Rapid Screening of Sulfuryl Fluoride as a Potential Phytosanitary Treatment for a Broad Selection of Fungi Relevant to Forestry

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Abstract

Increasing restrictions on the use of methyl bromide have created an urgent need to find suitable replacements that are effective in eliminating exotic pest establishments via trade in wood products. Efficacy data for established phytosanitary fumigants have primarily focused on arthropods and nematodes, whereas limited information exists for plant pathogens and fungi relevant to forestry. A rapid screening process was developed to evaluate efficacy and compare relative tolerance to fumigation treatments for a broad selection of relevant fungi under laboratory conditions. Sulfuryl fluoride (SF) fumigations were conducted in 10-liter glass chambers at six target concentrations (40 to 240 mg liter^{-1}) at 15°C and 20°C for 24-, 48-, and 72-hour exposure times against 23 fungal species represented with 35 isolates. Fungi were grown on sterilized barley grain and then distributed in felt-covered borosilicate glass tubes to allow uninhibited gas penetration during fumigation while minimizing the risk of fungal contamination. This allowed simultaneous testing of numerous species and isolates, followed by 100 percent recovery of controls without contamination. Results demonstrate that SF is an effective fumigant for a broad range of fungi. Several fungi and isolates were found to consistently be among the most tolerant to the fumigant treatment. These species may serve as a benchmark for screening SF in fumigations of logs and other wood products. Methods developed here will be useful in efficacy screening of other methyl bromide fumigant alternatives.

Exotic plant pests and pathogens are transported via international trade of various commodities. Several forest pests have caused significant economic and ecological losses in both North America and worldwide (Aukema et al. 2010, 2011; Leal et al. 2010; Liebhold et al. 2012; Lovett et al. 2016). Plants for planting have been identified as the most significant pathway for introduction of exotic tree pathogens; however, other wood products also pose a threat (Leal et al. 2010). Wood packaging in particular has been implicated in the introduction of several exotic insects and insect-vectored pathogens (Haack et al. 2002, 2010, 2014). Because of the volume of trade of commodities that include wood packing materials and other wood products, the transfer of pathogens and nematodes as well as insects remains a large concern that needs to be addressed.

Exotic tree pests can be particularly damaging when they become established in new countries because management strategies are often limited in forest systems and the cost of eradication programs is high and often unsustainable (Allen and Humble 2002, Brockerhoff et al. 2010). Several recent introductions of exotic insect species in North America threaten the future of native trees and have caused irreversible changes to forest ecosystems (Dodds and Orwig 2011, Herms and McCullough 2014). As a result, national plant protection organizations have become increasingly vigilant in preventing the introduction and establishment of

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exotic pest species through regulations and improvements in phytosanitary treatments and detection efforts.

Treatment of wood packing material in international trade is governed by the International Plant Protection Convention (IPPC), International Standard for Phytosanitary Measures No. 15 (ISPM-15). ISPM-15 currently includes treatment schedules for methyl bromide fumigation, conventional heat, and dielectric heat (Food and Agriculture Organization of the United Nations [FAO] 2016a). These treatments can be considered for wood products other than packing material, including for log exports. Finding alternative phytosanitary treatments for logs in the United States and Canada reflects the interests of the authors and their stakeholders that initiated the work. However, the information presented here is applicable worldwide and also for other wood commodities, including wood packaging, for which eradication of fungal pathogens also needs to be addressed.

Movement of logs and other forest products are not currently covered by broad international agreements and thus rely on bilateral trade agreements to establish effective treatments/measures. Log exports from the United States have typically relied on methyl bromide fumigation as a phytosanitary treatment for the oak wilt fungus and other pests of concern to foreign export markets (MacDonald et al. 1985). Heat treatment is currently viewed as an impractical phytosanitary measure for logs in North America because of its potential for damage or quality degradation owing to the long exposure time to heat and higher temperatures required to reach temperatures lethal to target organisms. Fumigation is therefore a preferred treatment for logs as it is unique in that it offers the opportunity for bulk treatment without affecting wood quality. However, because methyl bromide is an ozone-depleting compound there is a need to develop alternative fumigants.

