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Low-Temperature Methyl Bromide Fumigation of Emerald Ash Borer (Coleoptera: Buprestidae) in Ash Logs

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ABSTRACT Ash (*Fraxinus* spp.) logs, infested with fully developed, cold-acclimated larval and prepupal emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), were fumigated with methyl bromide (MeBr) at 4.4 and 10.0°C for 24 h. Concentrations × time dosages of MeBr obtained were 1,579 and 1,273 g-h/m^3 (24-h exposure) at 4.4 and 10.0°C after applied doses of 112 and 96 g/m^3, respectively. MeBr concentrations were simultaneously measured with a ContainIR infrared monitor and Fumiscope thermal conductivity meter calibrated for MeBr to measure the effect of CO_2 on Fumiscope concentration readings compared with the infrared (IR) instrument. The presence of CO_2 caused false high MeBr readings. With the thermal conductivity meter, CO_2 measured 11.36 g/m^3 MeBr per 1% CO_2 in clean air, whereas the gas-specific infrared ContainIR instrument measured 9.55% CO_2 as 4.2 g/m3 MeBr (0.44 g/m3 per 1% CO_2). The IR instrument was 0.4% as sensitive to CO_2 as the thermal conductivity meter. After aeration, fumigated and control logs were held for 8 wk to capture emerging beetles. No *A. planipennis* adults emerged from any of the fumigated logs, whereas 262 emerged from control logs (139 and 123/m^2 at 4.4 and 10.0°C, respectively). An effective fumigation dose and minimum periodic MeBr concentrations are proposed. The use of a CO_2 scrubber in conjunction with nonspecific thermal conductivity instruments is necessary to more accurately measure MeBr concentrations.

KEY WORDS *Agrilus*, *Fraxinus*, fumigation, quarantine, methyl bromide

Numerous invasive and destructive insect species that damage trees and shrubs have been introduced into the United States (Haack and Byler 1993, Mattson et al. 1994, Haack 2006), primarily as a result of containerized world trade. For example, emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a threat to eradicate North American ash (*Fraxinus* spp.) trees (Poland and McCullough 2006). It probably arrived in the United States in solid wood packing material originating from its native Asia and was discovered in southeastern Michigan in summer 2002 (Haack et al. 2002). Since then, it has been found in Ohio, Indiana, Illinois, Maryland, Pennsylvania, West Virginia, Virginia, Wisconsin, Missouri, New York, Minnesota, Iowa, and Kentucky (and in Ontario and Quebec, Canada; (http://pest.ceris.purdue.edu/searchpest.php?selectName=INAHQJA). Since its introduction, it has killed tens of millions of ash trees in Michigan, Ohio, and Indiana and in the other states. Emerald ash borer has probably cost municipalities, property owners, nursery operators, and forest products industries tens of millions of dollars (http://www.stopthebeetle.info/what-is-eab/). The regulatory agencies of these states may enforce quarantines and fines to prevent potentially infested ash trees, logs, and firewood from moving out of areas where emerald ash borer has been found.

The U.S. Department of Agriculture–Animal and Plant Health Inspection Service–Plant Protection and Quarantine (USDA–APHIS–PPQ) is working to find quarantine treatment alternatives to chipping and heat when treating emerald ash borer-infested and quarantined wood. Sulfuryl fluoride (SF) has been shown to be effective for emerald ash borer eggs and larvae within ash logs (Barak et al. 2010) but was more effective at temperatures of ≥15.6°C. Fumigation of solid wood packing materials (SWPM) with methyl bromide (MeBr) and SF has been documented to effectively eradicate the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Barak et al. 2005, 2006), although MeBr was more effective than SF at 4.4 and 10.0°C. Furthermore, MeBr and SF have been shown to be effective against the cerambycid *Chlorophorus annularis* (F.) in raw bamboo poles but bamboo required higher doses than SWPM and SF was again less effective than MeBr at lower temperatures (Barak et al. 2009, Yu et al. 2010). Soma et al. (1996) reported that essentially naked eggs of two forest pests, *Semanotus japonicus* Lacordaire (Cerambycidae) and *Cryphalus*

