The Influence of Staining Fungi on the Decay Resistance of Wood Treated with Alkylammonium Compounds

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SUMMARY

Although found to be very effective in laboratory tests, alkylammonium compounds (AAC's) have failed to perform as well in field stake tests. Examination of leachability showed that this was not the cause. The present study investigated the possibility that staining fungi, (which have been observed to rapidly infect the field stakes), degrade the AAC wood preservative.

Soil-blocks were treated with alkyldimethylbenzylammonium chloride, and sterilized, using gamma radiation. Half of the blocks were exposed to a mixed suspension of staining fungi, which had previously been isolated and identified from failed dialkyldimethylammonium chloride-treated stakes. After incubation for ten weeks they were conditioned, and one half leached in a static seven day leaching cycle. Half of the blocks not exposed to the staining fungi were also leached in a similar manner. All the blocks were then sterilized prior to exposing to one of three decay fungi, Lentinus lepideus, Gloeophyllum trabeum and Poria placenta.

Following the test, L. lepideus was found to be least tolerant to the AAC wood preservative, while P. placenta was the most tolerant fungus. The pre-exposure of the soil blocks to staining fungi greatly increased the toxic threshold and toxic limits of AAC to both G. trabeum and P. placenta. The increases were almost independent of leaching, suggesting that degradation of the AAC is taking place rather than rupture of the AAC-wood substrate bonding. The increase in the toxic threshold to ca 9 kg/m^3 would clearly have caused failure of the stake material in test at Westham Island, since the maximum concentration in that test was only ca 10 kg/m^3.

Keywords: alkylammonium compounds; soil block test; wood preservative; preservative degradation

INTRODUCTION

Considerable evidence has been generated during the past seven years to show that alkylammonium compounds (AAC's) have potential as wood preservatives. Initial laboratory studies by Butcher et al. (1977a) determined that dialkyldimethylammonium chlorides were effective wood preservatives and this has been confirmed in other studies which have suggested that didecyldimethylammonium chloride is the most effective of this type of AAC (Preston and Chittenden, 1982). However, alkyldimethylbenzylammonium chloride was observed to be equivalent to chromated-copper-arsenate when evaluated in the laboratory against both Basidiomycetes and soft-rot fungi (Butcher et al., 1977b).

While the laboratory tests have consistently provided positive results field testing of unmodified AAC's has failed to confirm their efficacy (Ruddick, 1981 and 1983). Possible causes of this loss in activity,

related to excessive leaching of the AAC in the field stake test, have been investigated (Ruddick and Sam, 1982), but failed to provide any explanation for the poor performance.

It had been noted previously that AAC treated stakes rapidly became discolored when placed in the test plot. A study was therefore conducted to determine whether the colonization of the treated stakes by staining fungi, could cause a reduction in the effectiveness of AAC's. In designing the experiment the potential of the staining fungi to either degrade the AAC, or to interfere with the 'AAC-wood substrate' bonding mechanism, were both examined.

MATERIALS AND METHODS

i) Preparation and treatment of test blocks

Test blocks (14 x 14 x 14 mm) prepared from ponderosa pine (Pinus ponderosa Laws) sapwood were oven dried at 103°C for 24 hours. They were then weighed and conditioned for seven days in the laboratory. A group of 504 test blocks were then selected, (four combinations of experimental variables x seven preservative concentrations x three test fungi x six replicates) for use in the study. The experimental variables were, exposure to staining fungi and leaching of the blocks following treatment. The conditioned blocks were vacuum impregnated with Barquat MB-80, an alkyldimethylbenzylammonium chloride (where the alkyl composition was 50 percent C_{14}; 40 percent C_{12} and 10 percent C_{16}) as described in the American Wood Preservers' Standard (AWPA M10-77). Treating solutions with concentrations (on a weight/weight basis) of 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28 percent, were prepared by diluting the commercial AAC solution with distilled water. Control blocks which were treated only with distilled water were also included. Following treatment the AAC preservative uptake was determined from the weight of the blocks following treatment. The blocks were then conditioned in the laboratory for 18 days, prior to sterilization with 25 Gy of gamma radiation during a 12 hour exposure.

ii) Exposure to staining fungi

A mixed suspension of staining fungi was prepared using identified cultures previously isolated from failed dialkyldimethylammonium chloride-treated stakes. The fungi were grown on a mixture of two percent malt, two percent agar (Difco) after which the agar and cultures were blended with 500 ml of sterile water. The fungi selected for the test are shown in Table 1.