Although quarantine and pre-shipment (QPS) uses of methyl bromide are still permitted, virtually all other uses are prohibited or scheduled for phase-out (United Nations Environment Programme [UNEP] 2006). Under the Montreal Protocol, the QPS exemption from the phase-out of methyl bromide is applicable to all member countries. Some countries still use significant amounts of methyl bromide, some very small amounts, whereas others, like the European Union, severely limit or prohibit use of methyl bromide for QPS altogether (UNEP 2014). In the United States the Environmental Protection Agency (EPA) regulates methyl bromide under the Clean Air Act. This presents additional challenges for timber exporters resulting from EPA permit requirements and annual limits on site source emissions of methyl bromide (US EPA 2016). Development of effective alternative fumigation treatments for logs and other wood products would allow for increased and pest-safe trade and significantly benefit the North American timber industry. Additionally, new methyl bromide alternative treatments could further reduce the global use of methyl bromide for wood packaging material and other QPS uses.

Several studies have demonstrated efficacy of sulfuryl fluoride (SF) against wood-inhabiting insects (Barak et al. 2006, 2010), pinewood nematode (Bonifácio et al. 2013, 2015), and oak wilt fungus (Schmidt et al. 1997, Tubajika and Barak 2011). Data to support SF as an approved ISPM-15 treatment for insects and nematodes have been submitted to IPPC and are currently under review (IPPC 2015). However, little is known about the efficacy of SF against a broader range of fungi. As invasive organisms continue to threaten North American forests and present potential trade barriers for exports of logs and other forest products, it is important to expand treatment options for timber exporters.

The goal of this study was to develop a screening method that could provide baseline efficacy data for SF across a broad range of wood-inhabiting fungi and fungus-like organisms from Class Oomycetes, such as *Phytophthora* spp., to determine the potential for SF fumigation for fungi beyond what is known for oak wilt. There is a need to develop methods to allow simultaneous work with biologically diverse pathogens that may not all grow well on common nutrient media. Testing must also be done aseptically to prevent contamination before assessing the survival of the test organisms. This article addresses these challenges and provides preliminary results on the efficacy of SF against a wide range of fungi and *Phytophthora* spp. Further work is needed to assess penetration and efficacy in larger-scale trials using commercial-size logs to demonstrate feasibility for international trade and replacement of methyl bromide for this purpose.

Materials and Methods

A selection of fungi and *Phytophthora* species (Table 1) was obtained from culture collections maintained at the FPInnovations Laboratory, Vancouver, British Columbia and grown on their preferred nutrient agar media and then on barley and wheat grains. For simplicity we will use the term ''fungi'' to cover both fungi and fungus-like organisms such as *Phytophthora* spp. The fungal cultures used in this study were selected to represent a wide variety of fungi with the potential to cause phytosanitary concerns for standing forest trees (both hardwoods and conifers), wood products, and decay in wood in service. These fungi were also available from the FPInnovations culture collection. Compared with laboratory work with insects and nematodes, fungi used in the screening process must be cultured and manipulated with good sterile techniques to avoid cross-contamination among test isolates and from air, which may affect survival data assessment.

In our preliminary tests, fungi were successfully grown on sterilized grain to produce well-colonized samples that were easier to manipulate and culture compared with growing each fungus on its preferred host species or nutrient media. Organically grown grains (Pro-form feeds, Unifeed, Chilliwack, British Columbia, Canada) were thoroughly rinsed in warm water to remove dust and debris, strained for 10 minutes and then soaked in room-temperature (~21.0°C) distilled water for 24 hours. Water containing the soaked grains was then brought to boil and immediately drained. The grains were then washed in cold water and allowed to drain for 15 minutes. This process allows grains to get thoroughly soaked but not become soggy. Grains remained firm and separate after inoculation and were easy to individually handle. Approximately 15 to 20 g of grain was added to 250-mL Erlenmeyer flasks, capped with cotton plugs and aluminum foil, and autoclaved for 30 minutes at 121°C at 15 psi. After cooling to room temperature, the flasks were inoculated with 10 plugs of colonized agar taken from the growing edge of individually grown fungal species and incubated at 25°C. The flasks were

