

Fungitoxicity of Methyl Iodide, Sulfuryl Fluoride, and Methyl Bromide to *Ceratocystis fagacearum* in Red Oak, Maple, Poplar, Birch and Pine Wood

Kayimbi M. Tubajika¹, Alan V. Barak²

¹United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST), Raleigh, USA; ²United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST), Buzzard Bay, USA.

Email: kayimbi.tubajika@aphis.usda.gov, Abarak@aphis.usda.gov, afbarak@verizon.net

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ABSTRACT

The threat of wood-inhabiting fungi to American hardwood forests, lumber industries, and tourism has enormous economic significance, and the aesthetic and dollar values of properties are potentially disastrous. The efficacy of methyl iodide (MeI) and sulfuryl fluoride (SF) for eradicating wood-inhabiting fungus, Ceratocystis fagacearum was assessed in wood blocks of birch, maple, poplar and red pine based on in-vitro experiments. In a series of replicated controlled experiments, wood blocks were inoculated with a 1g macerated mycelium/spores mixture of C. fagacearum and fumigated with 160 and 240 g/m³ of MeI, SF and methyl bromide (MeBr) as control) for 24, 48, and 72 hours. Analysis of variance showed that fumigant types, fumigant concentrations, and exposure time as well as their interactions ($C \times T$) had an effect on C. fagacearum recovery on tested wood species. Colonization of birch, maple, red pine, and poplar by C. fagacearum was significantly greater in non-fumigated samples than fumigated samples. C. fagacearum was greatly inhibited by MeI than SF in all wood species tested. Overall, the $C \times T$ products of $\leq 4.108 \text{ g·h/m}^3$ for MeI and $\leq 8.755 \text{ g·h/m}^3$ for SF were not effective in killing the fungus. These results suggest that longer treatment exposure time might achieve the goal of complete eradication of C. fagacearum and imply that MeI performed as well as MeBr in killing the fungus than SF under the conditions of this study with potential implications for quarantine use.

Keywords: Disease Control, Quarantine Treatment, Fumigation, Quercus Rubra

1. 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 woodinhabiting fungi [1-3]. Solid wood packing material (SWPM) is recognized as a major pathway for introduction of insects and pathogens into the United States which then subsequently infects indigenous tree (wood) species [4-6].

Currently, exported SWPM is disinfected by methyl bromide (MeBr) fumigation and conventional heat sterilization [7,8]. Methyl bromide has been used for nearly sixty years to control a wide range of pests and diseases in the production of high value crops such as strawberries, flowers, melons, peppers and tomatoes; foodstuffs associated with storage and certain commodities such as grain in trade to prevent the spread of pests and diseases as indicated in UNEP Kenya report [9].

Restrictions on MeBr use have increased interest in developing alternative treatments for SWPM [10-12]. Methyl iodide (MeI) and sulfuryl fluoride (SF) have been considered as alternatives to MB, however; research on these fumigants has been limited to few wood-inhabiting fungi and nematodes [7,8,13,14]. Therefore, scientific data are required to support quarantine treatments, especially with regulations for the reduction of MeBr use as a quarantine treatment. The objective of this study was to determine the fungitoxicity of methyl iodide, sulfuryl fluoride and methyl bromide to *Ceratocystis fagacearum*

in red oak, maple, poplar, birch and pine wood. Preliminary studies have been published [15,16].

2. Materials and Methods

2.1. Fumigants

2.1.1. Methyl Bromide

(MeBr) is an odorless, colorless gas that has been used as an agricultural soil and structural fumigant to control a wide variety of pests. However, because MeBr deplete the stratospheric ozone layer and is classified as a Class I ozone-depleting substance, the amount of MeBr produced and imported in The U.S. was incrementally reduced until the phase-out took effect on January 1, 2005, except for allowable exemptions. These exemptions include the Quarantine and Pre-shipment (QPS) exemptions, to eliminate quarantine pests, and the Critical Use Exemption (CUE), designed for agricultural users with no technical or economically feasible alternatives [17,18].

2.1.2. Sulfuryl Fluoride

(SF) is used as a structural fumigant and is easy to apply. It is nonflammable and noncorrosive and it offers high diffusion for rapid penetration and aeration [19]. It has been also considered as a potential alternative to MeBr. Similarly, research on MeBr as well as other fumigants has been limited to few wood inhabiting fungi and nematodes [19-21]. There is need for development of new treatments in order to support quarantine treatment.

