METHYL BROMIDE AS A QUARANTINE TREATMENT FOR *CHLOROPHORUS ANNULARIS* (COLEOPTERA: CERAMBYCIDAE) IN RAW BAMBOO POLES

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Methyl Bromide as a Quarantine Treatment for *Chlorophorus annularis* (Coleoptera: Cerambycidae) in Raw Bamboo Poles

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ABSTRACT At least 26 different species of insects of quarantine significance were intercepted from 1985 to 2005 on bamboo (*Bambusa* spp.) garden stakes from China. Three fifths of the live insects were cerambycids in nine genera, including *Chlorophorus annularis* F., the bamboo borer. The current APHIS–PPQ treatment is fumigation schedule T404-d, which requires high doses of methyl bromide (MeBr) for 24 h. No specific fumigation data exist for *C. annularis*. Chinese and American quarantine scientists cooperated in testing to determine whether this schedule, or lower doses, would be effective as a quarantine treatment for *C. annularis* infesting dried bamboo poles. A lower dose based on APHIS tests for solid wood packing (SWP) failed (3/511 survivors) at 56 g/m^3 for 24 h at 10.0°C. We therefore tested five progressive doses at five temperatures intermediate between the lower SWP schedule and the much higher applied doses (e.g., 120 g/m^3 for 24 h at 10.0°C) of schedule T404-d. Fumigations of infested bamboo poles conducted in 403.2-liter chambers with 52% vol:vol loading at doses of 48, 64, 80, 96, and 112 g/m^3 at 26.7, 21.1, 15.6, 10.0, and 4.4°C, respectively (20 total replicates, with 4 replicates per dose), had no survivors among 2,847 larvae, 140 pupae, and 122 adults. Control replicates (three) had a total of 455 live stages (397 larvae, 31 pupae, and 27 adults). Tests conducted with a sea/land cargo container loaded to 80% capacity with bamboo poles verified the ability of the schedule to maintain effective concentrations over 24 h in commercial-sized fumigations. We propose a new bamboo quarantine treatment schedule at reduced rates of applied MeBr.

KEY WORDS *Chlorophorus*, *Bambusa*, fumigation, quarantine, methyl bromide

The bamboo borer, *Chlorophorus annularis* F. (Coleoptera: Cerambycidae), is of particular interest, not only because it may infest U.S. bamboo (*Bambusa* spp.) and related species, but also because it has been found in imported garden stakes that would carry a significant risk of potentially aiding the spread of the beetle as well as putting it in proximity to possibly susceptible host material (Auclair and Kubilis 2006). In addition, at least 26 different species of live insects of quarantine significance were intercepted from 1985 through 2005 on dried bamboo garden stakes from China (Auclair and Kubilis 2006). Detections were made by inspectors at U.S. ports of entry: U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS; Anon. 2005a), by Smuggling Interdiction and Trade Compliance (SITC) Emergency Action Notification (EAN) (Anon. 2005b), and through determinations of live pests on products already distributed to locations in the United States. Three fifths of the live insects were long horned beetles (Cerambycidae) in nine genera, including *Chlorophorus* spp. At least 12 other subfamilies or genera were identified in 13 different families including Bostrichidae. Thus, bamboo is a potential source for the introduction of numerous pest species. Up to 1994, as many as 400 insect species that damage trees and shrubs have been introduced into the United States (Haack and Byler 1993, Mattson et al. 1994), primarily as a result of shipping commercial goods in container-based trade.

According to Weidner (1982), *C. annularis* is normally univoltine, but with drying bamboo, development may be extended 1 or more yr. Eggs are long and oval and are laid in clusters of ≈10–30 on air dried bamboo. The adult is 9.5–17.0 mm long and is densely clothed in yellowish pubescence. The elytra have dark brown or black markings (Fig. 1a). Larvae burrow under the epidermis, lengthwise, with tunnels densely packed with frass (Fig. 1b). There are five larval in-