A disposable aluminum tray 29 x 48 x 7.5 cm was filled with soil at a 50 percent M.C., to a depth of 5 cm. The tray was then sealed in an autoclavable plastic bag with a cotton plug to permit air flow, and sterilized in an autoclave at 121°C and 103 kPa, for one hour. The sterile tray was then transferred to a laminar flow air bench to cool to room temperature.

Half of the total number of test blocks were randomly selected for exposure to staining fungi, and placed, asceptically onto the soil in the tray. Using a standard pipette, 1 ml of the mixed fungal suspension was placed on the top of each test block. The remaining suspension was then poured over the blocks and onto the soil. The

Table 1

Fungal isolates used during exposure of AAC-treated blocks to staining fungi

15-1-10A	Botryodiplodia sp.
15-1-17B	Phialophora sp.
15-1-17C	Phialophora sp.
15-1-17D	Rhinocladiella sp.
15-2-1B	Penicillium/Paecilomyces sp.
15-2-9B	Fusarium sp.
15-2-22A	Verticillium sp.
15-3-29A	Unknown imperfect
15-3-27B	Sporothrix sp.

tray was resealed in the polythene bag and placed inside an incubator at 25°C and 75 percent R.H. After ten weeks the tray was removed from the incubator and mycelium and soil carefully scraped off the blocks. They were allowed to condition in the laboratory for three days.

iii) Leaching of non-stained and pre-stained blocks

Half of the blocks which had been exposed to staining fungi were submerged in a large beaker of distilled water which was placed in a dessicator for evacuation for 20 minutes using a water-aspirator. The beaker was removed from the dessicator and the blocks kept submerged for seven days. During this time the water was not changed. Half of the non-stained blocks were selected and leached in a similar manner.

iv) Exposure of non-stained and pre-stained blocks to wood-destroying fungi

Soil jars containing ca 250 ml of soil at 50 percent M.C. were infected with one of three fungi, Lentinus lepideus (Fr.) (WFPL 44D), Gloeophyllum trabeum (Fr.) Murr. (WFPL 47D) and Poria placenta (Fr.) Cke. (WFPL 120F). A ponderosa pine sapwood strip was placed on the surface of the soil, after which the jar was sealed with a screw-type metal lid. The lid contained a 5 mm hole, sealed with Gelman GA-8 2μ Metricel filter, to permit aeration (Smith, 1978). The jars were incubated at 25°C and 75 percent R.H. for three weeks.

All of the test blocks were oven dried at 65°C for 48 hours, and their weights recorded. They were then sterilized with 25 Gy of gamma radiation, after which they were placed on the ponderosa pine sapwood strip, three to a jar. The jars were then incubated at 25°C and 75 percent R.H. for 11 weeks.

The blocks were removed from the jars and any mycelium and soil adhering to the surface was carefully removed. They were then weighed, oven dried, and re-weighed. In this way both the final moisture content of the blocks and the loss in dry weight due to decay could be calculated.

RESULTS AND DISCUSSION

The results of the study are shown in Tables 2 - 5, and summarized in Figure 1. The toxic limits were determined as the highest preservative concentration, (based upon the treating solution concentration) which permitted a mean weight loss in excess of two percent and the lowest concentration which produced a mean weight loss of less than two percent. The toxic threshold was calculated for each sub-test (i.e., leached, non-leached, pre-stained and non-pre-stained), using the procedure described in the AWPA standard, (AWPA M10-77, 1977). Weight losses less than two percent were considered insignificant.

The results show that the tolerance of the fungi to the AAC wood preservative increases in the order of L. lepideus > G. trabeum > P. placenta. Considering first the blocks not exposed to staining fungi, the toxic thresholds for the leached, and non-leached blocks range from 0.9 kg/m^3 for leached blocks decayed by G. trabeum to 3.9 kg/m^3 for non-leached blocks exposed to P. placenta. For two fungi,