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fulvus Nijima (Scolytidae), were more tolerant than their larval stages to SF. In contrast to SF, MeBr at low doses was more effective at 15°C for eggs and pupae compared with larvae of several forest pest Coleoptera (Oogita et al. 1998). With emerald ash borer, eggs are laid on ash bark surfaces or in exposed cracks (Bauer et al. 2004), whereas larvae are at the cambial layer or in the sapwood. In a commercial fumigation then, eggs would receive a higher concentration × time product exposure (CxT) than larvae because higher and longer atmospheric exposures are needed to allow the fumigant to penetrate into logs.

The purpose of this research was to evaluate MeBr as a quarantine treatment for emerald ash borer at lower temperatures, where SF is least effective. This report provides fumigation data for MeBr fumigations with MeBr for 24 h at 4.4 and 10.0°C for control of emerald ash borer infesting ash logs. We also propose a fumigation schedule with required minimum concentrations and the required 24-h CxT exposure to be efficacious for emerald ash borer prepupae and larvae within ash logs. The accuracy of infrared (IR) compared with thermal conductivity monitoring of green logs with respect to CO_2 concentrations is documented, because CO_2 may give a false MeBr positive with the thermal conductivity technology.

Materials and Methods

Log Fumigation Chambers. Log fumigations were conducted in six, 432-liter Lexan chambers, described in detail by Barak et al. (2005, 2006). The chambers were kept in a 6.1-m-long refrigerated marine cargo container capable of setting temperatures ±0.1°C. Various fittings allowed gas infusion and monitoring of log temperatures and MeBr concentrations.

Wood Preparation. Thick-barked logs and large branches between 8 and 21 cm in diameter were harvested during fall 2008 from ash trees in Brighton, MI. Infestation was confirmed by inspection and dissection before harvest. Bolts were cut to lengths of 70–72 cm, to fit in emerald ash borer emergence barrels. Each log was weighed and measured for length and diameter to calculate its volume and bark surface area. Logs were cut and chilled ≈3–4 mo at 4.0°C to ensure that the emerald ash borers were in the overwintering prepupal stage. Recent research suggests cold treatment advances the rate of pupation and adult emergence. (Wang et al. 2010). Logs were stored until use in a refrigerated cargo container at 4.0 ± 0.1°C. Some logs were gradually acclimated to 10°C fumigation temperature 4–7 d before fumigation.

Bolts were initially dived into small-, medium-, and large-diameter groups. Ten to 12 representative ash logs (sizes distributed uniformly among replicates) were loaded in each fumigation chamber (Fig. 1B) and were selected to approximate a uniform replicate weight (≈116 kg) and load factor (≈35–36%, vol:vol) (Table 1). During selection, one of every seven logs was assigned to the control. Before each fumigation in February 2009, three ≈2.5-cm-thick sections were sawn from the middle of three representative log sections and were oven dried (ASTM 1992) to document mean wood moisture contents. Internal wood temperatures for each chamber were monitored by inserting a type T thermocouple into a ≈2.5-cm-deep, 3.5-mm-diameter hole in a large log (this approximates the depth of overwintering larvae in the wood). The hole was then plugged with electrician's duct putty. When the outer wood reached the fumigation temperatures (4.4 or 10.0°C), the chamber lids were put on, sealed all around with heavy duct tape, and tested for tightness with a digital manometer (Dwyer Instruments, Michigan City, IN). Container temperature set point was frequently monitored and adjusted to maintain the target wood temperature ±0.5°C.