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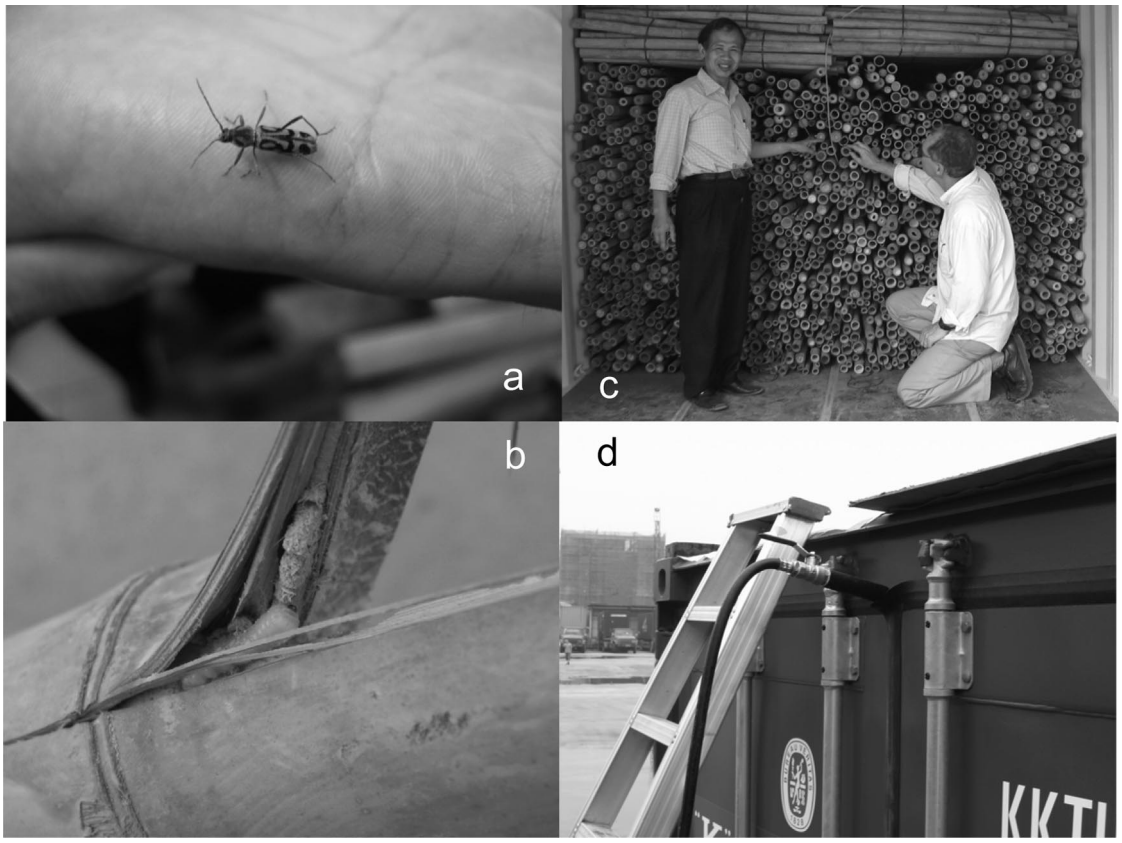

Fig. 1. (a) *C. annularis* adult on hand. (b) *C. annularis* larva in bamboo. (c) Container packed with bamboo poles prepared for fumigation at 80% load factor with center-load sample line. (d) Injection wand method of administering MeBr dose to closed cargo container through door seals.

stars, and mature larvae (14.7–20.8 mm in length) overwinter. Pupation occurs near the surface, with emergence from May to September. In South China (Shenzhen, Guangdong Province), the greatest emergence of adults occurs during May and June.

In China, near Hong Kong, the beetle "is found boring in dead stems where larvae eat out the nodes and emerge through small holes at intervals along the stem." Reported host plants include *Bambusa spinosa*, *B. chungii*, and *Phyllosstachys pubescens.* (Hill et al. 1982).

Chlorophorus annularis is widespread in China and Southest Asia (Anon. 1992, Xiao 1992) and damages bamboo in Fujian, Zhejiang, and Guangdong Provinces of China (Qian 1991). The reported distribution is as follows: China (Hainan Island, Koon 1999; Hong Kong area, Hill et al. 1982; northeast China, Koon 1999; south China, Hill 1983; Taiwan, Koon, 1999, Makihara et al. 1989); India (Assam, Punjab) (Duffy 1968, Koon 1999); and Indonesia (Java, Sumatra) (Duffy 1968).