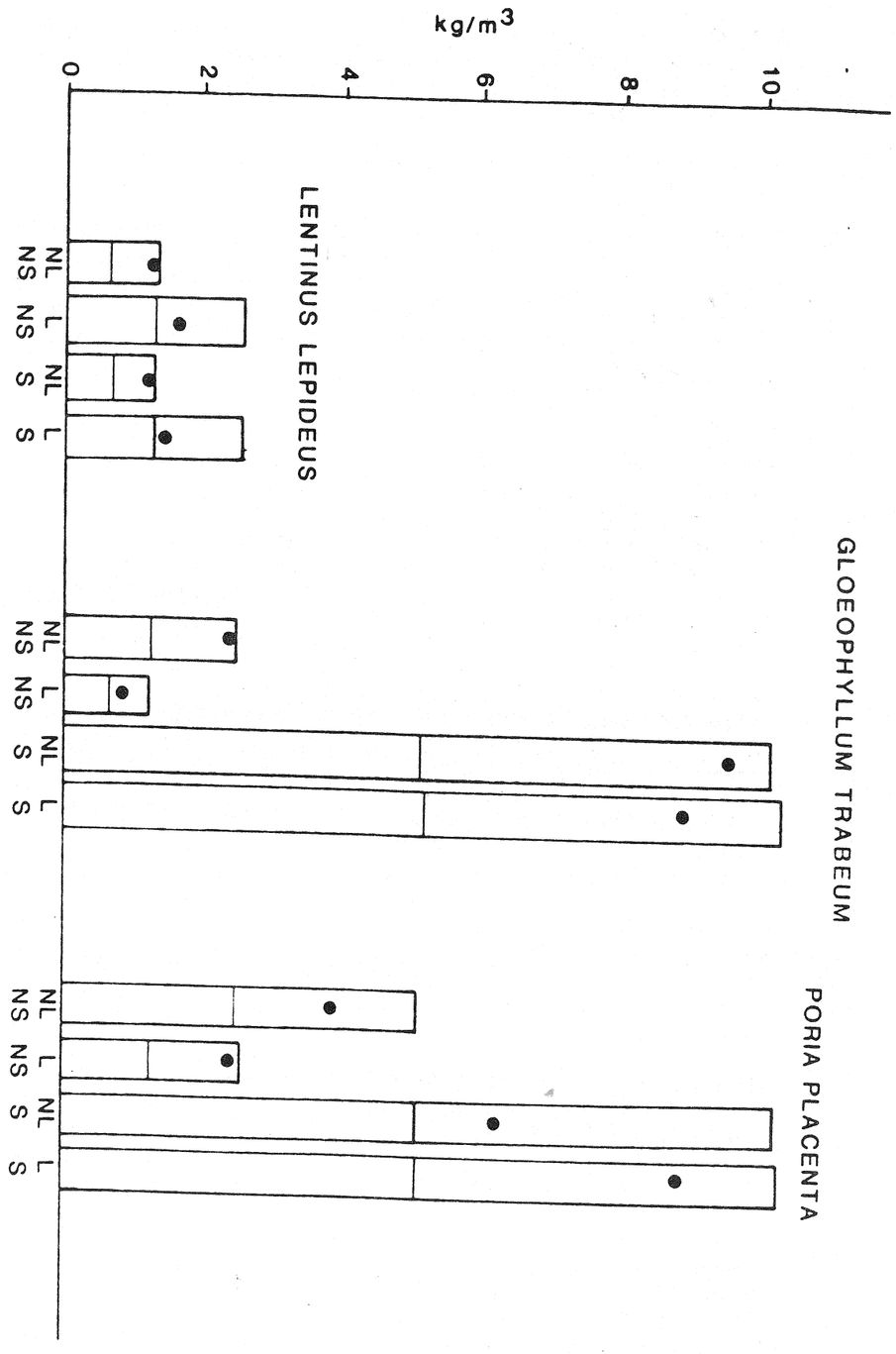

FIGURE 1 SUMMARY OF TEST RESULTS SHOWING BOTH TOXIC THRESHOLD AND TOXIC LIMIT FOR ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE-TREATED BLOCKS. TEST VARIABLES STUDIED WERE THE EFFECT OF:- LEACHING (L) VS. NON-LEACHING (NL); AND INFECTION BY STAINING FUNGI (S) VS. NO EXPOSURE TO STAINING FUNGI (NS).

Table 2

Test results for non-leached, non-stained ponderosa pine sapwood blocks treated with alkyldimethylbenzylammonium chloride

Fungus	Mean Weight Loss (%)	Preservative Retention (kg/m^3)	Toxic Threshold (kg/m^3)	Toxic Limits (kg/m^3)
Lentinus lepideus	49.4 (1.3)*	0.00	1.2	0.6-1.3
	26.0 (1.6)	0.32		
	16.7 (3.0)	0.63		
	1.7 (0.9)	1.28		
Gloeophyllum trabeum	54.1 (1.4)	0.00	2.5	1.3-2.5
	22.6 (4.5)	0.63		
	18.5 (5.5)	1.28		
	0.3 (0.1)	2.52		
Poria placenta	58.5 (1.1)	0.00	3.9	2.5-5.1
	41.9 (0.4)	1.28		
	11.9 (2.8)	2.50		
	1.0 (0.1)	5.06		

*Standard Error for weight loss data

Table 3

Test results for leached, non-stained ponderosa pine sapwood blocks treated with alkyldimethylbenzylammonium chloride

