

BIOASSAYING WOOD PRESERVATIVES WITH *ASPERGILLUS NIGER*

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ABSTRACT

The bioassay with *Aspergillus niger* is a simple way of determining the presence and amount of pentachlorophenol or tributyltin oxide in wood, but it does not appear useful for measuring strongly fixed chemicals such as chromated copper arsenate, borate, and fluoride. Its effectiveness in detecting other preservatives has not been reported. Accordingly, the bioassay was evaluated for its ability to detect various concentrations of 15 preservatives introduced into blocks of ponderosa pine. These results were compared with those from modified soil-block tests involving the same chemicals. Several of the formulations—three quaternary ammonium compounds, copper naphthenate, and Folpet—imparted some decay resistance to the blocks but were not detectable with the bioassay. The remaining chemicals were all detectable, although sensitivities varied. These results suggest that the *Aspergillus* bioassay can be useful for detecting many wood preservatives.

Keywords: *Aspergillus niger*, quaternary ammonium compounds, *Poria placenta*, copper naphthenate, soil-block test, zinc naphthenate, bioassays, copper-8 quinolinolate, Folpet, Azaconazole, iodopropynyl butylcarbamate, isothiazolone, chlorothalonil.

INTRODUCTION

Chemical assays of wood preservatives are increasingly complex and expensive. While these techniques are useful when working with certain substances, their application is often limited because of the expense involved. In such cases, bioassays can provide appraisals of preservative penetration and retention at much lower costs.

The bioassay with *Aspergillus niger* has been recommended as an easily reproducible method for appraising the retention of pentachlorophenol (Penta) or tributyltin oxide (TBTO) in wood (Scheffer and Gollob 1982; Schmidt 1984; Scheffer and Morrell 1986). When wood treated with one of these chemicals is placed on petri plates seeded with *Aspergillus*, the chemical diffuses from the wood and inhibits production of the fungus' dark black spores. However, the reliability of this bioassay with preservatives other than Penta, TBTO, chromated copper arsenate, boron, or fluorine is unknown (Moreschi 1982; Scheffer and Gollob 1982; Dost and Scheffer 1983; Moreschi and Willeitner 1984). The present study was conducted to assess this reliability with 15 additional formulations.

MATERIALS AND METHODS

Sapwood blocks, 1 × 1 × 0.5 cm (grain length), of ponderosa pine (*Pinus ponderosa* L.) were prepared for an *Aspergillus* bioassay of wood preservatives. Each block was oven-dried at 54 C for 24 hours and weighed (nearest 0.001 g) before being treated with one of 15 test chemicals diluted in water or toluene to concentrations shown in Table 1. Each concentration of each chemical was applied to 18 sapwood blocks. For maximum penetration of the test chemicals, the treat-

TABLE 1. Preservatives evaluated by the *Aspergillus* bioassay.^a

Chemical formulation (abbreviation)	Carrier
N(trichloromethylthio)phthalimide (Folpet)	Toluene
Copper-8 quinolinolate (Cu-8-Q) (A)	Water ^b
Cu-8-Q (B)	Toluene ^b
Cu-8-Q (C)	Water
[[2-(2,4-dichlorophenyl)-1,3-dioxolan-2-yl]methyl] 1H-1,2,4 triazole (Azaconazole)	Water
3 iodopropynyl butylcarbamate (IPBC)	Water ^b
Dodecyldimethylbenzylammonium salt of naphthenic acid (NWD)	Water
Dodecyldimethylbenzylammonium salt of naphthenic acid (NSS)	Toluene
Trimethyl coccammonium chloride (AC-50)	Water
2(thiocyanomethylthio)benzothiazole (TCMTB)	Water ^b
TCMTB + methylene bis(thiocyanate) (MBT)	Water ^b
Copper naphthenate (Cu naph)	Toluene
Zinc naphthenate (Zn naph)	Water ^b
Chlorothalonil (Napcocide)	Toluene
4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (Isothiazolone)	Toluene

^a Each chemical was tested at 0.05, 0.10, 0.20, 0.50, 1.0, and 3.0% active ingredient.