USDA–APHIS–PPQ (Plant Protection and Quarantine) has in place Schedule T-404d (Fig. 2) for the methyl bromide (MeBr) fumigation of bamboo in containers for wood-boring insects (Anon. 2004). PPQ wood packing fumigation schedules for phosphine and sulfuryl fluoride were recently rescinded because of failed fumigations conducted during the progress of studies in China and in previous in-house tests. There was a need for scientific data to support quarantine treatments, especially with worldwide desire to eliminate or reduce MeBr use as a quarantine treatment because of its ozone depletion potential. MeBr is currently regulated internationally through acceptance

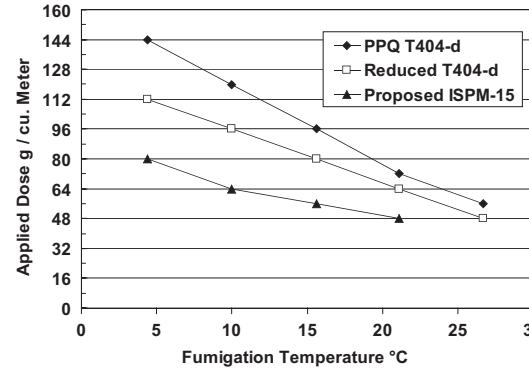

Fig. 2. Relation of proposed ISPM Rule 15 doses for ALB, proposed doses for *C. annularis*, and currently used PPQ T404-d MeBr fumigation schedule for wood borers.

of the Montreal Protocol of 1998 (UNEP 1998) and through the International Standards for Phytosanitary Protection (ISPM) Rule 15 () with regard to solid wood packing material (SWPM).

The presence of two insect species of quarantine significance, the other being *Heterobostrychus aequalis* Waterhouse (Bostrichidae), caused this commodity to be considered high risk, thus requiring mandatory fumigation treatment. We report here a series of fumigations to determine the effectiveness of a fumigation schedule previously recommended for wood boring beetles in SWPM (Barak et al. 2005, 2006) for *C. annularis* in bamboo or to develop a fumigation schedule as effective, or at lower doses than APHIS, PPQ T404-d, at several temperatures.

Materials and Methods

Bamboo infested with *C. annularis* was obtained locally in Shenzhen from construction sites, where dried bamboo poles have a high risk of infestation and reinfestation by natural populations of *C. annularis.* The bamboo was divided into piles of small, medium, and large diameter. Small pieces were ~4 cm and large as much as 8 cm in diameter. The bamboo was cut to length (≈116 cm) to fit the fumigation chambers. Certain pieces with good indication of high infestation were set aside to be selectively added to the typical batches of bamboo to increase insect numbers within a replicate. The typical replicate used 57–60 individual bamboo stems. Replicate batches of bamboo to be fumigated were weighed and separated in bags for temperature conditioning and temporary storage. In addition, two pieces of bamboo with intact internodes, free from cracks and splits, were artificially infested with field-collected larvae by drilling a small hole in each internodal cavity, inserting one larva, and taping the hole with gas proof tape. This produced a situation in which fumigant gas would need to entirely penetrate the bamboo walls to the interior, thus potentially reducing the exposure of the larvae to fumigant gas.

Fumigations were conducted in four 403.2-liter chambers constructed of tempered glass of ≈8.0 mm thickness, which measured 0.56 m high by 0.6 m wide by 1.2 m long internally. Each chamber end was fitted with two ≈6.35-mm (0.25 in) brass ball valves with hose barbs, used to draw a vacuum, introduce the fumigants, and to monitor concentrations. Three additional brass 0.25-in (6.35 mm) Swagelok compression fittings were added to provide access for thermocouple wires and electricity to the small 220-V equipment ventilation fans inside the chambers to facilitate gas circulation.

All chambers were previously tested for tightness by drawing a differential pressure of about −50 mmHg and observing a decrease no greater than to −25 mmHg in at least 1 min. The ability to hold vacuum was essential for infusion of the dose and maintaining the fumigant concentration.

The four chambers were kept in a 6.1-m-long refrigerated marine cargo container capable of holding a set point of ±0.1°C. To conduct a fumigation, the infested bamboo along with the pieces with inserted larvae were added to each chamber to give a typical load factor of ≈52% and were essentially similar in weight (Table 1). Loading factor was determined by measuring the circumference and length of four replicate bundles and calculating volume. Internal bamboo temperatures for each replicate were monitored by inserting a type T thermocouple into a hole drilled into the center of the thickest wall of a large piece, which was plugged with electrician's putty. When the bamboo reached the desired temperature, the chamber lids were put on, and the vacuum was tested.

Table 1. Composition of replicates of bamboo (*Bambusa* spp.) used in fumigations in 403.2-liter glass fumigation chambers

Fumigation parameters		Bamboo weight (kg ± SEM) (mean of four replicates)	Representative mean load factor percent V/V (range, four replicates)
Applied dose (g/m^3)	Target temperature		
48	26.7°C	46.3 ± 0.13	52.5 (52–54)
64	21.1°C	46.10 ± 0.07	52.5 (52–54)
80	15.6°C	45.88 ± 0.09	52.5 (52–54)
96	10.0°C	45.05 ± 0.41	52.5 (52–54)
112	4.4°C	43.20 ± 0.49	52.5 (52–54)

Fumigations were conducted during Jan.-Feb. and May 2008 in Shenzhen, China.