2.1.3. Methyl Iodide

(MeI) degrades in sunlight with resulting low ozone depletion potential, chemically reacts as an alkylating agent and possesses a lower melting point for increased worker safety. It is reported to be a good soil fumigant and it has also been reported to kill live parenchyma cell in logs [13,21]. Although, MeI and SF have been considered as alternatives to MeBr, researches on these fumigants have been limited to few wood-inhabiting fungi and nematodes [13].

2.2. Fungi

The fungal species commonly associated with wood degradation, *Ceratocystis fagacearum* was chosen for this study because of its common occurrence, economic importance and previous work on fumigation with MeBr and SF [21,22]. The fungus was grown on oak wilt medium [23] for two weeks at 27°C or until almost complete colonization of the plates.

2.3. Wood Block Tests

In a series of controlled experiments, wood blocks of birch, maple, poplar and red pine (2.5 \times 2.5 \times 1.0 cm)

were inoculated with a 1 g 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/m³); 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.

2.4. Fumigation

All fumigations were conducted at room temperature $(21^{\circ}C \pm 2^{\circ}C)$. 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/m³. 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 ($C \times T$) product, which was used to express dosage. After fumigation, the glass jar lids were removed and the wood filled chambers were aired out in a fume cabinet for 24 hr. Untreated wood were aired in a separate fume cabinet. Woods were sampled aseptically from the jar and cultured for the presence of the pathogen.

2.5. Pathogen Isolation

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 before and after fumigation treatments. Precautions were observed to prevent crosscontamination of samples and aseptic procedures were used.

2.6. Data Analysis

Experiments were analyzed separately for each fumigant and combined when treatments-by-experiments were not significantly different. The experiment was carried out in a completely randomized design with four replicates. Each wood block was considered as a replicate and the experiment was conducted twice. The graphical plots of data on percent fungal recovery versus $C \times T$ were done. Fungal recovery (%) was measured by number of wood block sections with visible growth after 2 weeks of incubation/total blocks inoculated ×100. Fungal recovery and $C \times T$ 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.

3. Results

Analyses showed no significant test-by-treatments interaction for the fungal growth, therefore, data from duplicate tests were combined for final analysis. Analysis of variance indicated statistically significant differences among fumigants, fumigant concentrations and exposure time as well as their interactions effected *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 (**Figures 1** and **2**). 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** and **2**, **Figures 1** and **2**).

Complete absence of the pathogen was achieved after birch and red pine samples were exposed to 160 g/m³ concentration of MeI for 48h or after birch, red pine, maple, and poplar samples were exposed to 160 g/m³ of MeI for 72 h (C × T products of 5, 491-11, 704 g·h/m³) (**Table 1**). In samples fumigated with SF, complete absence of the pathogen was achieved after maple samples were exposed to 160 g/m³ for 72h (C × T product of 11,316 g·h/m³). SF killed the fungus in birch, red pine, maple, and poplar samples fumigated at 240 g/m³ concentration for 24 h (C × T products of 5817 - 16,466 g·h/m³) (**Table 2**).

Methyl bromide killed C. *fagacearum* in red pine, maple, and poplar samples exposed at 160 g/m³ for 24 h. No survival of the pathogen was observed in all tested wood species treated with MeBr at 240 g/m³ for 24 h (C × T product of 9529 - 13,532 g·h/m³) (**Table 3, Figure 3**). Colonization of birch, maple, red pine, and poplar by *C. fagacearum* was greater in non-fumigated samples than fumigated samples (**Table 4, Figures 2** and **3**). *C. fagacearum* was greatly inhibited by MeI than SF in all wood species tested (**Tables 1** and **2**). Overall, the C × T products of ≤ 4.108 g·h/m³ for MeI and ≤ 8.755 g·h/m³ for SF were not effective in killing the fungus.

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

Fumigant Conc. (g/m ³)	Exposure time (h)	Conc. × time $(g \cdot h \cdot m^3)^y$	Percent pathogen recovery ^x				
Funingant Conc. (g/m)	Exposure time (ii)	$conc. \wedge time (general) =$	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	

^xPercentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto oak wilt medium. ^yThe average concentration was multiplied by the period of exposure in hours to obtain the concentration time product ($C \times T$) used to express dosage. ^zMean of duplicate experiments (200 isolations total).