^b These chemicals produced wide, unmeasurable zones of effect at the six test concentrations and were tested at 0.01% active ingredient.

ment blocks were subjected to a vacuum for 20 minutes, after which the vacuum was released and then reapplied for 15 more minutes. The blocks were then blotted dry and reweighed to determine uptake of the solution. They were then oven-dried at 54 C for 24 hours, reweighed, and stored at room temperature for 21 days to allow the solvent to evaporate and any chemical fixation to occur.

Half of the 18 blocks treated with a given concentration of a chemical were used in an *Aspergillus* bioassay. In each of three petri dishes, three blocks were placed on the surface of potato dextrose agar seeded with a spore suspension of *A. niger*. The spore suspension had been prepared by flooding an actively growing culture of the test fungus with distilled water and spraying this suspension over the agar. [A previous study has shown that the *A. niger* bioassay is insensitive to spore concentration within a wide range of densities (Scheffer and Morrell 1986).] The dishes and blocks were incubated at room temperature for one week or until sufficient sporulation had developed. Then the distance between each block and the point where *A. niger* was unable to sporulate was measured to the nearest millimeter. This zone, called the zone of effect (ZOE), was compared with such zones produced by blocks treated only with water or toluene, and the zones and concentrations were plotted on semi-log paper.

Results of the bioassay were compared with the ability of the same chemicals to protect blocks from decay in a modified soil-block test previously developed by Scheffer (unpublished). In this test, 57-ml bottles were half filled with loamy garden soil, and a 25- × 25- × 0.5-mm feeder strip was placed on the soil surface. Twenty ml of distilled water was added to the bottles, which were capped and autoclaved for 45 minutes at 121 C. The bottles were cooled overnight, re-autoclaved for 15 minutes at 121 C, and cooled before inoculation with the test fungus, *Poria placenta* (Fr.) Cke. (Madison 698). They were incubated at 28 C until the feeder strips were thoroughly colonized; then the treated blocks were sterilized for 10 minutes at 100 C and two were placed on each feeder strip. Thereafter, the bottles were incubated for 10 weeks at 28 C; then the blocks were

TABLE 2. Ability of chemicals in sapwood blocks to inhibit sporulation by *Aspergillus niger*.

Chemical ^a	Concentration (%)						
	0.01	0.05	0.10	0.20	0.50	1.0	3.0
 <i>Aspergillus</i> ZOE ^b (mm)						
Arquad C-50 (W)	—	0	0	0	1.6	0.2	2.5
NSS (T)	—	0	0	0	0	0	0
NWD (W)	—	0	0	0	0	0	1.3
IPBC (W)	11.9	23.4	24.3	26.2	29.9	30.4	35
TCMTB (W)	3.1	3.1	23.3	35	35	35	35
TCMTB + MBT (W)	9.5	20.0	30.4	35	35	35	35
Cu naph (T)	—	0	0	0	2.0	2.2	2.7
Zn naph (W)	5.5	11.6	11.6	9.8	9.9	10.7	11.7
Cu-8-Q (A) (W)	2.1	9.0	11.3	11.0	12.4	13.8	17.8
Cu-8-Q (B) (T)	0	10.4	13.1	12.0	11.0	11.0	12.0
Cu-8-Q (C) (W)	—	3.9	11.4	9.0	7.0	5.4	7.4
Folpet (T)	—	7.3	8.8	10.6	12.4	15.4	12.2
Isothiazolone (T)	—	7.1	9.4	10.4	11.8	10.2	11.7
Napcocide (W)	—	0	0	0	0	0	0
Azaconazole (W)	—	0.7	1.6	9.7	28.0	15.9	27.7

^a See Table 1 for chemical formulations. Carriers are designated as W for water and T for toluene.

^b ZOE = zone of effect.

removed, scraped clean, weighed, oven-dried overnight at 54 C, and reweighed to determine the weight lost because of fungal exposure. These weight losses were compared with the sizes of the corresponding ZOE's in the bioassay.