Table 1.—List of species and isolates used in the test in alphabetic order, and their survival when exposed to different sulfuryl fluoride (SF) dosages for 24, 48, and 72 hours at 15°C and 20°C.[a]

Species (isolate)	Fumigation time (h)	Survival at the SF dosage (mg liter^{-1}) listed:						
		Control	40	80	120	160	200	240
Ceratocystis fagacearum (CMW2039)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y/0
	72	Y	Y	Y	Y	Y	Y/0	Y/0
C. fagacearum (C520)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	0/Y	0	0/Y	0/Y
	72	Y	Y/0	Y	Y/0	Y/0	0	0
C. polonica (93-208/115)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	0	0/Y	Y	0
	72	Y	Y	Y	0	0/Y	0	0
Chalara fraxinea (CBS 122504)	24	Y	Y	Y	y	0	0	0
	48	Y	Y	0/Y	0/Y	0/Y	0/Y	0/Y
	72	Y	0/Y	0	0	0	0	0
Fomitopsis pinicola (030) (Deck 3-D14)	24	Y	Y	Y	Y/0	Y	0	0
	48	Y	Y	Y	Y/0	0	0	0
	72	Y	Y	0	0	0	0	0
Geosmithia morbida (1272)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72	Y	Y	Y	Y	Y/0	Y/0	Y/0
G. morbida (1259)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72	Y	Y	Y/0	Y	Y/0	Y/0	0
G. morbida (1223)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y/0	Y
	72	Y	Y	Y	Y/0	0	0	0
G. obscura (CBS 121749)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	0
	72	Y	Y	Y	0	0	0	0
Gloeophyllum sepiarium (FT1C46C)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y/0	Y/0	m/0	Y/0
	72	Y	Y	0	0	0	0	0
Heterobasidion annosum (AU 299-1)	24	Y	Y	Y	Y	Y	0	0
	48	Y	Y	0	0	0	0	0
	72	Y	0/Y	0/Y	0	0	0	0
H. annosum (034) (MKRF-4)	24	Y	Y	Y	Y	Y	Y/0	Y/0
	48	Y	Y	Y	Y	0/Y	0	0/Y
	72	Y	Y	Y/0	0/Y	0	0	0
H. occidentalis (AU 250-1)	24	Y	Y	Y	Y	Y	Y/0	Y/0
	48	Y	Y	0	0	0	0	0
	72	Y	Y	0	0	0	0	0
Hyphoderma praetermissum (044) (Deck 5)	24	Y	Y	Y	Y	Y	Y	Y/0
	48	Y	Y	Y	Y	Y	Y	0/Y
	72	Y	Y	Y/0	0	0	0	0
Lachnellula willkommii (CBS 172.35)	24	Y	Y	Y	Y	Y	Y	Y
	48							
	72							
L. willkommii (CBS 200.66)[b]	24							
	48							
	72	Y	Y	0	0	0	0	0
Leptographium longiclavatum (55R140E2-2)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72	Y	Y	Y	Y/0	Y/0	Y	0
L. wageneri (C731)	24	m/Y	m/Y	m/Y	m/Y	m/Y	m/Y	m/0
	48	Y	Y/0	0/Y	0	Y/0	m/0	0
	72	Y	Y/0	Y/0	0	0	0	0
L. wageneri (#91)	24	Y	Y	Y	Y	Y	Y	Y/0
	48	Y	Y	Y	0/Y	0/Y	0/Y	0
	72	Y	Y	0/Y	0	0	0	0
L. wingfieldii (25/3LK)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y/0
	72	Y	Y	Y/0	Y	Y/0	Y	Y/0
Mycosphaerella populorum (01-74C)	24	Y	Y	Y	Y	Y	Y	y
	48	Y	Y	Y	0/Y	Y	0/Y	0/Y
	72	Y	Y	0	Y/0	0	0	0

Table 1.—Continued.