The fumigant doses were computed volumetrically by calculating the volume of the required grams of MeBr gas after compensating for ambient pressure and temperature according to the "ideal gas law" ($V = nRT/P$). The MeBr was transferred to 10- or 22-liter gas bags (Calibrated Instruments, Hawthorne, NY) using a 2-liter gas-tight syringe (Hamilton, Reno, NV) from a reservoir bag to a dose bag through a three-way valve (The Swagelok Company, Solon, OH). To introduce the fumigant to the chambers, a slight vacuum was created with a vacuum pump and measured with a digital manometer (Dwyer Instruments, Michigan City, IN) until pressure was ≈20–32 mmHg below ambient atmospheric, enough to accommodate the gas volume plus an additional 10 mmHg to speed gas infusion. The gas bag was attached, and the valve was opened, thus allowing the gas to quickly flow into the chamber. After all gas was infused, the valve was left open only long enough to equalize the remaining pressure differential. The concentration × time value (CxT) was computed for each fumigation.

Fumigant concentrations in the test chambers were monitored at intervals of 5 min and 0.5, 1, 2, 4, 8, and 24 h after gas introduction, using a Spectros Instruments infrared MeBr monitor (Spectros Instruments, Hopewell, MA) The instruments were calibrated against a standard gas mixture made to a concentration of ≈60 g/m^3 MeBr. Accuracy was tested in the laboratory and found to be typically within 1% from 20 to >400 g/m^3.

Initial fumigations were begun at two doses (48 g/m^3 at 21.1°C and 56 g/m^3 at 15.6°C) known to kill the wood boring cerambycid *A. glabripennis*, in solid *Populus* spp. wood in experiments conducted previously in Lanzhou, China, during 2002–2003 (Barak et al. 2005,