RESULTS AND DISCUSSION

Not all of the test chemicals inhibited sporulation by *Aspergillus* (Table 2); NSS (in toluene), NWD (in water), Arquad C-50, Cu naph, and Napcocide had little influence on the ZOE in the bioassay, although blocks treated with these chemicals lost less weight than did the untreated controls (Table 3, footnote c). The bioassay measures the ability of chemicals to migrate from impregnated wood into the surrounding medium and inhibit sporulation by *Aspergillus niger*. Dost and Schef-fer (1983) have shown that chemicals such as chromated copper arsenate that are strongly bound to the wood cannot migrate into the medium and do not perform well in these bioassays. Two of the formulations that performed poorly in the bioassay were quaternary ammonium compounds (NSS and NWD), both of which bind themselves strongly to wood. Similarly, Napcocide did not produce a ZOE in the bioassay, and neither did Cu naph at low concentrations, although it did do so at high concentrations. Apparently, these compounds are too strongly bound to migrate into the surrounding medium or are unable to diffuse into the medium because of their low solubility in water.

All of the remaining compounds produced measurable ZOE's at the lowest concentration and larger ZOE's as the concentration increased. Generally, at a given concentration, weight loss was inversely related to ZOE, although TCMTB and TCMTB + MBT both produced complete inhibition of *A. niger* at concentrations above 0.1%.

In previous studies in which the *Aspergillus* bioassay was used to detect penta or tributyl-tin oxide, ZOE and chemical concentrations were well correlated (Schef-fer and Gollob 1982), a relation that suggests the bioassay could be used routinely

TABLE 3. Weight loss in sapwood blocks treated with chemicals and exposed to *Poria placenta* in a modified soil-block test.

Chemical ^a	Concentration (%)						
	0.01 ^b	0.05	0.10	0.20	0.50	1.0	3.0
	Average weight loss (%) ^c						
Arquad C-50 (W)	—	8.31	5.30	1.78	0	0	0
NSS (T)	—	34.71	20.35	23.98	21.60	9.30	0
NWD (W)	—	29.66	8.69	2.82	2.94	0	0
IPBC (W)	15.48	31.12	0.79	0	0	0	0
TCMTB (W)	—	44.30	15.30	1.15	1.40	0	0
TCMTB + MBT (W)	30.01	1.15	1.20	0	0	0	0
Cu naph (T)	—	27.42	23.13	11.32	1.97	0	0
Zn naph (W)	58.61	58.96	46.47	13.94	1.94	0	0
Cu-8-Q (A) (W)	60.23	49.06	27.41	10.39	1.51	1.00	0
Cu-8-Q (B) (T)	56.81	18.84	1.80	1.30	0	0	0
Cu-8-Q (C) (W)	—	4.12	2.44	0	0	0	0
Folpet (T)	—	54.92	15.37	3.61	1.71	0	0
Isothiazolone (T)	—	2.21	0.97	0	0	0	0
Napcocide (W)	—	38.99	27.75	12.91	9.00	0	0
Azaconazole (W)	—	3.07	3.40	0	0	0	0

^a See Table 1 for chemical formulations. Carriers are designated as W for water and T for toluene.

^b Chemicals that produced high ZOE's (>30 mm) at the 0.05% concentration were tested at 0.01% as well.

^c Averages based on results of 9 blocks per chemical concentration. Weight losses were 56.01% for blocks treated with toluene and 58.4% for those treated with water.

to determine preservative retentions. In our study, however, many of the tested chemicals produced gradual increases in ZOE's with increased chemical concentrations, responses that would be incompatible with the determination of retention. Our results do suggest, however, that the assay could be used to detect penetration of some preservatives, particularly of colorless chemicals difficult to detect visually or expensive to assay by conventional chemical techniques. While additional testing might permit more accurate determinations of these chemicals, present results indicate that *A. niger* is most useful for detecting the presence of chemicals with slight water solubility.

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