Efficacy of Various Fumigants in the Eradication of Decay Fungi Implanted in Douglas-fir Timbers

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ABSTRACT

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Vapam proved to be the most effective of 12 fumigants in killing test tube cultures of *Poria placenta*, *P. carbonica*, *P. xantha*, *Fibroporia vaillantii*, *Lentinus lepideus*, *Antrodia serialis*, *Serpula incrassata*, and *Gloeophyllum trabeum* implanted in Douglas-fir timbers. Of 11 other fumigants tested, Busan 40, Mylone, and sodium bisulfite were the next most effective. Fumigant toxicity was generally greatest during the first 4 mo following

treatment. Vapam continued to be effective up to 16 mo at 0.61 m from the base of fumigation, after which its lethality dropped markedly. The variation in fumigant efficacy between timbers could be attributed to checking in the timbers. Differences in fungal sensitivity to the fumigants were observed.

Additional key words: acetaldehyde, 2-bromopyridine, ethanolamine, isopropylamine, sec-butylamine.

The fumigants Vapam and chloropicrin effectively control *Poria carbonica* Overh. and *P. placenta* (Fr.) Cke. in large, heavily checked, Douglas-fir wharf timbers (10). However, because decay fungi vary in tolerance to different fumigants (4,11,13), we did not know whether fumigation would successfully eradicate other important fungi that decay Douglas-fir wood products. This study was initiated primarily to provide this information. Other objectives of the study were to determine the efficacy of other untested chemicals for use as eradicants of wood decay fungi, the extent and speed of penetration of toxic amounts of test fumigants through horizontally oriented Douglas-fir timbers, and the longevity of toxic concentrations of fumes in that wood.

MATERIALS AND METHODS

Preparation of test timbers. Twenty-five new Douglas-fir timbers, $0.20 \times 0.20 \times 4.88 \text{ m}$ (8 in. \times 8 in. \times 16 ft), treated with ammoniacal-copper-arsenate for aboveground use, were numbered and placed on concrete blocks (Fig. I) in a secluded area of Naval Submarine Base Bangor, Bremerton, WA. Elevating the timbers on cinder blocks lessened the decay hazard and made easier subsequent work on the heavy timbers. A cluster of holes (each hole 2.54 cm in diameter and about 17 cm deep) were drilled in the top at the midpoint of each timber for fumigant containment (Fig. 2). The number of holes drilled varied according to the amount of fumigant being applied to a given timber. On one side face of each timber, at distances of 0.30, 0.61, and 1.22 m (1, 2, and 4 ft) in both directions

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from the midpoint or centerline (CL), four 1.9-cm-diameter (3/4 in.) inoculation holes, about 15 cm deep, were drilled in a vertical row (Fig. 2).

Fumigants. Vapam (33% sodium N-methyldithiocarbamate) was included both to gather more information on its efficacy against test fungi in horizontal timbers, and to use as a reference to gauge the efficacy of new fumigant candidates. The latter included Busan 40 (41% potassium N-hydroxymethyl-N-methyldithiocarbamate) in water; Mylone (99% 3,5-dimethyltetrahydro-1,3,4,-2Hthiadiazine-2-thione); sodium bisulfite; acetaldehyde; 2bromopyridine; 2-chloropyridine; 2,6-dichloropyridine; 2fluoropyridine; 3-fluoropyridine; 2,6-difluoropyridine; ethanolamine; isopropylamine; sec-butylamine; tertiary butylamine; trichloroethylene; tetrachloroethylene; 1,2-dibromotetrachloroethane; and 2,3,5,6-tetrachloronitrobenzene. To determine which of these candidates would be included in field trials, they were first tested in the laboratory. This was accomplished by suspending eight white pine blocks, each of which had been infected with one of eight important Douglas-fir wood decay fungi (for their identities, see the section on preparation of inoculum) into a flask containing one of the fumigant candidates. The flask was plugged and after 1 wk, the blocks were transferred to test tubes containing 2% malt extract agar. If no fungal growth ensued, the fungus was presumed to have been killed by the fumes and the fumigant responsible was included in subsequent field trials. Ammonium bifluoride was not included in laboratory screening trials, but it was included in the field trials because of its efficacy in on-site protection of piling from decay (9). The chemicals chosen for inclusion in field trials, and their rate of application, are described in Table 1.