Log Fumigation Trials. The fumigant used was Q-Label MeBr (100%, Chemtura Corp., Middlebury, CT) for all trials. Fumigations were conducted at 4.4 and 10.0°C for 24 h during February 2009, at applied doses of 96 and 112 g/m^3, respectively. These dosages were selected based on results of previously reported solid wood packing fumigations infested with Asian longhorned beetle (Barak et al. 2006) and with Chinese bamboo (Poaceae) poles and stakes infested with *C. annularis* (Daojian Yu et al. 2010). The fumigant dose, based on the known volume, was computed volumetrically by calculating the volume of the required grams of MeBr gas by using the ideal gas law ($V = nRT/P$), after measuring gas temperature and current atmospheric pressure. The desired quantities of fumigant were infused into the chamber from 10- and 20-liter gas bag reservoirs (Calibrated Instruments Inc., Hawthorne, NY) by drawing a slight chamber vacuum, as described by Barak et al. (2005, 2006).

Fumigant Monitoring. Fumigant concentrations in the test chambers were measured at six intervals (0.1, 0.5, 1, 2, 4, 8, and 24 h after gas introduction) with a ContainIR infrared fumigant monitor (Spectros Instruments, Hopewell, MA) (Fig. 1C) Accuracy was tested in the laboratory and found to be typically within 1% from 0 to 40,000 ppm. The concentrations also were simultaneously monitored using a calibrated thermal conductivity Fumiscope (Key Chemical and Equipment Co., Clearwater, FL) to compare readings and ascertain the effect of evolved CO_2 on the readings. Both instruments are approved by APHIS for quarantine fumigation monitoring (Anonymous 2004). The ContainIR instrument was selective for MeBr and therefore not influenced by CO_2, unlike the nonselective thermal conductivity which measures total sample density (fumigant, CO_2 plus other organic tree volatiles). In the laboratory, ≈2,500-ml mixtures of 0.5–5.0% CO_2 in air (vol:vol) were prepared and supplied to a calibrated Fumiscope to ascertain the effect of various concentrations of CO_2 on the thermal conductivity instrument calibrated with MeBr prepared at 56 g/m^3.

After 24-h exposures, each fumigation chamber was opened and aerated ≈30 min by the use of the cargo container's large air exchange capacity. Each fumigant dose and exposure period was replicated six times per temperature. Untreated controls were replicated once per exposure period and temperature.