Fungus	Mean Weight Loss (%)	Preservative Retention (kg/m^3)	Toxic Threshold (kg/m^3)	Toxic Limits (kg/m^3)
Lentinus lepideus	47.1 (1.3)*	0.00	1.6	1.3-2.6
	3.6 (3.2)	0.64		
	3.1 (1.3)	1.29		
	-0.6 (0.1)	2.55		
Gloeophyllum trabeum	61.3 (1.4)	0.00	0.9	0.7-1.3
	29.8 (6.2)	0.33		
	10.9 (2.6)	0.65		
	0.3 (0.3)	1.28		
Poria placenta	60.7 (0.9)	0.00	1.9	1.3-2.6
	56.0 (0.6)	0.65		
	27.5 (4.4)	1.28		
	0.1 (0.1)	2.58		

*Standard Error for weight loss data

Table 4

Test results for non-leached, stained ponderosa pine sapwood blocks treated with alkyldimethylbenzylammonium chloride

Fungus	Mean Weight Loss (%)	Preservative Retention (kg/m^3)	Toxic Threshold (kg/m^3)	Toxic Limits (kg/m^3)
Lentinus lepideus	47.6 (1.0)*	0.00	1.2	0.6-1.3
	35.6 (2.5)	0.32		
	23.7 (2.5)	0.63		
	0.9 (0.1)	1.29		
Gloeophyllum trabeum	61.1 (1.5)	0.00	9.6	5.1-10.2
	18.2 (1.9)	2.57		
	11.6 (1.8)	5.14		
	0.8 (0.4)	10.15		
Poria placenta	60.7 (1.1)	0.00	6.3	5.1-10.2
	48.7 (2.0)	1.30		
	17.2 (3.7)	2.61		
	2.4 (1.1)	5.10		
	0.7 (0.1)	10.18		

*Standard Error for weight loss data

Table 5

Test results for leached, stained ponderosa pine sapwood blocks treated with alkyldimethylbenzylammonium chloride

Fungus	Mean Weight Loss (%)	Preservative Retention (kg/m^3)	Toxic Threshold (kg/m^3)	Toxic Limits (kg/m^3)
Lentinus lepideus	44.5 (2.1)*	0.00	1.40	1.3-2.60
	32.0 (2.4)	0.33		
	16.8 (1.3)	0.63		
	2.2 (0.3)	1.30		
	0.0 (0.1)	2.60		
Gloeophyllum trabeum	63.2 (0.9)	0.00	9.0	5.2-10.3
	14.9 (1.7)	2.63		
	7.2 (0.7)	5.20		
	0.2 (0.1)	10.25		
Poria placenta	62.8 (0.3)	0.00	9.1	5.1-10.3
	36.5 (1.3)	2.63		
	5.6 (2.2)	5.13		
	0.9 (0.1)	10.29		

*Standard Error for weight loss data

G. trabeum and P. placenta, the leached blocks were less readily decayed than the non-leached blocks. This may be explained by the removal of soluble nutrients during the leaching process. The fungus, L. lepideus, did not exhibit this effect presumably due to its low tolerance for AAC's.

When a pre-staining step was added to the test, for two of the fungi, the toxic threshold increased markedly, while for L. lepideus, no change in the effectiveness was observed (Tables 2 - 5). The toxic threshold of the non-leached blocks increased almost four times for G. trabeum, from 2.5 kg/m^3 to 9.6 kg/m^3. The corresponding increase for P. placenta was less, being almost doubled from 3.9 kg/m^3 to 6.3 kg/m^3 by the action of the staining fungi.

The comparison of the toxic thresholds for leached, non-stained and pre-stained blocks showed even greater differences with that of G. trabeum increasing ten times, from 0.9 kg/m^3 to 9.0 kg/m^3. The toxic threshold against P. placenta was raised from 1.9 kg/m^3 to 9.1 kg/m^3 (Tables 3 and 5).

The observation that the blocks exposed to attack by staining fungi did not show enhanced decay by the fungus L. lepideus would suggest that this fungus is insensitive to changes in low concentrations of the AAC. For both other test fungi, the considerable increases in both the toxic threshold and toxic limits would indicate that the staining fungi have been able to degrade the AAC. The actual degradation process is unknown. However, it does not appear to be simply an interference with the bonding of the AAC to the wood substrate, since the loss in effectiveness is present for both the leached and non-leached test blocks. Further, for the blocks exposed to staining fungi, the toxic limits are identical in both the leached and non-leached tests.

From the results it is proposed that the staining fungi are able to degrade the AAC's, resulting in their loss in efficacy. Further studies are planned to confirm the results of this investigation, and will include the measurement of the alkylammonium compound (MB-80) levels in both leached and non-leached test blocks following exposure to staining fungi.

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