All timbers were fumigated during June 1981, except for timbers 24 through 33 which were treated in October 1982. When solid chemicals were used, water was immediately added to the fumigation holes. All holes were tightly sealed with rubber stoppers following treatment (Fig. 1).

Preparation of inoculum. Test tubes, 16×25 mm in size and containing 2% malt extract agar, were each inoculated with one of the eight test fungi used in this study. When growth was well established in these tubes, a $1.3 \times 1.3 \times 3.5$ -cm white pine stick, previously soaked in distilled water and then steam sterilized, was aseptically placed into each tube. The cultures were incubated until the fungi became established in the sticks. The fungi found to be most commonly associated with decay of Douglas-fir structures (2,3) were used as test fungi. These were, in order of apparent frequency of occurrence in such structures, as follows: Poria placenta (Fr.) Cke. (MAD-698), Poria carbonica Overh. (MD-141), Lentinus lepideus Fr. (MAD-534), Poria xantha (Fr.) Cke. (MAD 5096-35), Antrodia serialis Fr. (FP-104443-sp.), Fibroporia vaillantii (DC:Fr.) Parm. (FP-90877-R), Serpula incrassata (Berk. & Curt.) Donk (MAD-563), and Gloeophyllum trabeum (Pers.:Fr.) Murr. (MAD-617).



Fig. 1. Douglas-fir timbers in place on cinder blocks at the test site. Note the rows of rubber stoppers sealing the inoculation and fumigation holes.

Inoculation of timbers. A test tube, containing a culture of one of the above fungi and capped with a cotton plug, was inserted completely into each inoculation hole which was then sealed with a rubber stopper (Fig. 1). Test fungi were distributed in the timbers as shown in Fig. 2. In alternate timbers, the cultures were placed into holes in the order of 2, 1, 4, 3, and 6, 5, 8, 7.

Inspections. At 4-mo intervals, the test tubes were removed from each timber and replaced with a fresh test tube culture. The removed test tubes were transported to the laboratory where the decayed wood insert was transplanted to a sterile tube of malt agar. These tubes were incubated at 27 C for 6 wk and subsequently inspected for signs of growth, i.e., viability.

RESULTS

The results of fumigation tests conducted over a 20-mo period are provided in Table 2. Cultures implanted in the control timbers generally survived therein for 4-mo incubation periods during all seasons of the year.

In timbers that had received Vapam, most of the cultures died at both 0.30 and 0.61 m from the CL by the fourth month following treatment. Most fresh cultures installed at 8-, 12-, and 16-mo intervals in three of the timbers also died. However, culture replacements in one timber generally survived. In all four timbers, survival of replacement cultures was above 50% by the 20-mo

TABLE I. Fumigants tested against cultures of wood-decay fungi in Douglas-fir timbers

Fumigant	Amount per timber						
Control	None						
Vapam	473 ml						
2,6-Dichloropyridine	227 g						
Ammonium bifluoride	227 g						
Ammonium bifluoride	454 g						
2-Bromopyridine	237 ml						
Busan 40	473 ml						
Mylone	227 g						
Sec-butylamine	150 ml						
Isopropylamine	300 ml						
Acetaldehyde	125 ml						
Sodium bisulfite	454 g						
Ethanolamine	300 ml						

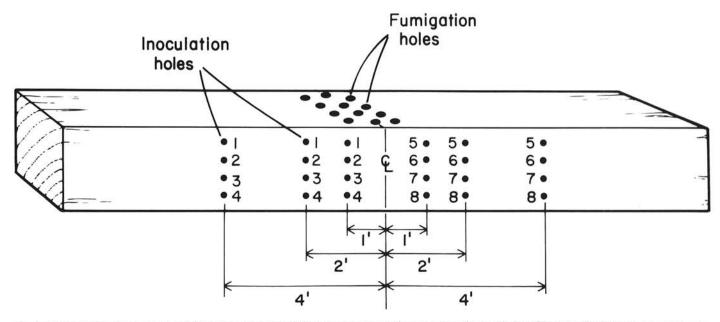


Fig. 2. Location of the fumigant-containing holes, and those bearing test tube cultures, in a representative test timber of Douglas-fir. Decay fungi added to the numbered holes are as follows: Poria placenta (No. 1); P. carbonica (No. 2); Lentinus lepideus (No. 3); P. xantha (No. 4); Antrodia serialis (No. 5); Fibroporia vaillantii (No. 6); Serpula incrassata (No. 7); and Gloeophyllum trabeum (No. 8).

