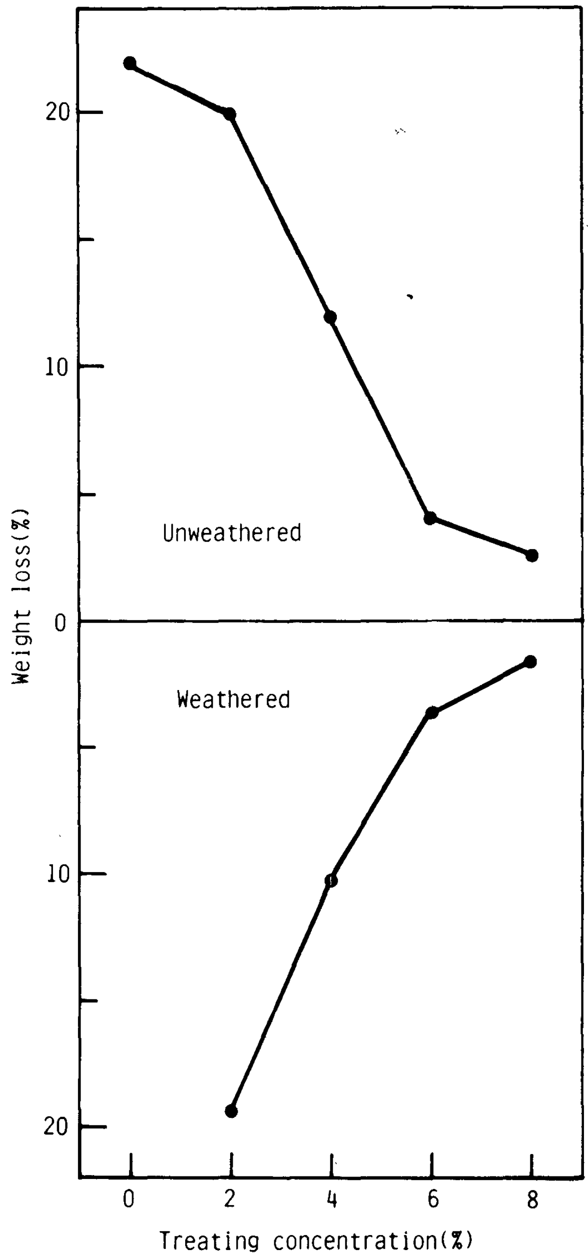

Fig. 4. Fungicidal performance of *Cryptomeria japonica* sapwood brush-treated with TBP-OH against *Tyromyces palustris*

With the increase in retention of the chemical, decay tended to be restrained. It was supposed that the glue-additive treatment could prevent decay at retentions higher than 3.0 kg/m^3, since TBP-OH was thoroughly effective against decay fungi at 3.0 kg/m^3 in the JIS decay test (see Table 3). However, mean percentage weight loss unexpectedly exceeded 10% even at the highest retention of 5.0 kg/m^3. This was probably induced by the fact that active ingredient incorporaterd in the glue-line would not come out to exude into the adjacent veneers. Similar phenomenon was observed for the plywoods treated with other fungicides[4)].

Table 4. Fungicidal effectiveness of TBP-OH (value of efficiency determined in JWPA decay test)

Treating conc. (%)	Fungus-timber species combination					
	Coriolus versicolor-Fagus crenata		*Tyromyces palustris-Cryptomeria japonica*		*Serpula lacrymans-Pinus densiflora*	
	Weathering		Weathering		Weathering	
	No	Yes	No	Yes	No	Yes
1	—*	—	—	—	99	97
2	42	34	9	11	99	97
4	66	63	46	53	—	—
6	65	74	81	84	—	—
8	81	89	88	89	—	—

*: Not tested

Table 5. Fungicidal performance of plywoods treated with TBP-OH (percentage weight loss)

Retention (kg/m^3)	*Coriolus versicolor* min.—mean—max.	*Pycnoporus coccineus* min.—mean—max.
1.0	21.1 — 32.0 — 39.4	3.9 — 8.6 — 15.0
3.0	22.1 — 27.8 — 31.9	3.4 — 4.7 — 6.8
5.0	6.2 — 10.8 — 16.4	1.9 — 2.5 — 3.0
Untreated controls	27.0 — 32.6 — 40.3	3.5 — 8.8 — 13.4

As demonstrated in the present investigation, the treated plywoods were attacked by white rot fungi under the conditions which were favorable for the growth of the fungi. On the other hand, bending creep tests under the slow progress of fungal attack using the same treated materials showed that if the conditions were not favorable for a decay fungus, simulating flooring situation, protective effect was noticeable even at 1.0 kg/m^3 and satisfactory at 5.0 kg/m^3 [5].

These results would suggest that especially for board materials, decay tests should be designed in consideration of where and how long the materials are in service.

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