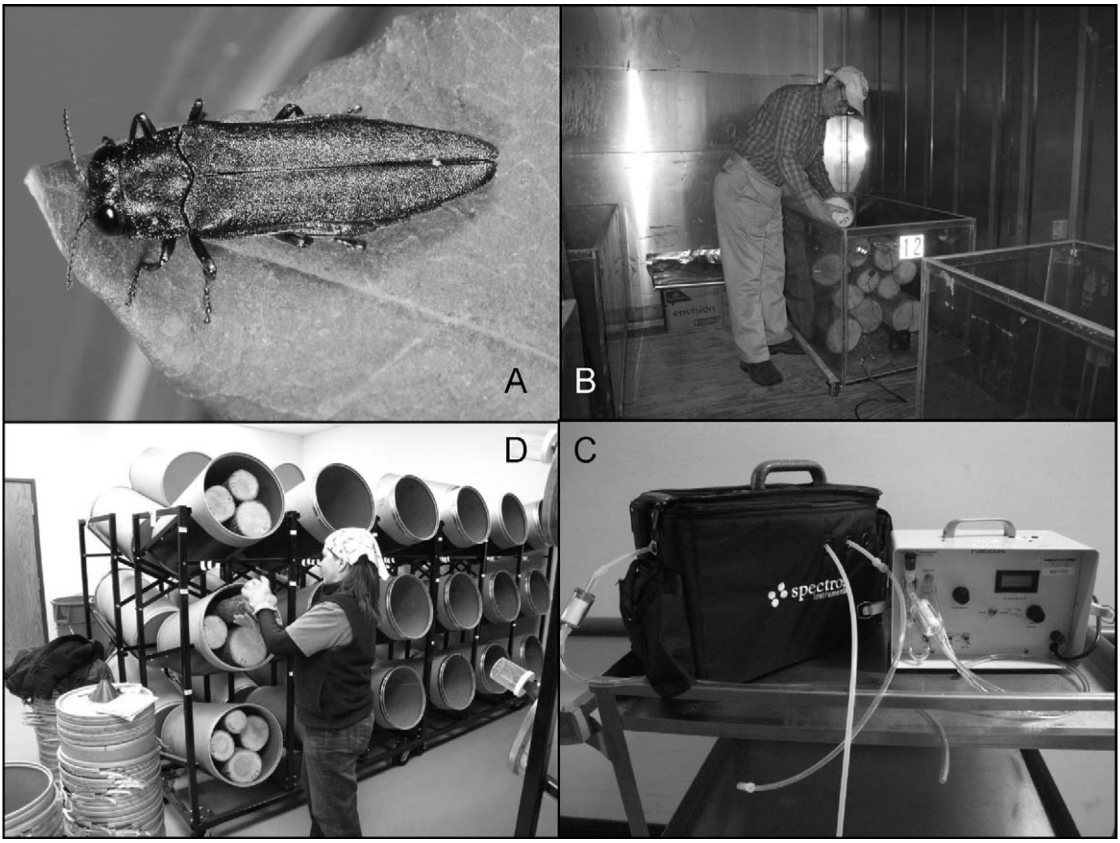

Fig. 1. (A) Emerald ash borer adult on ash leaf. (B) Loading 432-liter fumigation chambers with infested logs. (C) Approved ContainIR infrared monitor and Fumiscope thermal conductivity instruments used to monitor MeBr concentrations. (D) Loading fiber drums for rearing out emerald ash borer adults from infested, fumigated lash logs.

Emerald Ash Borer Emergence Assays. Logs were placed three to five per modified fiber drum (39.4 cm in diameter by 77.5 cm in height) (Barrels Boxes and More, Bolton, CT) and maintained in drum racks at 20–23°C and ambient relative humidity (Fig. 1D). Numbers of beetles emerging from logs were recorded for up to 8 wk after fumigation. Emergence per square meter of bark area was calculated. Control emergence was multiplied by six (for six replicates) to estimate expected emergence from fumigated logs.

All results were tabulated and displayed with Excel (Microsoft 2002). Statistical analysis (means, SEM, paired *t*-test) was performed using Statistix 9 (Analytical Software 2008), and binomial confidence limits were calculated using an online calculator (Sauro 2005).

Results

Log Chamber Trials and Fumigant Monitoring. The applied doses, periodic readings, and accumulated CxT products for fumigations at 4.4 and 10.0°C are shown in Table 1. Log weights, loading factors, and adult emergence for the fumigations are shown in Table 2. Average log weights per fumigation were between 116.5 and 116.8 kg (range, 115.6–117.3) at 10.0 and 4.4°C, respectively, with average chamber loading factors of 34.7 and 34.8% (vol:vol), respec-

Table 1. Periodic MeBr concentrations during ash log fumigations in 432.6-liter chambers at 4.4 and 10.0°C for 24 h, February 2009, Brighton, MI

Temp (°C)	Time (h)	Applied dose (g/m^3)	Replicates (*n*)	MeBr concn at time indicated, avg ± SEM							CxT ± SEM (g-h/m^3)
				5 min.	0.5 h	1 h	2 h	4 h	8 h	24 h	
4.4	24	112	6	127.0 ± 0.6	108.0 ± 0.5	98.6 ± 0.3	88.1 ± 0.4	76.8 ± 0.5	65.6 ± 0.5	50.1 ± 0.3	1,579 ± 9.9
10.0	24	96	6	107.0 ± 3.2	89.7 ± 1.2	80.5 ± 1.1	71.3 ± 0.9	62.0 ± 0.5	53.1 ± 0.5	39.7 ± 0.3	1,273 ± 10.8

There were six treatment replicates and one control replicate, with 10–12 logs per replicate.