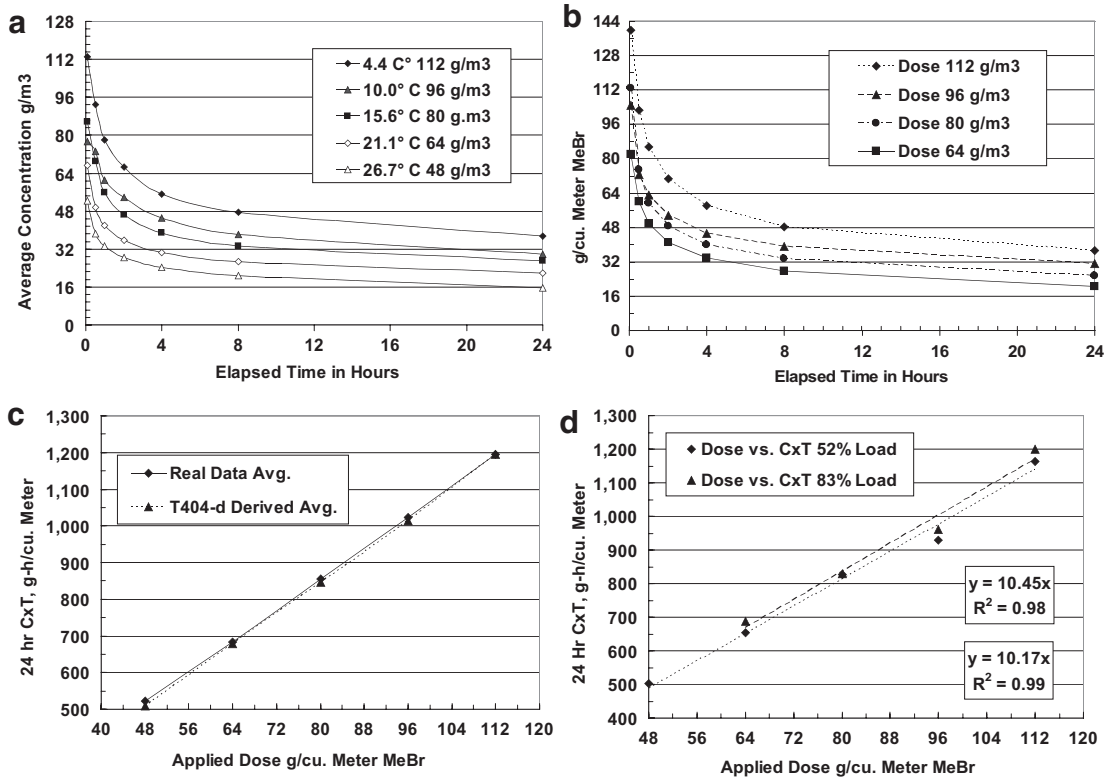

Fig. 3. (a) Average sorption curves for bamboo at five temperature and doses, with a 52% loading factor. (b) Sorption curve at 15.6°C, with four doses and an 83% loading factor. (c) Relation of CxT products with 52 compared with 83% loading factor. (d) Comparison of average CxT's calculated from replicated real bamboo fumigation with 52% load factor, with CxT's derived from minimum concentrations as a percent of applied doses from T404-d schedule, at the five doses tested.

2006). Based on results of these initial fumigations, the final confirmatory fumigations were conducted at doses of 48, 64, 80, 96, and 112 g/m^3 at temperatures of 26.7, 21.1, 15.6, 10.0, and 4.4°C, respectively, with a loading factor of 52% vol./vol. (Fig. 2). Because the sorption characteristics were similar at all temperatures, we also tested sorption and periodic concentrations with a higher load factor of 82% at one temperature, 15.6°C, at four doses (64, 80, 96, and 112 g/m^3), with one replicate each because of limited bamboo material of good quality.

Ambient outdoor temperatures during the work during 11 January to 3 February 2008 ranged between ≈8 and 12°C, so that insects were acclimated to cooler temperatures. To increase cold acclimation and potentially increased insect tolerance to fumigation, bags of prepared replicates were held in the refrigerated container so that they were subject to the next lower fumigation temperature for an additional 2–4 d.

Additional fumigations at a higher temperature (26.7°C, 48 g/m^3, 24 h) were similarly conducted during May 2008, during which time temperatures were warmer and pupae naturally would be found, although pupae are less tolerant than larvae. The bamboo was stored in a cold room at ≈7–8°C to prevent premature adult emergence, until near fumigation time. The bamboo was removed from storage 4 d before fumigation and allowed to naturally warm to 26–27°C before the fumigation. These fumigations were conducted in the chambers in an air-conditioned room. A batch of bamboo of similar weight served as the control. Four replicates were fumigated.

After fumigation, and after 2–3 h of aeration, the bamboo was placed, by replicate, into woven polypropylene bags for a postfumigation holding period of 4–5 d. After this time, the bamboo was carefully split by hand, and all stages found were counted and evaluated. Larvae were considered dead if they were limp and had no movement. Larvae that were turgid or had body movement were considered as alive. We did not conduct egg fumigations, because the work of Oogita et al. (1998) indicated that naked eggs of two common wood-infesting cerambycids (*Callidiellum rufipenne* Motschulsky and *Monochamus alternatus* Hope) were susceptible to MeBr at low levels from 5 to 15 g/m^3 for 24 h and that larvae, not adults, were the most tolerant stage tested. Furthermore, the eggs of *C. annularis* are fragile, being laid naked on the surface or in cracks of bamboo, thus being more susceptible to fumigation and also not likely to survive commodity handling or commerce.

Bamboo moisture content was determined from taking 24 crosscuts from the centers of randomly selected pieces of representative sizes (mean circum-

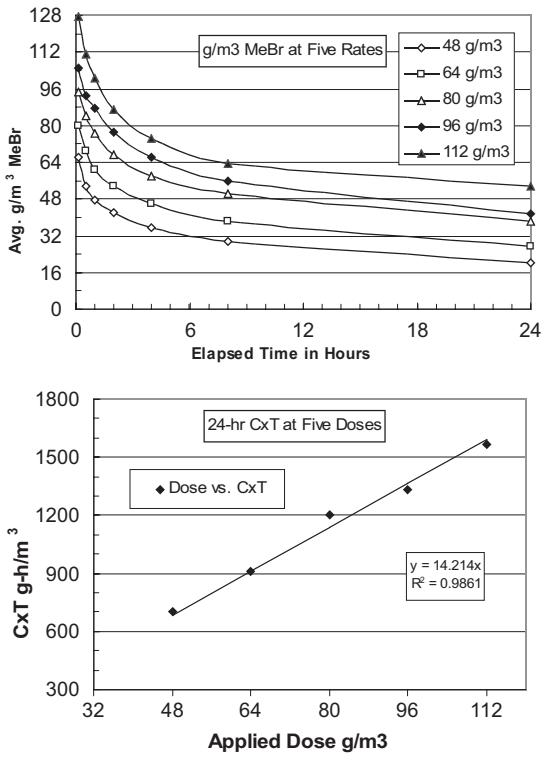

Fig. 4. Sorption curves and accumulated CxT exposure for bamboo poles fumigated at five doses over a 24-h period. Bamboo was fumigated in a standard height 6.1-m (standard, 20 ft) cargo container loaded to 80% height with cut and bundled bamboo poles.

