EFFICACY OF SULFURYL FLUORIDE AND METHYL BROMIDE AGAINST WOOD-INHABITING FUNGI.

Tubajika, K.M. and Barak, A. V.
USDA APHIS CPHST, Otis Pest Survey, Detection and Exclusion Laboratory.
Cape Cod, MA 02542

INTRODUCTION

The threat of wood-inhabiting fungi to American hardwood forests, lumber industries, tourism, and the esthetic 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 infect indigenous wood species. Currently, exported SWPM is disinfected using methyl bromide (MB) fumigation and conventional heat sterilization. Restrictions on MB use have increased interest in developing alternative treatments of SWPM. Sulfuryl fluoride has been considered as one of the alternatives to MB and research on this fumigant as well as other fumigants has been limited to few wood-inhabiting fungi and nematodes. There is a need for good scientific data to support quarantine treatments, especially with pressure to reduce MB use as a quarantine treatment. The purpose of this study was to conduct a comprehensive trial to confirm the efficacy of SF and MB in eradicating wood-inhabiting fungi.

MATERIALS AND METHODS

Fungi

Diverse fungal genera and species commonly associated with wood degradation, such as *Irpex lacteus*, *Serpula lacrymans*, *Postia placenta*, *Armillaria mellea*, *Gloeophyllum trabeum, Ganoderma lucidum*, *Heterobasidium annosum, Leptographium wingfieldii, Ceratocystis fimbriata*, *Ceraoscystis polonica,* and *Ceratocystis fagacearum* from red oak and poplar wood species were chosen for this study because of their status as, or similarity to, species of concern from the USDA pest risk assessment (Table 1).

Wood preparation

Wood block tests

In a series of experiments, blocks of red oak and poplar sapwood (10 x 10 x 25 cm) were inoculated with a 1g macerated mycelium/spores mixture of *I. lacteus, S. lacrymans, P. placenta, A. mellea, G. trabeum,* and *G. lucidum* fungi and allowed to grow for 6 weeks in an incubator set at 27^0C. Red oak and poplar blocks were inoculated by dipping each block one face down 2-cm-deep into fungal inoculum (Dipping inoculation technique). Identical red oak and poplar wood blocks were left untreated as controls. Wood was then incubated at 27°C for a minimum of 30 days.

Soil block tests

For uniform colonization of wood by these test fungi and a better determination of their efficacy to fumigant, we used a modified AWPA soil block tests (AWPA, 1994). Wood blocks (2.5 x 2.5 x 1.0 cm) were cut, air-dried, oven-dried, and autoclaved for 25 min at 121^{o}C (250^{o}F). After cooling, blocks of each type were pressure-soaked with sterile distilled water (1h at 880 MPa) and were individually added aseptically to cylindrical 500 ml culture bottles with metal screw lids which contained infested soil. Samples were allowed to grow for 6 weeks in an incubator set at 27^{o}C. The control blocks were not inoculated and not fumigated, they were used to detect background contamination (negative controls) and identical samples were inoculated with these fungi but were not fumigated (positive controls). Two sterile white birch feeder strips were sandwiched between the infested soil and test samples, and samples at the time of inoculation had an average moisture content of 28% (red oak) and 18% (poplar).

Fumigation

All fumigations were conducted at room temperature (21±2^{o}C). Fumigations were performed in sealed 2.2 L glass jars with MB (100% pure) and SF (99.8%) separately at concentrations of 16, 32, 48, 64, 80, 96, and 112 g/m$^{3.}$ Fumigants were injected as pure neat gases with a gas-tight syringe (Hamilton, Reno, Nevada 89502) into the chambers after first withdrawing an equivalent volume of air. Fumigant concentrations in the test chambers and control chamber were monitored at intervals of 0.5, 1, 2, 4 and 24 hours. 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 MB in killing tested fungi was determined by attempts to isolate the pathogen from wood shavings. 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 treatment.

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 by 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

The fungi, identified as *I. lacteus, S. lacrymans*, *P. placenta*, *A. mellea*, *G. trabeum, I. lacteus, G. lucidum, H. annosum, L. wingfieldii, C. polonica, C. fimbriata*, and *C. fagaceurum* were re-isolated from inoculated controls and in fumigated samples. Microscopic examination of test fungi from inoculated wood species confirmed the presence of these fungi originally used as inoculum. No other fungi or bacteria contamination was observed during experimentation.