Species (isolate)	Fumigation time (h)	Survival at the SF dosage (mg liter^{-1}) listed:						
		Control	40	80	120	160	200	240
Ophiostoma clavigerum (M33)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y/0	Y/0	Y/0
	72	Y	Y	Y	Y/0	0	0	0
O. clavigerum (SWGIIAW2-2)[b]	24							
	48							
	72	Y	Y	Y	Y/0	Y/0	Y/0	0
O. montium (GW51EW)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72							
Pachnocybe ferrigunea (53) (Deck 4-4D-4B-b)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72	Y	Y	Y	Y	Y/0	Y/0	0/Y
Phellinus sulphurascens (061) (CFS 543)	24	Y	Y	Y	Y/0	Y/0	Y/0	0
	48	Y	Y	Y	Y	Y	Y	0/Y
	72	Y	Y	Y	Y	0	0	0
Phytophthora alni subsp. *multiformis* (P16202)	24	Y	Y	Y	Y	Y	0	0
	48	Y	Y/0	Y	0/Y	0/Y	0/Y	0
	72	Y	Y	0	0	0	0	0
P. quercina (CBS 782.95)	24	Y	Y	Y	Y	0/Y	0	0
	48	Y	Y	Y	Y	Y	0/Y	0/Y
	72	Y	Y	Y/0	0	0	0	0
P. ramorum (CBS 101553)	24	Y	Y	Y	Y	0	0	0
	48	Y	Y	Y	Y	0/Y	0/Y	0/Y
	72	Y	Y	0/Y	0	0	0	0
P. ramorum (CBS 101329)	24	Y	Y	Y	0	0	0	0
	48	Y	Y	Y	Y	Y	Y	0/Y
	72	Y	Y	0	0	0	0	0
P. ramorum (SOD-04-002) (NA1)[b]	24							
	48							
	72	Y	Y	0	0	0	0	0
P. ramorum (SOD-03-002) (EU1)[b]	24							
	48							
	72	Y	Y	0	0	0	0	0
P. ramorum (SOD-04-25165) (NA2)[b]	24							
	48							
	72	Y	Y	0/Y	0	0	0	0
Rosellinia necatrix (CBS 349.36)	24	Y	Y	Y	Y/0	Y/0	0/Y	0
	48	Y	Y/0	Y	0	Y/0	0	0
	72	Y	Y/0	Y/0	0	0	0	0
R. necatrix (ATCC 28386)	24	Y	Y	Y	Y	Y	Y	Y
	48	Y	Y	Y	Y	Y	Y	Y
	72	Y	Y	0	0	0	0	0

[a] Data including a forward slash represent different survival outcomes for the two temperatures, respectively. Y= survived; 0 = no growth after 1 month; m = missing data. Species treated on August 29, 2015 and assessed on September 3, 12, and 23, 2015.

[b] These isolates added for 72-hour fumigation.

shaken at weekly intervals to prevent the grains from sticking to each other and facilitate mycelial fragmentation and further colonization of grains.

