Methyl Iodide and Sulfuryl Fluoride as Quarantine treatments for Solid Wood Packing Material.

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INTRODUCTION

The threat of wood-inhabiting fungi to American hardwood forests, lumber industries, tourism, and the aesthetic and dollar values of properties, is potentially disastrous. Biodegradation of wood is accomplished in part by insects and marine borers, but the greatest degree of deterioration and product devaluation is caused by wood-inhabiting fungi. Solid wood packing material (SWPM) is recognized as a major pathway for introduction of insects and pathogens into the US which then subsequently infects indigenous tree (wood) species. Currently, exported SWPM is disinfected by methyl bromide (MeBr) fumigation and conventional heat sterilization. Restrictions on the use of MeBr have increased interest in developing alternative treatments for SWPM. Methyl iodide (MeI) and sulfuryl fluoride (SF) have been considered as alternatives to MeBr, however; research on these fumigants have been limited to few wood-inhabiting fungi and nematodes. Therefore, scientific data are required to support quarantine treatments, especially with regulations for the reduction of MeBr use as a quarantine treatment. The purpose of this study was to conduct a comprehensive trial to assess the efficacy of MeI and SF for eradication of wood-inhabiting fungi.

MATERIALS AND METHODS

Fungi

The fungal species commonly associated with wood degradation, *Ceratocystis fagacearum* was chosen for this study because of its common occurrence, economic importance, and similarity in biological activity to other pathogen species causing damage based on USDA assessment.

Wood block tests

In a series of controlled experiments, wood blocks of birch, maple, poplar, and red pine (2.5 x 2.5 x 1.0 cm) were inoculated with a 1g macerated mycelium/spores mixture of *C. fagacearum* Identical wood blocks were left untreated (or non-fumigated) as controls. Wood was then incubated at 27°C for a minimum of 30 days. A factorial experiment [3 fumigants (methyl iodide, MeI; sulfuryl fluoride, SF; and methyl bromide, MeBr); 2 fumigant concentrations (160 and 240 g/m^3); 4 wood types (birch, red pine, maple, and poplar); and 3 exposure times (24, 48, and 72 hours)] was arranged in a completely randomized design with four replications. The experiment was replicated twice.

Fumigation

All fumigations were conducted at room temperature (21±2^{o}C). Fumigations were performed in sealed ca. 10.0 L glass fumatoria jars with 100% pure liquid MeI, 99.98 pure SF, and 100% pure MeBr in separate containers at concentrations of 160 and 240 g/$m^{3.}$ Each fumitorium was fitted with a small 12V DC fan to Mix and circulate the fumigant gas. Fumigants were injected as pure neat liquid (MeI) or gases (SF) and Mebr with a gas-tight syringe (Hamilton, Reno, Nevada 89502) into the chambers through a 0.25 in. compression fitted with a 10 mm silicone septa after first withdrawing an equivalent volume of

air. The liquid MeI was injected onto a piece of filter paper, from which it was allowed to evaporate. Fumigant concentrations in the test chambers and control chamber were monitored at intervals of 0.5, 2, 4, 24, 48, and 72 hours. Fumigant concentrations were monitored with a Sapphire infrared gas analyzer (Thermo-Fisher, Franklin, MA). Sample of 2.5 ml volume were directly injected into the analyzer, which was fitted with a closed loop tube, which resulted in a sample dilution factor of 905. A custom low-ppm application was developed by this laboratory. Wood was sampled aseptically from the jars and cultured for the presence of the pathogen as described below. The time-weighted concentration (g/m^3) was multiplied by the period of exposure, in hours, to obtain the concentration time (C x T) product, which was used to express dosage.

Pathogen detection

The efficacy of SF or MeI in killing tested fungus was determined by attempts to isolate the pathogen from wood shavings. The effectiveness of SF and MeI in killing the fungus was compared to the standard fumigant, MeBr. After the completion of the fumigation and subsequent incubation, samples obtained at 10 different locations on wood block surfaces were quickly transferred using flame-sterilized tweezers onto amended malt yeast agar and oak wilt medium. All isolations of suspected test fungi were sub-cultured and subsequently compared with the reference test fungi used as controls. Pathogen isolation attempts were made prior to and after fumigation treatments.

Data analysis

Experiments were analyzed separately and combined when treatments by experiments were not significantly different. Data were plotted by percent pathogen recovery versus C x T. Percent fungal recovery was measured as percent of wood block sections with visible growth after 2 weeks. Percent pathogen recovery and C x T data from the fumigant treatments were subjected to the General Linear Models procedure of SAS (SAS Institute, Cary, NC). Treatment means were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$.