Table 2. Fumigation parameters, chamber loading factors, and observed and expected emergence of *A. planipennis* from ash logs fumigated with MeBr at 4.0 and 10.0°C for 24 in 432-liter chambers, February 2009, Brighton, MI

Temp (°C)	Duration (h)	Dose (g/m^3)	Cumulative CxT product (g-h/m^3), mean ± SEM	Log parameter: kg load, mean ± SEM	Log parameter: Load % (vol:vol), mean ± SEM	Log parameter: Bark area, total m^2	Adult emergence: Emergence, total (n/m^2)	Adult emergence: Total[a], expected	Binomial CL $P = 0.95$ $P = 0.99$
4.4	24	0	Control = 0	116.7	0.360	3.654	139 (38.0)		
	24	112	1,579 ± 9.86	116.1 ± 0.203	0.348 ± 0.003	22.676	0	862	0.9965–1.0 0.9947–1.0
10.0	24	0	Control = 0	116.8	0.351	3.880	123 (31.7)		
	24	96	1,273 ± 10.8	116.5 ± 0.265	0.347 ± 0.002	23.333	0	740	0.9960–1.0 0.9938–1.0

[a] Total expected emergence was control emergence emerald ash borer adult emergence per square meter bark times total square meters bark in combined six replicates.

tively, at the two temperatures. Mean moisture content was determined to be 28.1% (26.8–30.1) wet basis. Sorption and CxT curves are shown in Fig. 2.

The response of the Fumiscope to a span (0.5–5.0%, vol:vol) of prepared concentrations of CO_2 in air is illustrated in Fig. 3A. It was clear that, when calibrated for MeBr, the Fumiscope returned a false indication of 11.36 g/m^3 MeBr per percent CO_2 in laboratory ambient air. The CO_2 concentration was therefore estimated to be up to 0.314 and 0.909% after 24 h at 4.4 and 10.0°C, respectively. In the laboratory, 9.55% CO_2 registered as 4.2 g/m^3 MeBr with the ContainIR instrument. Considering that, we would expect the error in 10.0°C ash log fumigations to amount to approximately +0.40 g/m^3 after 24 h, with equivalent load factors and wood moisture content. A comparison of chamber concentrations monitored with the ContainIR compared with the Fumiscope used without a CO_2 scrubber is shown in Table 3. The effect was significantly severe at the higher temperature and increased over time. Fumigations conducted at higher temperatures or for longer durations would be expected to have even greater false MeBr concentration if the commodity being fumigated evolves CO_2 due to respiration.

Adult Emerald Ash Borer Emergence. No emerald ash borer adults emerged from any of the fumigated logs. In comparison, 262 emerald ash borer emerged from combined nonfumigated control logs. In previous tests (Barak et al. 2010), dissecting logs postfumigation to determine mortality was not useful, because high mortality from injury made it impossible to determine whether stages were killed by fumigation or injury due to dissection. The estimated potential emergence from the fumigations was 740 and 862 emerald ash borer adults at 10.0 and 4.4°C from 23.3 and 22.7 per m^2 bark area, respectively. The fumigation dosages tested (96 and 112 g/m^3 MeBr, respectively) resulted in 100% control of emerald ash borer infesting test logs.

Discussion

Fumigations with SF have been shown previously to be less practical at low temperatures with cold-acclimated larvae of Asian longhorned beetle (Barak et al. 2006) and more difficult to apply at very high doses, therefore the associated higher cost relative to MeBr. MeBr thus was an attractive treatment at 4.4–9.9 and 10.0°C or warmer. It has been shown that, with sawn *Populus* spp. timber, doses of 80 and 64 g/m^3 MeBr for 24 h at 4.4 and 10.0°C, respectively, were required to provide quarantine level control of Asian longhorned beetle (Barak et al. 2005). Furthermore, the required