SF fumigations were conducted in 10-liter glass jars modified for use as fumigation chambers as described by Tubajika and Barak (2011). Jars were cleaned and decontaminated by wiping them with 99 percent ethanol before use. Borosilicate glass tubes (16 mm outer diameter by 1.22 m; Corning Glass Works, Corning, New York) were cut into 10-cm lengths to be used to hold the fungi-inoculated grains during fumigation. One side of each tube was first covered with a piece of felt cloth held in place by a rubber band, and then the tubes were autoclaved for 30 minutes at 121°C at 15 psi. After the tubes had cooled, three pieces of colonized grain of each fungus were aseptically placed inside individual tubes, and the other end was covered with a sterilized piece of felt cloth and secured with a sterile rubber band (Fig. 1). Each fungal isolate was represented by three colonized pieces of grain in one borosilicate tube for each treatment combination. This allowed the large amount of isolates and treatment combination to be tested at the same time in this initial experiment. Subsequent work will require greater replication of the same isolate in several borosilicate tubes as well as in several jars to allow for statistical analysis. The tubes containing the test fungi were then aseptically placed inside the fumigation jars and the jars were then sealed with high-vacuum grease (Dow Corning Corp., Midland, Michigan). The method of using felt-covered ends on glass tubes was tested before fumigation and the felt cloth covers were

Figure 1.—Test methodology and apparatus used to simultaneously screen various fungal isolates exposed to six different gas target concentrations and a control (no fumigation). From top left to bottom right: colonized barley grains with individual fungi; distribution of grains into individual tubes; sealed tubes containing three grain pieces inoculated with a test fungus; 10-liter test jars filled with tubes containing different test isolates and petri dishes with pinewood nematode-infested wood block; and simultaneous and automatic measuring of gas concentrations in six jars using Agilent 490 micro GC.

Table 2.—Sulfuryl fluoride applied doses, concentration by time products, and cumulative percent reduction in headspace concentration on the basis of periodic gas chromatography measurements for fumigations conducted over 24, 48, and 72 hours at 15°C and 20°C.

Treatment duration (h)	Applied dose (mg liter^{-1})	15°C		20°C	
		h-mg liter^{-1}	% reduction	h-mg liter^{-1}	% reduction
24	40.1	1,044.9	4.3	1,019.2	7.3
	80.1	1,755.5	16.1	1,900.2	3.3
	120.2	2,760.8	6.4	2,787.7	2.5
	160.3	3,555.3	10.8	3,683.4	9.1
	200.3	4,675.2	4.4	4,753.1	1.5
	240.4	5,599.2	5.3	5,620.0	1.8
48	40.8	1,904.9	5.3	2,096.7	9.5
	81.7	3,523.8	10.3	3,934.3	2.8
	120.3	5,554.0	7.1	5,825.3	10.2
	161.2	7,520.5	3.1	8,009.4	2.7
	202.0	8,809.2	11.9	9,732.9	3.6
	245.0	10,732.3	9.5	11,711.2	5.3
72	40.2	2,812.7	7.8	2,698.3	6.8
	80.5	5,681.8	12.0	5,669.7	6.9
	120.7	8,580.3	9.9	9,025.8	5.9
	160.9	11,847.4	6.1	12,052.6	4.1
	201.1	13,404.7	16.1	14,751.4	7.7
	241.4	17,961.9	2.4	17,079.4	8.0

found not to impede gas flow into the tubes. During the fumigation, the jars were held at constant temperature in an environmental chamber (Fig. 1). Temperature inside the chamber was monitored and recorded at 5-minute intervals using a temperature and humidity data logger (Lascar Electronics, Part No. EL-USB-2-LCD).

The efficacy of SF for control of test fungi was evaluated for three time exposures, 24, 48, and 72 hours, and at two temperatures, 15°C and 20°C. There were 23 fungal species and some were represented by several isolates (Table 1). Six concentrations of SF and an untreated control were tested in each experiment. Initial-dose SF concentrations were 40, 80, 120, 160, 200, and 240 mg liter^{-1}. Doses were calculated volumetrically with milligram per liter calculations corrected for temperature and atmospheric pressure at the start of the fumigation using a source cylinder of 99.8 percent purity SF (Profume, Dow AgroSciences). Treatments were established by removing a volume of air from each sealed jar equivalent to the volumetric amount of SF needed to achieve the treatment concentration increased for additional ~20 percent to create a vacuum in the jar and allow for introduction of the fumigant. Jars were equilibrated to near-atmospheric pressure immediately after introduction of SF. Each jar was equipped with a 40-mm 12-V fan set on the underside of the lid to circulate gas during the fumigation.