RESULTS & SUMMARY

The percents of *C. fagacearum* recoveries from fumigated and non-fumigated control wood samples are shown in Tables 1-4 and Figures 1-3. Analysis of variance shows that fumigant, fumigant concentrations, and exposure time as well as their interactions had an effect on *C. fagacearum* recovery on tested wood species. There was no difference in response of these fumigants on wood species (Tables 1-3). Pathogen recovery was greater at 24 hr than at 72 hr after fumigation (Figs. 2-3). The percent of pathogen recovery from wood exposed to MeI and SF for 24 h ranged from 0% (red pine) to 6% (birch); 3% (poplar) to 24% (maple); and 0% (red pine, poplar) to 5% (maple), and this depended on the fumigant concentration (Tables 1, 2, Figs. 2-3). Complete absence of the pathogen was achieved after birch and red pine samples were exposed to 160 g/m^3 concentration of MeI for 48h or after birch, red pine, maple, and poplar samples were exposed to 160 g/m^3 of MeI for 72 h (CXT products of 5,491-11,704) (Table 1).

In samples fumigated with SF, complete absence of the pathogen was achieved after maple samples were exposed to 160 g/m^3 for 72h (CXT product of 11,316). SF killed the fungus in birch, red pine, maple, and poplar samples fumigated at 240 g/m^3 concentration for 24 h (CXT products of 5,817-16,466) (Table 2).

MeBr killed *C. fagacearum* in red pine, maple, and poplar samples exposed at 160 g/m^3 for 24 h. No survival of the pathogen was observed in all tested wood species treated with MeBr at 240 g/m^3 for 24 h (C X T product of 9,529-13,532) (Table 3, Fig. 1). Colonization of birch, maple, red pine, and poplar by *C. fagacearum* was greater in non-fumigated samples than fumigated samples (Table 4, Figs. 1, 3). *C. fagacearum* was greatly inhibited by MeI than SF in all wood species tested (Tables 1, 2). Overall, the C x T products of ≤ 4,108 for MeI and ≤ 8,755 for SF were not effective in killing the fungus.

The results from this study suggest that longer treatment time might achieve the goal of complete eradication of *C. fagacearum* and imply that MeI performed as well as MeBr in killing the fungus in some wood species by exposure time combination. Overall, MEI was most effective in killing the fungus than SF under the conditions of this study.

Table 1. Percent *Ceratocystis fagacearum* recovered from cultured wood samples following fumigation with methyl iodide

Fumigant Conc. (g/m^3)	Exposure time (h)	Conc.x time (g.h.m^3)[y]	Percent pathogen recovery[x]				
			Birch	Red pine	Maple	Poplar	Mean
160	24	2,827	6.23 ± 2.55[z]	5.32 ± 3.02	5.11 ± 2.34	5.24 ± 1.32	5.48 ± 1.27
160	48	5,491	0.00 ± 0.00	0.00 ± 0.00	1.12 ± 0.92	2.06 ± 0.98	0.79 ± 0.48
160	72	7,840	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
240	24	4,108	1.17 ± 0.06	0.00 ± 0.00	2.03 ± 0.08	0.00 ± 0.00	0.80 ± 0.02
240	48	7,805	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
240	72	11,704	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean			1.23 ± 0.23	0.89 ± 0.16	1.04 ± 0.31	1.22 ± 0.59	

[x]Percentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto amended malt yeast agar and oak wilt medium.
[y]The average concentration was multiplied by the period of exposure in hours to obtain the concentration time product (C x T) used to express dosage.
[z]Mean of duplicate experiments (200 isolations total)

Table 2. Percent *Ceratocystis fagacearum* recovered from cultured wood samples following fumigation with sulfuryl fluoride

Fumigant Conc. (g/m^3)	Exposure time (h)	Conc.x time ($g.h.m^3$)[y]	Percent pathogen recovery[x]				
			Birch	Red pine	Maple	Poplar	Mean
160	24	3,860	24.86 ± 1.57[z]	16.23 ± 1.54	24.13 ± 1.92	19.87 ± 2.64	21.22 ± 1.90
160	48	7,574	4.02 ± 1.46	4.12 ± 1.92	9.80 ± 1.96	6.42 ± 2.08	6.09 ± 1.80
160	72	11,316	0.89 ± 0.10	1.61 ± 0.34	0.00 ± 0.00	1.24 ± 0.56	0.94 ± 0.25
240	24	5,817	5.23 ± 2.07	4.17 ± 2.24	5.08 ± 1.72	3.02 ± 1.26	4.38 ± 1.66
240	48	8,755	1.06 ± 0.84	1.80 ± 0.89	2.56 ± 0.78	2.19 ± 0.98	1.90 ± 0.85
240	72	16,466	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean			6.01 ± 0.99	4.66 ± 1.34	6.93 ± 0.98	5.46 ± 1.22	

[x]Percentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto amended malt yeast agar and oak wilt medium.
[y]The average concentration was multiplied by the period of exposure in hours to obtain the concentration time product (C x T) used to express dosage.
[z]Mean of duplicate experiments (200 isolations total).