Fumigant Conc. Exposure time (g/m ³) (h)	Exposure time	Conc. × time	Percent pathogen recovery ^x					
	$(g \cdot h \cdot m^3)^y$	Birch	Red pine	Maple	Poplar	Mean		
160	24	3860	24.86 ± 1.57^{z}	16.23 ± 1.54	24.13 ± 1.92	19.87 ± 2.64	21.22 ± 1.90	
160	48	7574	4.02 ± 1.46	4.12 ± 1.92	9.80 ± 1.96	6.42 ± 2.08	6.09 ± 1.80	
160	72	11316	0.89 ± 0.10	1.61 ± 0.34	0.00 ± 0.00	1.24 ± 0.56	0.94 ± 0.25	
240	24	5817	5.23 ± 2.07	4.17 ± 2.24	5.08 ± 1.72	3.02 ± 1.26	4.38 ± 1.66	
240	48	8755	1.06 ± 0.84	1.80 ± 0.89	2.56 ± 0.78	2.19 ± 0.98	1.90 ± 0.85	
240	72	16466	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		

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

^xPercentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto oak wilt medium. ^yThe average concentration was multiplied by the period of exposure in hours to obtain the concentration time product ($C \times T$) used to express dosage. ^zMean of duplicate experiments (200 isolations total).

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

Fumigant Conc. Exposure time (g/m ³) (h)	Exposure time	Conc. × time	Percent pathogen recovery ^x					
	(g.h.m ³) ^y	Birch	Red pine	Maple	Poplar	Mean		
160	24	3363	2.46 ± 1.05^z	3.92 ± 1.32	5.08 ± 1.43	3.62 ± 1.19	3.78 ± 1.22	
160	48	6174	1.12 ± 0.00	0.00 ± 0.00	2.98 ± 0.16	1.13 ± 0.38	1.31 ± 0.06	
160	72	9388	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
240	24	4873	1.32 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.01	
240	48	9529	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	

^xPercentage of samples removed from fumigated wood that showed *C. fagacearum* growth after transfer onto amended malt yeast agar and oak wilt medium. ^yThe average concentration was multiplied by the period of exposure in hours to obtain the concentration time product ($C \times T$) used to express dosage. ^zMean 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				

^yPercentage of samples removed from nonfumigated wood that showed fungal growth after transfer onto oak wilt medium. ^zMean of duplicate experiments (100 isolations total).

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

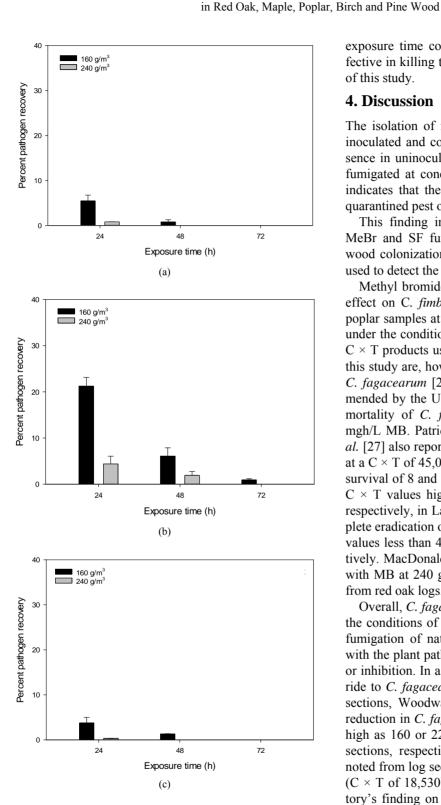


Figure 1. Percent pathogen recovery from cultured birch, red pine, maple, and poplar samples fumigated with 160 g/m³ and 240 g/m³ of methyl iodide (a), sulfuryl fluoride (b), and methyl bromide (c). Data are averaged across wood species.

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exposure time combination. Overall, MeI was most effective in killing the fungus than SF under the conditions of this study.

4. Discussion

The isolation of fungus from wood samples previously inoculated and colonized by C. fugacearum and the absence in uninoculated controls as well as wood samples fumigated at concentrations as high as 80 and 96 g/m³ indicates that these fumigants may be effective against quarantined pest of SWPM.

This finding indicates that the efficacy of the MeI, MeBr and SF fumigation may depend on the level of wood colonization of the tested fungi and the technique used to detect the pathogen in wood.