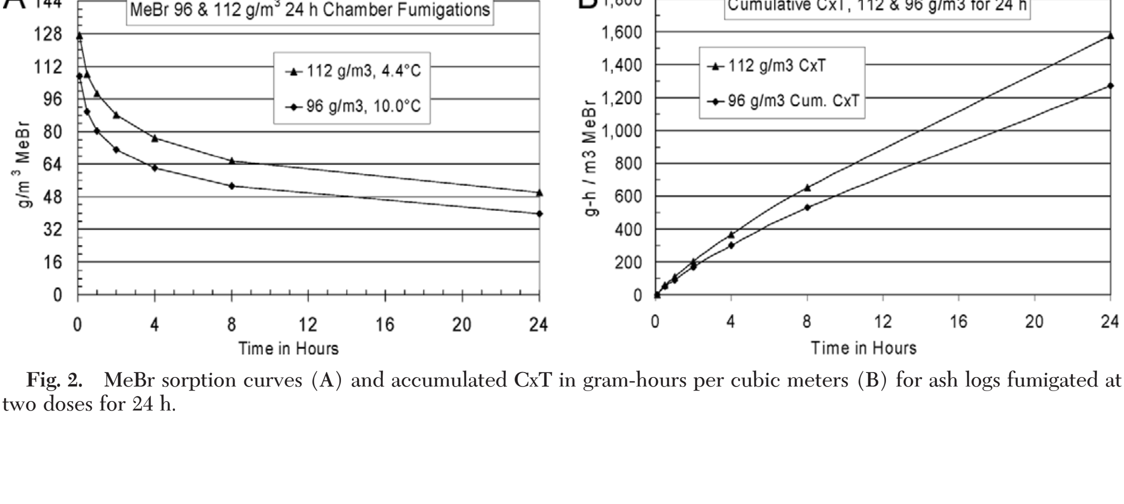

Fig. 2. MeBr sorption curves (A) and accumulated CxT in gram-hours per cubic meters (B) for ash logs fumigated at two doses for 24 h.

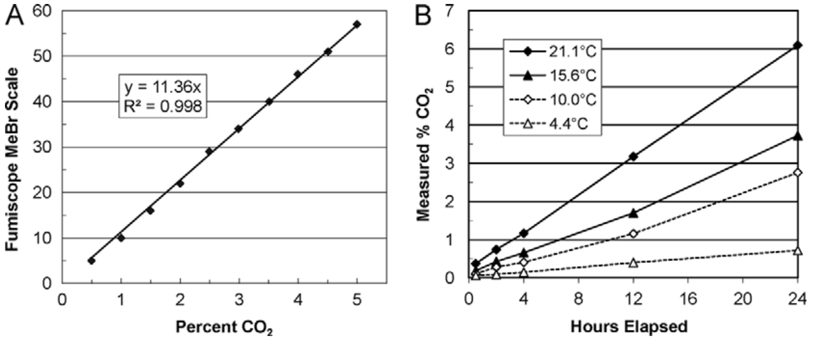

Fig. 3. (A) Correlation of Fumiscope reading when calibrated for grams per cubic meter MeBr versus percent CO_2 in ambient air. (B) CO_2 evolution from green *Populus* spp. SWPM under MeBr and SF fumigation for 24 h.

wood-borer schedule of APHIS–PPQ (Schedule T404d; Anonymous 2004), if used for emerald ash borer in logs, would require doses of 144 and 120 g/m^3 at 4.4 and 10.0° for 24 h, respectively. Doses of 112 and 96 g/m^3 for 24 h at these temperatures provided control of cerambycids in bamboo (with a heavy cortex); so, it was reasonable to test these same intermediate doses with emerald ash borer, which remain just under the bark and not deep into logs. Also, the larvae, not the eggs (as with SF) of several forest pest Coleoptera were relatively more tolerant of MeBr (Oogita et al. 1998) where doses of 5 and 10 g/m^3 MeBr a5 15°C for 24 h was 100% effective against eggs of *S. japonicus* (Lacordaire), *Callidiellum rufipenne* (Motschulsky), *Monochamus alternatus* Hope, *Cryphalus fulvus* Nijima, and *Pissodes nitidus* Roelofs. This could result in a maximum CxT of 240 g-h/m^3, whereas in our tests at 4.4 and 10.0°C we obtained CxT values of 1,273 and 1,579 g-h/m^3 (5.3–6.6-fold greater) at temperatures only 5–10.6°C lower.