Table 3. Percent *Ceratocystis fagacearum* recovered from cultured wood samples following fumigation with methyl bromide

Fumigant Conc. (g/m^3)	Exposure time (h)	Conc.x time ($g.h.m^3$)[y]	Percent pathogen recovery[x]				
			Birch	Red pine	Maple	Poplar	Mean
160	24	3,363	2.46 ± 1.05[z]	3.92 ± 1.32	5.08 ± 1.43	3.62 ± 1.19	3.78 ± 1.22
160	48	6,174	1.12 ± 0.00	0.00 ± 0.00	2.98 ± 0.16	1.13 ± 0.38	1.31 ± 0.06
160	72	9,388	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
240	24	4,873	1.32 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.01
240	48	9,529	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
240	72	13,532	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean			0.82 ± 0.14	0.66 ± 0.18	1.48 ± 0.24	0.77 ± 0.23	0.00 ± 0.00

[x]Percentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto amended malt yeast agar and oak wilt medium.
[y]The average concentration was multiplied by the period of exposure in hours to obtain the concentration time product (C x T) used to express dosage.
[z]Mean of duplicate experiments (200 isolations total)

Table 4. Percent *Ceratocystis fagacearum* recovered from cultured wood samples non fumigated with sulfuryl fluoride and methyl iodide

Exposure time (h)	Percent pathogen recovery[y]				
	Birch	Red pine	Maple	Poplar	Mean
24	84.26 ± 3.57[z]	98.84 ± 2.13	94.66 ± 3.07	96.73 ± 1.51	93.62 ± 2.22
48	98.24 ± 1.86	98.38 ± 2.52	96.01 ± 1.386	97.18 ± 2.12	97.45 ± 1.73
72	97.79 ± 2.11	99.03 ± 2.68	97.48 ± 1.83	98.86 ± 3.67	98.29 ± 1.98
Mean	93.43 ± 2.36	98.75 ± 2.12	96.05 ± 2.15	97.57 ± 2.04	

[y]Percentage of samples removed from nonfumigated wood that showed fungal growth after transfer onto amended malt yeast agar and oak wilt medium.
[z]Mean of duplicate experiments (100 isolations total).

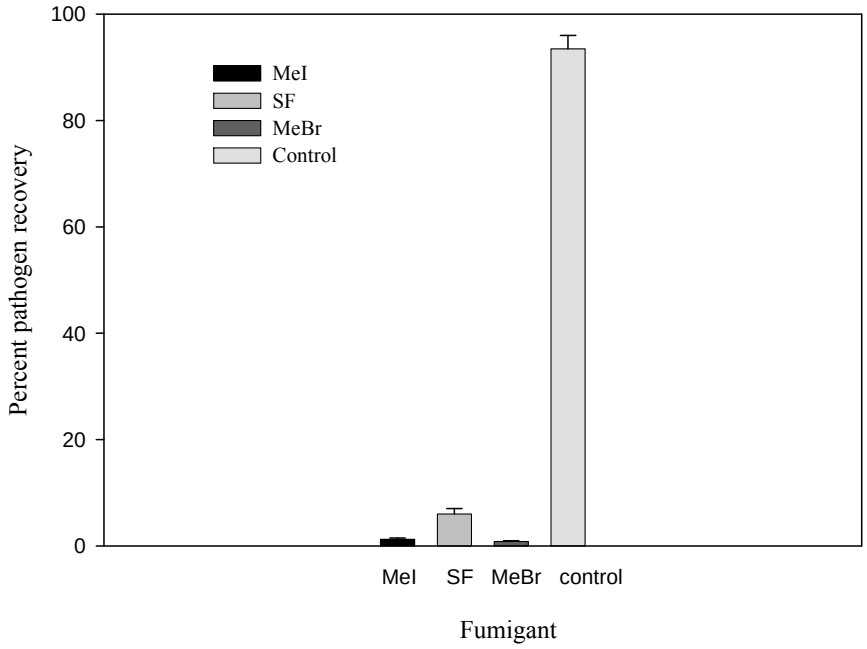

Figure 1. Percent *Ceratocystis fagacearum* recovery from cultured birch, red pine, maple, and poplar samples folowing fumigation with Methyl iodide, sulfuryl fluoride, methyl bromide, and from nonfumigated controls. Data are averaged across wood species, exposure time, and fumigant concentrations.