Headspace concentrations were measured via gas chromatography (GC) at regular intervals throughout the duration of the fumigation. An Agilent 490 micro-GC with thermal conductivity detector and a Poraplot Q column was used (Agilent Technologies Inc., Santa Clara, California). The column and sample line temperatures were 100°C and 110°C, respectively. Headspace samples were drawn through a 1-m-long by 0.12-mm-inside diameter peek tubing over a 60-second sampling time (~5-mL sample volume) using a six-position stream selector valve (Valco Instruments Inc., Product No. EUTA-6CSD6MWE). Initial readings were made ~2 minutes after introduction of SF, and subsequent readings were made at 30 and 60 minutes and then at 2-hour intervals in the 24-hour fumigation and at 4-hour intervals in the 48- and 72-hour experiments. Concentration by time products (CT) were calculated using Riemann sums with the gas concentration measurement as the midpoint for each interval. After fumigation, the jars were aseptically aerated in a Class II biological safety cabinet (NuAire Inc). The individual tubes were then removed, and the felt cloth from one side was removed. Three grains from inside each tube were aseptically placed on 1 percent malt extract agar (MEA), which had been previously shown to support growth of all test fungi used in this project. Fungi were assessed at 1, 2, and 4 weeks by observing growing colonies from the individual grains and comparing morphology with the same fungi from the control jar and grown on 1 percent MEA plates. Although it was assumed that the test organism was dead if not growing after 4 weeks under optimal conditions, it still does not guarantee that the organism was completely killed; this needs to be studied further in subsequent testing, including the use of vital stains or other techniques such as RNA-based detection to confirm actual death of a fungus. The growth (morphology, speed, and observation of any unusual growth patterns) was noted in comparison with the growth of fungi from the untreated control grains, but no further detailed assessment of stunted growth was conducted, apart from assessing growth at 4 weeks posttreatment under optimal conditions. Although complete mortality is used regularly to assess treatment efficacy, it is important to note that ISPM No. 5 (FAO 2016b) indicates that successful phytosanitary treatments may not necessarily result in killing pests, but may inactivate, remove, devitalize, or render them infertile, which are often called ''sublethal effects'' and are generally poorly understood with respect to fungi.

Results and Discussion

The use of individual felt-covered glass tubes was effective in preventing test fungus contamination from airborne mould spores and cross-contamination from the other fungi in each chamber. After fumigation, all grains taken from the control jar grew (100% recovery) into colonies that exhibited morphology identical to the original cultures grown on 1 percent MEA before inoculation onto grain. There was no contamination in any of the agar plates and each piece of plated grain was associated with uniform fungal colony growth.

Survival of fungi posttreatment for 24-, 48-, and 72-hour fumigation durations at 15°C and 20°C is presented in Table 1. The data demonstrate that there were differences in tolerance to SF under the various treatment combination tested.

Temperatures measured in the environmental chamber during fumigation were ±1°C from the target temperatures (15°C and 20°C). Sorption or loss of SF as measured by headspace concentrations was minimal because of small load factors and lack of sorptive material in the chambers during fumigation. Resulting CT products ranged from ~1,000 h-mg liter^{-1} in the lowest rate in the 24-hour fumigation to >17,000 h-mg liter^{-1} at the highest rates in 72-hour treatments (Table 2).