The subject of quarantine level doses for less than ideal hosts has been discussed by Follett and McQuate (2001) and Landolt et al. (1984), and they suggest that Probit 9 control levels (99.99683%) originally developed for tropical fruit flies may not be essential. This view also has been put forth with MeBr and SF for cerambycids in bamboo (Barak et al. 2009, Yu et al. 2010) and with SF for emerald ash borer in ash logs (Barak et al. 2010). Logs should not be considered as high a risk compared with easily reinfested tropical fruit with pests with short life cycles and high fecundity. The long, seasonal life cycle of emerald ash borer can allow other regulatory steps, such as transportation or harvesting windows and required timely processing.

APHIS fumigations, unlike traditional pesticide application, or cold/hot treatments, have an additional level of security not readily apparent. APHIS treatment schedules prescribe a fumigant dose in grams per cubic meter (ounces per 1,000 cubic feet) based on commodity temperature, with doses decreasing stepwise as commodity temperature increases in increments of ≈5.6°C. Therefore, a dose required at 4.4°C is used until the commodity temperature reaches 9.9°C. It is highly probable that most fumigation will be conducted at higher temperatures than the specified minimum at which the treatment has been demonstrated to be effective.

The importance of measuring and compensating for the evolution of CO_2 from green wood under fumigation has been shown here. Fumiscope readings, if CO_2 is not scrubbed or accounted for, would overestimate MeBr concentrations, and thus a potential failure of the treatment could be possible. In previous work in China with green *Populus* spp. SWPM (Barak et al. 2005, 2006), more detailed unpublished data (Fig. 3B) show the degree of CO_2 evolution that was measured during MeBr and SF fumigations at four temperatures with only ≈25–27% (vol:vol) wood load factor. In addition, volatiles other than CO_2 could potentially contribute to higher false concentrations if nonselective thermal conductivity monitoring instruments are used.

USDA–APHIS requires that 8-20 mesh Ascarite II (Thomas Scientific, Swedesboro, NJ) an efficient CO_2 scrubber, be required whenever a thermal conduc-

Table 3. Simultaneous fumigant concentrations at 10.0 and 4.4°C after 8 and 24 h by using the Fumiscope thermal conductivity meter without a CO_2 scrubber, compared with the ContainIR infrared monitor

Applied dose (g/m^3), temp (°C)	Fumiscope, g/m^3 ± SEM	ContainIR, g/m^3 ± SEM	Fumiscope minus ContainIR, g/m^3 ± SEM	Paired *t*-test, df = 5
96 g/m^3, 10.0°C				
8 h	53.0 ± 0.58	53.05 ± 0.50	−0.05 ± 0.21	$t = -0.24$, $P = 0.8166$
24 h	50.0 ± 1.16	39.67 ± 0.32	10.33 ± 0.97	$t = 10.7$, $P = 0.0001$
112 g/m^3, 4.4°C				
24 h	53.67 ± 0.62	50.10 ± 0.31	3.57 ± 0.44	$t = 8.16$, $P = 0.0004$

Table 4. Recommended MeBr doses and periodic minimum concentrations for eradication of *A. planipennis* in ash logs based on data presented in this study

Fumigation parameter		Min. concn (g/m^3) at					MeBr exposure, calculated CxT, $g\text{-}h/m^3$
Fumigation temp (°C)	Applied dose (g/m^3)	0.5 h	2 h	4 h	8 h	24 h	
≥10.0	96	90	71	62	53	40	1,273
4.4–9.9	112	108	88	77	66	50	1,579

Observed concentrations were rounded to nearest whole grams per cubic meter at the selected times. This CxT value was calculated from the actual experimental values.

tivity instrument is used to monitor quarantine concentrations where CO_2 evolution may be significant. The effect of CO_2 would be more important at higher temperatures or longer duration fumigations. Scrubbing CO_2 may not be essential for fumigations monitored with a gas-specific infrared instrument, such as the ContainIR used here.

Based on these results, we propose here an effective MeBr fumigation schedule for emerald ash borer in ash logs with bark, at temperatures of ≥4.4°C (Table 4). This treatment will allow safe interstate movement of ash logs from locales known to be infested by emerald ash borer. This proposed schedule is not officially recognized by APHIS–PPQ until its publication in the PPQ treatment manual.

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