Analyses showed no significant test-by-treatment interactions for the fungal colonization, therefore, data from duplicate tests were combined for final analysis. Analysis of variance indicated statistically significant main effects of wood species, fumigants, and fumigant concentrations on fungal recovery ($P < 0.0001$). The effect of interactions of wood species, fumigants, and fumigant concentrations on fungal recovery was also statistically significant ($P < 0.001$).

Colonization of red oak and poplar by test fungi was greater in control samples than fumigated samples (Tables 2, 3). Of the fungi tested, *I. lacteus, P. placenta, A. mellea, G. trabeum,* and *G. lucidum* were in some instances more susceptible to MB and SF (Fig. 1). No survival of these fungi was observed at concentrations 80 g/m^3 or higher within 24hr for MB (C x T product of 1350) and SF (C x T product of 2002).

In soil block assays, *H. annosum, L. wingfieldii*, *C. polonica, C. fimbriata*, and *C. fagaceurum* were more resistant to MB- and SF fumigation (Fig. 2). All tested fungi were recovered at all concentrations for both the fumigants. The C x T products of 2643 for MB and 2804 for SF were not effective in killing the fungi.

MB and SF fumigation did not have an effect on *H. annosum, L. wingfieldii*, *C. polonica, C. fimbriata*, and *C. fagaceurum* at C x T products as high as 2600 $g.h.m^3$ in conditions of this study. However, the C x T products used to effectively kill *C. fagaceurum* in this study are however lesser than previously reported for *C. fagaceurum* (Jones, 1963; Patridge, 1961; Schmidt 1983; Schmidt et al. 1982; MacDonald et al. 1985) but higher than those recommended by the USDA APHIS PPQ. This study together with other studies showed clearly that 80 g/m^3 of MB or SF are not effective in killing all wood-inhabiting fungi.

Additional studies are aimed at determining the penetration of SF and other fumigants throughout logs at different concentrations and temperatures in different wood species and pathogenic fungi combinations.

References: AWPA, 1999; Jones, 1963; MacDonald et al. 1985. Patridge, 1961; Schmidt, 1983; Schmidt et al. 1982.

Table 1: Wood-inhabiting fungi evaluated for sensitivity to sulfuryl fluoride and methyl bromide.

Species	Isolate Number	Source	Cultural Media
Irpex lacteus	ATCC 60993		MYEA[y]
Serpula lacrymans	ATCC 36335		MYEA
Postia placenta	Mad 698R		MYEA
Armillaria mellea	ATCC 11113		MYEA
Gloeophyllum trabeum	ATCC 11539		MYEA
Ganoderma lucidum	B611	Durham, NH	MYEA
Heterobasidion annosum	B614	Durham, NH	MYEA
Heterobasidion annosum	B572	Syracuse, NY	MYEA
Heterobasidion annosum	B323	Durham, NH	MYEA
Leptographium wingfieldii	C445	Sweden,	MYEA
Ceratocystis fagacearum	C1108	Sac City, IA	Bernett medium[z]
Ceratocystis fagacearum	C1694	Ames, IA	Bernett medium
Ceratocystis polonica	C1226	Russia,	Bernett medium
Ceratocystis fimbriata	C1317	North Carolina	MYEA

[y]Malt yeast extract agar (2%)
[z]Barnett semiselective medium (Oak wilt medium) (Barnett, 1953)

Table 2. Effect of methyl bromide and sulfuryl fluoride on wood-inhabiting fungi in 10 x 10 x 25 cm red oak and poplar wood blocks[z].

		Percent pathogen recovery		
Wood type	Wood size	Control	Methyl bromide	Sulfuryl fluoride
Poplar	10 x 10 x 15cm	84.23 ± 3.571Aa	28.38 ± 0.098Ba	24.54 ± 0.091Ba
Red Oak	10 x 10 x 15cm	88.1 7± 6.076Aa	18.75 ± 0.079Bb	20.77 ± 0.083Ba

[z]Calculated from the means for six concentrations of fumigant and controls lacking fumigant. Within rows, means followed by the same A, B, or C are not significantly different ($P = 0.05$); within columns, means followed by the same a or b are not significantly different ($P = 0.05$) according to the Fisher's least significant difference test.

Table 3. Effect of methyl bromide and sulfuryl fluoride on wood-inhabiting fungi in 2.5 x 2.5 x 1.0 cm red oak and poplar wood blocks[z].