TABLE 2. Viability of fungus cultures inoculated into Douglas-fir timbers at different distances from fumigation sites and after five time intervals following application of fumigants

				Time of assay (mo) following fumigation and numbers and viability of cultures ^b											
Fumigant		Distance of		4	8	12	16	20							
	culture from fumigant		Timber	Culture numbers	Culture numbers	Culture numbers	Culture numbers	Culture numbers							
and amount	m	feet		1 2 3 4 5 6 7 8	1 2 3 4 5 6 7 8	1 2 3 4 5 6 7 8	1 2 3 4 5 6 7 8	1 2 3 4 5 6 7 8							
Control (no fumigant)	0.30 0.61 1.22	1 2 4	C-1 C-2 C-3	+++++++++++++++++++++++++++++++++++++++	+++++++	+++++++	+++++++++++++++++++++++++++++++++++++++	++++++							
Vapam ^c 473 ml	0.30	1	1 2 3 4	+	+	+	++ + +++++++	+++							
	0.61	2	1 2 3 4			++	+	-++-+++							
	1.22	4	1 2 3 4	+ + + + - + - + + + + + - + + +	-+++-++ +++	+ + + + + + + + + + + + + + + + + + +	+ - + + + - + + + + + + + + + + +	++++							
2,6-Dichloropyridine 227 g	0.30	1	5 6 7 8	- + + + - + - + + - + + +	+ + + + - + + + + + + + + + -	+++	+ + + + - + - + + + + + + + + + - + + + + + + + +								
	0.61	2	5 6 7 8	-++++-+ -+++-+-+ +-+++		+-+++	+ + + + - + + + + + + + + + + + + - + + + + + + + +								
	1.22	4	5 6 7 8			+-+ +++++++	-++++++ +++++++ +-++++++								
Ammonium bifluoride 227 g	0.30	1	9 10	-++++ ++-++	- + + + + + + + + + + + - + + +	+++++++++++++++++++++++++++++++++++++++	++-++ +++++++								
	0.61	2	9 10	-++++++ -+++-+-+			+++++++								
Ammonium bifluoride 454 g	0.30	1	11 12		++++++++++++		++++++++								
	0.61	2	11 12	+ - + + + +			++++++++								
2-Bromopyridine 237 ml	0.30	1	13 14 15 16	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		+++++++++++++++++++++++++++++++++++++++									
	0.61	2	15	- + + + - + + + + + + + - + + + + - + + + - + +		- + + + + M + + + + + + + + +	+ + + + + + + + + + + + + + + + + + +								
Busan 40 ^c 473 ml	0.30	1	18 19	+	++ ++-++ +++	-+++ +-++-	++-++++++++								
	0.61	2	18 19	-+++-+-+ -++++-	+ + - + + + + + + + + + + + +	+ + +	M M M M M M M M M + + + + + + + + + + +								

TABLE 2. (continued). Viability of fungus cultures inoculated into Douglas-fir timbers at different distances from fumigation sites and after five time intervals following application of fumigants