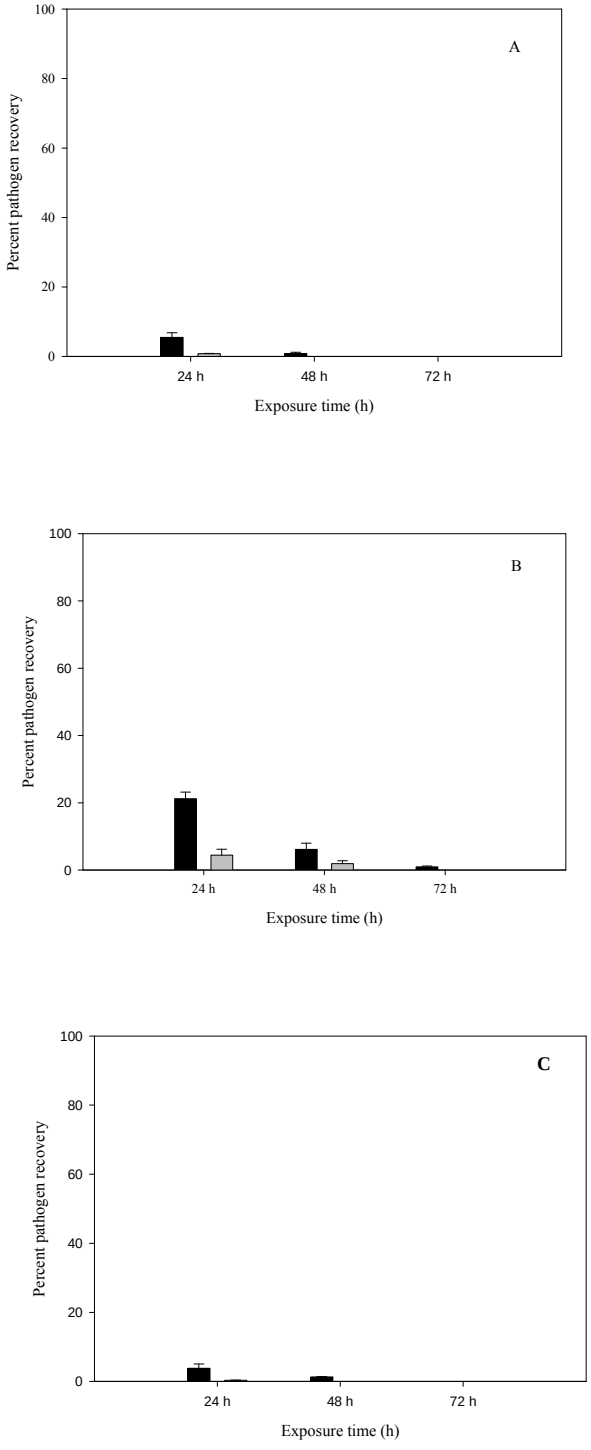

Figure 2. Percent pathogen recovery from cultured birch, red pine, maple, and poplar samples fumigated with methyl iodide **(A)**, sulfuryl fluoride **(B)**, and methyl bromide **(C)**. Data are averaged across wood species.

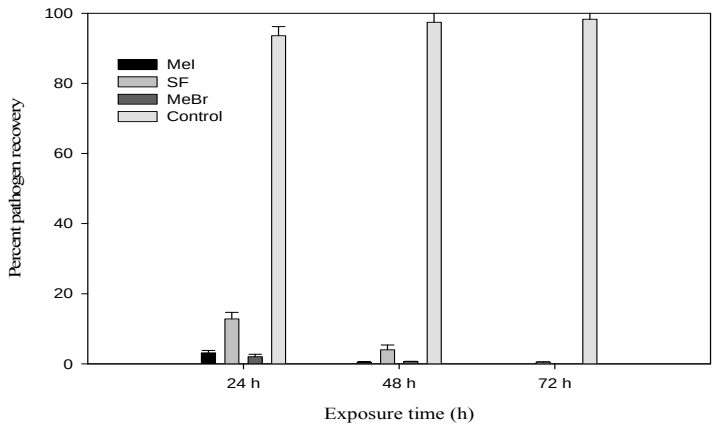

Figure 3. Percent pathogen recovery from cultured birch, red pine, maple, and poplar nonfumigated and fumigated samples with methyl iodide, sulfuryl fluoride, and methyl bromide. Data are averaged across wood species.