		Percent pathogen recovery		
Wood type	Wood size	Control	Methyl bromide	Sulfuryl fluoride
Poplar	2.5 x 2.5 x 1.0cm	98.59 ± 1.406Aa	79.69 ± 1.992Ba	83.25 ± 1.949Ba
Red Oak	2.5 x 2.5 x 1.0cm	100.00 ± 0.000Aa	73.44 ± 2.641Ba	94.50 ± 1.089Ab

[z]Calculated from the means for six concentrations of fumigant and controls lacking fumigant. Within rows, means followed by the same A, B, or C are not significantly different ($P = 0.05$); within columns, means followed by the same a or b are not significantly different ($P = 0.05$) according to the Fisher's least significant difference test.

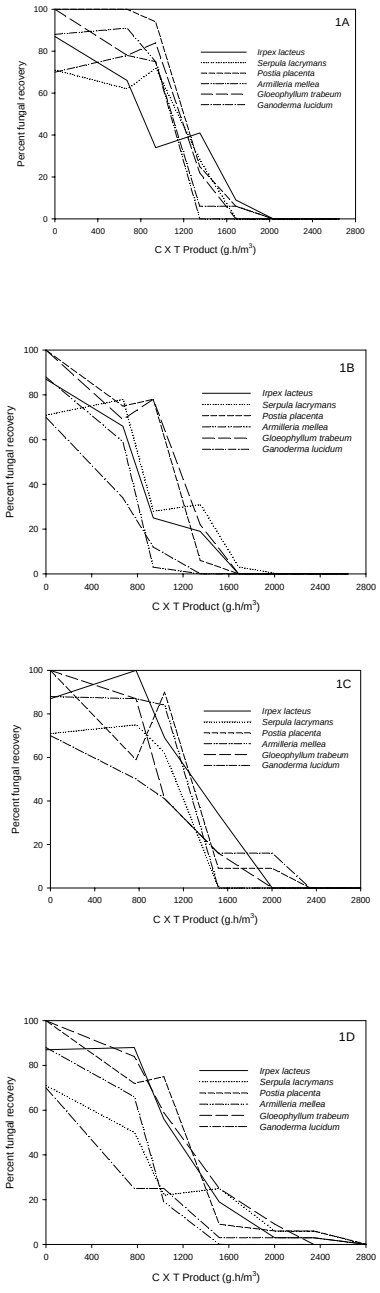

Figure 1. C x T Product-percent fungal recovery for *Irpex lacteus*, *Serpula lacrymans*, *Postia placenta*, *Armillaria mellea*, *Gloeophyllum trabeum, Ganoderma lucidum* fumigated with methyl bromide **(A, B)** and sulfuryl fluoride **(C, D)** in poplar **(A, C)** and red oak **(B, D)** wood blocks.

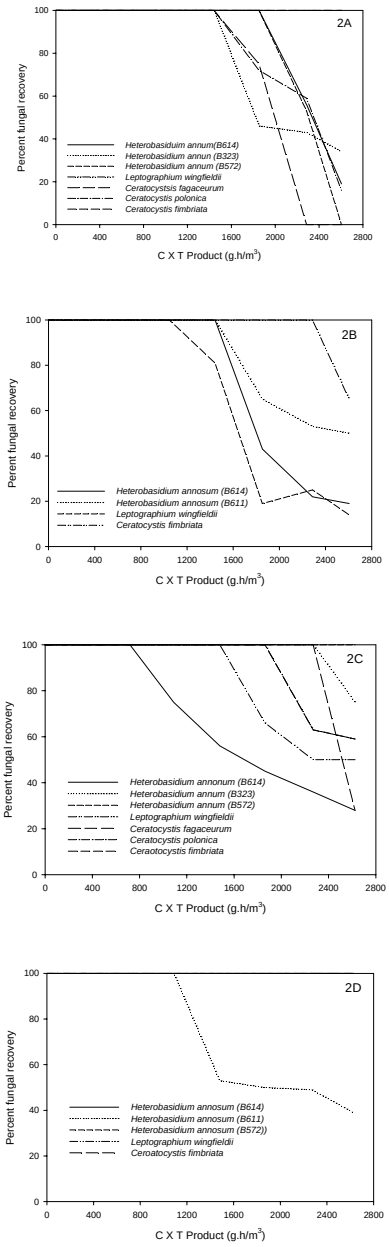

Figure 2. C x T Product-percent fungal recovery for *H. annosum, L. wingfieldii*, *C. polonica, C. fimbriata*, and *C. fagaceurum* fumigated with methyl bromide **(A, B)** and sulfuryl fluoride **(C, D)** in poplar **(A, C)** and red oak **(B, D)** wood blocks.