				Time of assay (mo) following fumigation and numbers and viability of cultures											ь							
Fumigant	Distance			Culture numbers			Culture numbers				Culture numbers				Culture numbers					Culture numbers		
	culture f fumiga		Timber																			
	m f	feet	number	1 2 3 4 5	6 7 8	1	2 3 4	1 5	6 7	8	1 2 3	3 4	5 6	7 8	1 2	3 4	1 5	5 7 8	1	2 3	4 5	6 7 8
Busan 40 473 ml	1.22	4		+ + + + - + + + + + + + + + + + + + + +	+ + + + - +	+ - +	+ + - + + - + + -	+	+ + + + + + -	+ -	+ + -	+ + + + + + + +	+ + + + - +	++++++	+++++	+ -	+ - + + + +	+ + + + + + +	+ + +			
Mylone 227 g	0.30	1	22	++- +-	+	-	- + -	-		+ -		-+		-+	M M	MI	M -		-			
	0.61	2	21 22 23	+ - + + + + + + + + + + + + -	+ - +						++-	+ +		-+	++	+ -		M M N - + + M M N	F			
	1.22	4	21 22 23	+ + + + - + + + + + + + + + -	+++					-	+ + -	+ +	- +	++	M M	M	м м	M M N M M N M M N	1			
Sec-butylamine 150 ml	0.30	1	24 25	+ + + + - + - + + -		- 7	200															
	0.61	2	24 25	++++-					7.1													
	1.22	4	24 25	+++++																		
Isopropylamine 300 ml	0.30	1	26 27	++++-																		
	0.61	2	26 27	++++-																		
Isopropylamine 300 ml	1.22	4	26 27	- + + + - + + + + +					7													
Acetaldehyde 125 ml	0.30	1	28 29	-++++ ++++	+-+	+	++-	+ +		+												
	0.61	2		+ - + + + -	-++	+	+++		+ +	+												
	1.22	4	28 29	+ - + + -	+++	+	++-		+ +	+												
Sodium bisulfite 454 g	0.30	1	30 31	+	- + -	-	- + -	-	- +	-												
	0.61	2	30 31	+++++	-++	+	+ + +	-	+ +	+												
Ethanalani	1.22	4	30 31	+++++	-++	+	+ + +	+ :	+ +	+												
Ethanolamine 300 ml	0.30	1	32 33	+++-+	+ - +	+	+++	+	+ M	M												
	0.61	2		- + + + - + + + + -	+	+	+ + +	M	+ M	M												
	1.22	4		++++++																		

^a Fumigants were poured into 1-in. holes clustered about the center of the timber, the number of holes depending on the amount of chemical.

^b Culture No. 1 = Poria placenta; No. 2 = P. carbonica; No. 3 = Lentinus lepideus; No. 4 = P. xantha; No. 5 = Antrodia serialis; No. 6 = Fibroporia vaillantii; No. 7 = Serpula incrassata; No. 8 = Gloeophyllum trabeum. Viability: - = no growth, culture presumed dead; + = positive growth.

^c Vapam = 33% sodium N-methyl-dithiocarbamate.

^dM = culture tube missing.
^eBusan = 41% potassium *N*-hydroxymethyl-*N*-methyldithiocarbamate in aqueous solution.

Mylone = 99% 3,5-dimethyltetrahydro-1,3,4,2-*H*-thiadiazine 2-thione.

inspection, indicating that a significant reduction in toxic quantities of Vapam had occurred between 16 and 20 mo after fumigation. Cultures implanted 1.22 m from the CL were generally little affected by Vapam.

Based on data collected 4 mo after fumigation, 2,6-dichloropyridine appeared to be effective primarily against A. serialis, S. incrassata, and, to a lesser degree, F. vaillantii. Few culture replacements died beyond 4 mo after fumigation.

Ammonium bifluoride behaved similarly to 2,6-dichloropyridine. It was effective generally against the same fungi during the first 4 mo following fumigation. It differed in that it was more effective against F. vaillantii and S. incrassata than against A. serialis. After 4 mo, ammonium bifluoride was ineffective against most isolates.

Most of the cultures survived in 2-bromopyridine-treated timbers regardless of the time lapse following treatment; hence this chemical was deemed to be ineffective as a fumigant.

Busan 40 approached Vapam in effectiveness during the first 4 mo after treatment, but only at 0.30 m from the CL. During this period, complete eradication of implanted cultures occurred only in one timber at both 0.30 and 0.61 m from the CL. At 8 and 12 mo after fumigation, the effectiveness of Busan 40 decreased markedly, although over half of the cultures removed during those periods were killed. By the 16th mo, only about one-fourth of the cultures were killed.

Mylone appeared less effective than Busan 40 at 0.30 m from the CL during the first 4 mo of fumigation, but it became more effective 8 and 12 mo following treatment. Also, although vandals destroyed about two-thirds of the cultures to be inspected at 16 mo, all those remaining were dead; hence, Mylone continued to appear effective up to that time.