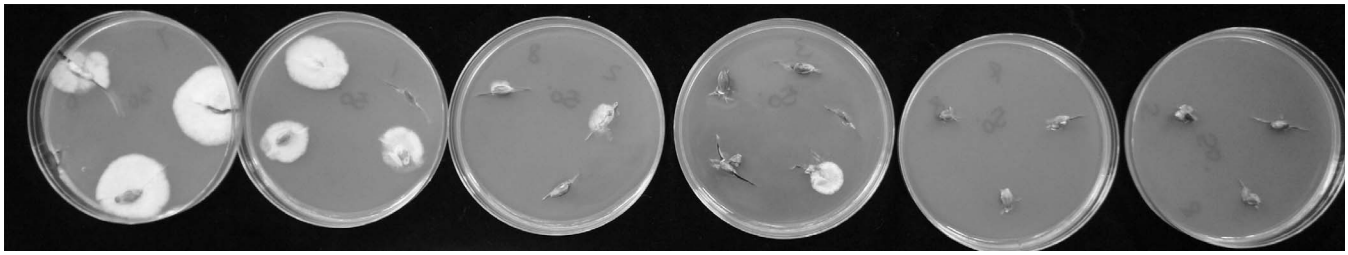

Figure 2.—Assessment of Hymenoscyphus pseudoalbidus *(*Chalara fraxinea*) survival after 14 days of incubation after exposure to 40, 80, 120, 160, 200, and 240 mg liter^{-1} (left to right) of sulfuryl fluoride at 20°C for 24 hours.*

The 24-hour fumigation period was generally not long enough to kill (or apparently kill) the majority of the test fungi, even at the highest concentrations. Only *Phytophthora ramorum* (sudden oak death fungus) and *Phellinus sulphurascens* were killed at the 120-mg liter^{-1} initial dose in 24 hours. *Hymenoscyphus pseudalbidus* (*Chalara fraxinea*), *Fomitopsis pinicola*, *Heterobasidion annosum, H. occidentalis, Phytophthora alni*, and *P. quercina* were killed at 200 mg liter^{-1}, whereas *Leptographium wageneriі* and *Hypoderma pratermissum* required 240 mg liter^{-1} to be killed within 24 hours. All other fungi survived across all concentrations.

Efficacy improved when the exposure time was increased to 48 hours. Overall, more fungi were killed; however, only 9 of 29 isolates were killed at both test temperatures at the highest dosage rate (240 mg liter^{-1}), whereas 22 isolates were killed at one of the test temperatures at the highest concentration.

The most effective SF fumigation schedule was at 72 hours, where 29 of 33 treated fungal isolates were killed at both temperatures at 240 mg liter^{-1}. The remaining four isolates were recovered from one of the two temperatures; however, the higher temperature was not consistently more effective.

Differences in survival among isolates of the same fungal species were also observed. For example, *Geosmithia morbida* isolate (isolate 1272) was recovered from the most severe treatment in the 72-hour exposure at 15°C; however, the *G. morbida* test isolate 1223 was killed at much lower dosage rates (Table 1).

Figure 2 shows growth of *H. pseudoalbidus* (*C. fraxinea*) on 1 percent MEA media plates 14 days posttreatment for control and treatments of increasing concentrations of SF in 24-hour fumigation exposures. No colony regrowth of *H. pseudoalbidus* was observed at the highest concentrations of 200 and 240 mg liter^{-1}. Other fungi were able to successfully regrow posttreatment with no observed colony morphology change as a result of the SF fumigation, particularly in the shortest duration we tested (24 h; Fig. 3). This indicates that there is likely a wide range of tolerance to SF treatment for wood-inhabiting fungi.

Our results generally suggest that there is potential for SF to be used as a quarantine treatment for wood fungi. However, CT products greater than those produced in this study will likely be required to achieve quarantine-level control as penetration of the fumigant into wood products was not considered in this study. Additional work using commercial-size wood samples may require an increase in CT to allow lethal concentrations of SF to penetrate logs and provide effective control.