Methyl bromide and sulfuryl fluoride did not have an effect on C. fimbriata and C. polonica in red oak and poplar samples at C \times T products as high as 2000 g·h/m³ under the conditions of this study (Data not shown). The $C \times T$ products used to effectively kill C. fagacearum in this study are, however, less than previously reported for C. fagacearum [22,24-27] but higher than those recommended by the USDA [28]. Jones [24] reported a-100% mortality of C. fagacearum with a C × T of 45,000 mgh/L MB. Patridge [26] and Schmidt [26]; Schmidt et al. [27] also reported a-100% mortality of C. fagacearum at a C × T of 45,000 mgh/L. Rhatigan et al. [29] reported survival of 8 and 12% of H. annosum and L. wageneri at $C \times T$ values high as 3010 and 4750 mgh/L of MeBr, respectively, in Larch heartwood. They estimated a complete eradication of *H. annosum* and *L. wageneri* at $C \times T$ values less than 4000 and 6000 mgh/L of MeBr, respectively. MacDonald et al. (1985) reported that fumigation with MB at 240 g/m³ for 72 h eradicated C. fagacearum from red oak logs.

Overall, C. fagacearum was resistant to MeBr or SF in the conditions of this study. It is not known however, if fumigation of natural wood products naturally infected with the plant pathogens would result in fungal mortality or inhibition. In a study on fungitoxicity of sulfuryl fluoride to C. fagacearum in vitro and in wilted red oak log sections, Woodward and Schmidt reported 15 and 7% reduction in C. fagacearum isolation at concentrations as high as 160 or 220 g/m³ of SF for 72 h, in red oak log sections, respectively. No C. fagacearum growth was noted from log sections fumigated with 280 g/m³ for 72 h $(C \times T \text{ of } 18,530)$. Woodward and Schmidt [19] laboratory's finding on eradication of C. fagacearum with SF were in contrast with field data obtained by Schmidt et al. [30] showing the presence of the fungus after MeBr fumigation. They suggested longer treatment time to in crease the chance of obtaining complete eradication of

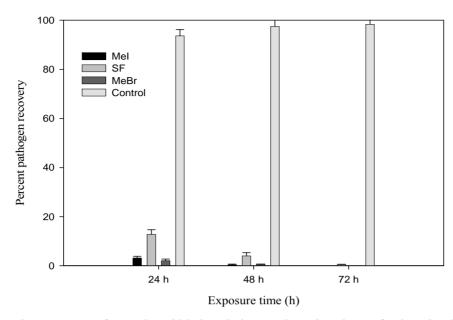


Figure 2. Percent pathogen recovery from cultured birch, red pine, maple, and poplar nonfumigated and fumigated samples with methyl iodide, sulfuryl fluoride, and methyl bromide. Data are averaged across wood species.

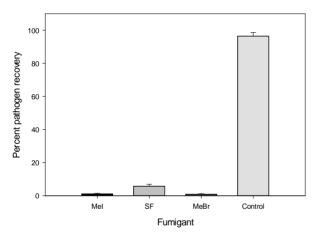


Figure 3. Percent *Ceratocystis fagacearum* recovery from cultured birch, red pine, maple and poplar samples following fumigation with methyl iodide, sulfuryl fluoride, methyl bromide, and nonfumigated controls. Data are averaged across wood species, exposure time and fumigant concentrations.

the fungus in the logs. This suggests that the differences in SF treatment effects may have been accounted for by moisture or other treatment conditions of the two experiments.

The ineffectiveness of MeBr or SF to kill *C. fagacearum* in red oak and poplar samples may possibly due to their deep penetration into wood, to exposure time used in this study, wood characteristics, sorption, or to other unknown factors. It is probable that SF at concentrations less than 240 g/m³ is not sufficient to deplete energy lev-

els of fungal cells to delay hyphal elongation and/ or to disrupt cell metabolism as observed in SF-insect systems reported elsewhere [31,32]. Kawakami *et al.* [13] suggested that when bulk wood of lower moisture content (which is more sorbent of MeBr) is fumigated, such fumigation will require higher doses than indicated here to achieve the requisite $C \times T$ product.

This study documents on the effect of MeI, SF and MeBr fumigation on a number of wood-inhibiting fungi. We have demonstrated that a concentration of 240 g/m³ MeBr or SF (highest concentration used in this study) is not effective or adequate to control all wood fungi tested. This study together with previous studies by Rhatigan *et al.* [29,32-34] showed clearly that the MeBr fumigation is not effective in killing *C. fagacearum* as tested in this study. Additional studies are aimed at determining the penetration of MeI and SF fumigants throughout logs at different concentrations and temperatures in different wood species and fungi combinations.

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