Sodium bisulfite, which was placed into test timbers later than the aforementioned chemicals, was tested for only 8 mo. It was similar to Mylone in effectiveness at 0.30 m from the CL and was similar to Mylone and Busan 40 in its limited effectiveness beyond that distance. Unlike these fumigants, however, sodium bisulfite failed to eradicate *L. lepideus* and *S. incrassata* in either of the two timbers tested (Table 2).

Acetaldehyde, sec-butylamine, isopropylamine, and ethanolamine, although effective fumigants in laboratory tests, were ineffective in field application.

DISCUSSION

Fumigants have been found by Scheffer and Graham (12) to vary in efficacy between different Douglas-fir poles. Some of the factors influencing the success of fumigation of wood include its permeability, grain direction, temperature, moisture content, and the presence or absence therein of decay pockets or checks (1). Excessive checking, resulting in the escape of fumigant, is thought to be the reason for the lack of control evidenced 8, 12, and 16 mo after fumigation in timber 4 (Table 2). Although toxic concentrations of Vapam were present during the first few months of fumigation, resulting in eradication of all cultures except one at 0.61 m from the CL, the fumes then apparently dissipated to the point where toxic amounts were present in only one or two localized areas in the timber. In some cases, movement or buildup of fumes was seemingly impeded in isolated sectors of the timbers, e.g., in timber 1, at 0.30 m from the CL, P. xantha continually escaped exposure to toxic quantities of Vapam. These situations could have been due to grain direction or local checking.

Wood decay fungi vary in sensitivity to fumigants (11,13). In a study of seven decay fungi, *L. lepideus* was most tolerant and *G. trabeum* and *Coniophora puteana* least tolerant to exposure to fumes of chloropicrin, Vapam, and Vorlex (11). We noted a greater sensitivity of *A. serialis*, *P. vaillantii*, and *S. incrassata* to 2,6-dichloropyridine and ammonium bifluoride and, to a lesser extent, to sec-butylamine and isopropylamine. *L. lepideus* and *S. incrassata* were least sensitive to sodium bisulfite (Table 2).

Based on numbers of implanted cultures killed, toxic concentrations of Vapam remained throughout much of three of the four Douglas-fir timbers that we had treated 12 mo earlier (Table 2). However, by 16 mo, the toxic quantities had decreased markedly. This agrees with the results of Scheffer and Graham (12)

who found that no residual vapors remained 20 mo after treatment of Douglas-fir pole sections with Vapam. In the present work, Mylone differed in that toxic concentrations at 0.30 m from the CL appeared to increase with time. On the basis of only two test periods, i.e., 4 and 8 mo after fumigation, sodium bisulfite appeared to be maintaining its toxicity with time. However, as treatment with sodium bisulfite was accomplished in October, rather than in June as with Vapam and Mylone, the cooler temperatures prevailing may have slowed action of the chemical.

Fumigation with chemicals like Vapam has proven to be an effective way of controlling decay in Douglas-fir poles (5-8,12) and wharf members (10). Furthermore, we show in the present work that, despite the variable sensitivity of some fungi towards fumigants, Vapam and other test fumigants are capable of eliminating the known major Douglas-fir wood decay fungi in horizontal timbers at a distance of 0.61 m from the point of treatment. Thus, fumigators of infected Douglas-fir waterfront and other structural timbers may be more confident of success in eradication of the most frequently encountered fungal causes of decay in these timbers. Those planning to use fumigants in control of wood decay fungi should be aware, however, of both the inherent and fungus-induced variability in wood that may affect efficacy of treatment. They should take steps to circumvent fumigation failures, particularly in bearing timbers that are difficult to replace by applying fumigants near common infection sites and at more frequent intervals linearly in the infected wood than are suggested by fumigant penetration studies.

CAUTION

Vapam is registered with the U.S. Environmental Protection Agency and is used extensively for controlling interior decay in poles (6). However, Vapam and the other materials used in these field trials are hazardous and extreme care must be employed, particularly where these chemicals might spill or leak into the surrounding environment. Furthermore, fumigants should not be used on timbers located within structures or other poorly ventilated areas.

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