The results from this study are comparable with other studies that have evaluated SF for efficacy against the oak wilt fungus, *Ceratocystis fagacearum*. Woodward and Schmidt (1995) found that *C. fagacearum* was completely

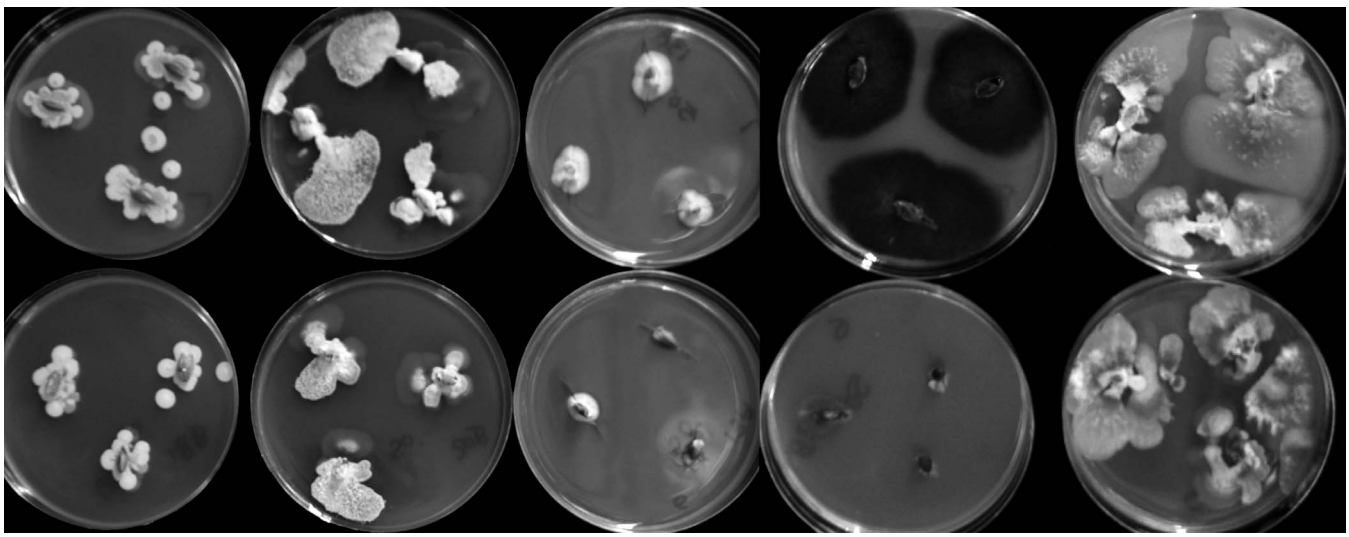

Figure 3.—Assessment of colony morphology and survival of fungi: Rossellinia necatrix, Geosmithia morbida, Lachnellula willkommii, Leptographium wageneri*, and* Pachnocybe ferruginea *after 14 days of incubation from grains taken out of control jar (top row) and from jar exposed to 240 mg liter^{-1} (bottom row) of sulfuryl fluoride at 20°C for 24 hours.*

controlled at CT values above 18,000. Schmidt et al. (1997) reported complete control at SF CTs of 27,400 and 35,010 h-mg liter^{-1} at temperatures of about 10°C but they did not evaluate efficacy of lower rates. Nematodes and insects are controlled using much lower treatment schedules. Pinewood nematode in wood blocks requires CTs ranging from 1,400 to 3,000 h-mg liter^{-1} in fumigations at temperatures from 15°C to 30°C (Bonifácio et al. 2013, 2015). Reports on control of wood-boring insects are variable across species, but CTs required for quarantine control are generally similar to those for pinewood nematode and CTs range from ~300 to 3,000 h-mg liter^{-1} (e.g., Mizobuti et al. 1996; Barak et al. 2006, 2010; Yu et al. 2010).

This laboratory-based method is useful for simultaneous screening of a large number of isolates to directly compare their response to the fumigation treatments under identical conditions. This test identifies the approximate toxic threshold concentration for a target organism, and provides a basis to compare treatment tolerances across fungal species and isolates and aid in identifying which are the most tolerant. Results on the effect of SF on a variety of fungi are only a preliminary indication and the next stage of work needs to use higher replication to confirm these results and verify mortality at various CT values. For development of a new universal wood treatment, these results would need to be tested under field conditions with actual wood products and logs of different sizes to confirm that lethal dosage is met throughout the profile of treated wood.

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