ference, 17.92 ± 0.98 [SEM] cm; mean length, 116.22 ± 0.096 cm; mean wet weight, 0.91 ± 0.095 kg). Wet basis moisture content was determined according to ASTM test method D-4442-92 (ASTM 1992).

A series of commercial-sized fumigations were also conducted in a new 6.1-m-long standard height general cargo container filled with bamboo poles (Fig. 1c and d). The purpose was only to confirm that efficacious periodic concentrations could be maintained with Chinese commercial fumigation techniques in a container in good condition. We purchased a quantity of similarly sized bamboo poles from a local bamboo market and cut the poles into lengths of 3.0 and 2.5 m for forming into 138 bundles, as well as 90 bundles of poles of 1 m length. The container was loaded to ≈80% of its internal height. The container had an internal volume of 33.2 m^3; therefore, ≈26.6 m^3 of bundled bamboo with a combined weight of 3,900 kg was loaded into the container. The weight to volume ratio was therefore 117.47 kg/m^3 compared with 112.37 kg/m^3 volume with the small chamber tests. Gas sample lines (6.35 mm OD and 4.8 mm ID polyethylene) were placed in three locations within the filled container, following traditional recommendations (front-high, center-middle of load, and low-back near the floor). A large electric fan of >33-m^3/min capacity was placed on top of the load near the back doors to maximize MeBr circulation. The sample lines and fan cord were set in a small quantity of putty and placed through the door frame, and the doors were closed. This formed a tight seal of the door gasket around the lines. The lines terminated at a monitoring station ≈10 m from the container. Air vents were sealed with tape. MeBr was applied from a pressurized cylinder using the Methyl Bromide Carburetor, which is a microprocessor-controlled digital applicator/scale combination with an electrically heated, oil-filled volatilizer (Guangzhou Import & Export Commodity Inspection Technology Institute, Guangzhou, China). Desired doses were entered into the digital control panel, which automatically opened and closed an electronic valve once the desired amount of gas was dispensed from the cylinder. The volatilizer was preheated to 90°C before gas introduction. A gas introduction line attached to the volatilizer outlet, fitted terminally with a small diameter metal wand, was used to inject the MeBr between the door seals just behind the fan (Fig. 1d). Fumigations were conducted at ambient temperature (27–32°C) at doses of 48, 64, 80, 96, and 112 g/m^3. The end of the introduction was considered the start time of the fumigation. MeBr concentrations were monitored with all three sample lines after 5 min and at 0.5, 1, 2, 4, 8, and 24 h after introduction. After 24 h, the container was opened and aerated continually for at least 24 h, after which there was no detectable MeBr through the middle-load sample line. The container was readied for fumigation at the next higher dose.

Results were tabulated and displayed with Microsoft Excel (Anon. 2000), and statistical analysis (means and SEM) was performed using Statistix 8 (Analytical Software, Tallahassee, FL).

Table 2. Periodic methyl bromide concentrations obtained at five temperatures and five doses during 24-h fumigations of bamboo stakes in 403.2-liter glass chambers

Fumigation parameters		MeBr concentration [mean (SEM)] at time from start loading factor 52% vol./vol.						Concentration time product (g-h/m^3) [mean (SEM)]	Bamboo temperature (°C) (mean ± SEM)
Target temperature (mean ± SEM)	Applied dose (g/m^3)	0.5 h	1 h	2 h	4 h	8 h	24 h		
26.7°C	48	38.43 (0.45)	33.15 (0.09)	28.58 (0.23)	24.45 (0.21)	20.83 (0.37)	15.75 (0.26)	503.1 (7.04)	26.3 ± 0.12
21.1°C (20.94 ± 0.054 °C)	64	49.53 (1.10)	42.03 (1.06)	35.63 (1.090)	30.65 (1.01)	26.75 (0.89)	21.73 (0.70)	653.9 (20.40)	20.9 ± 0.05
15.6°C (15.58 ± 0.043°C)	80	69.03 (0.59)	55.88 (1.36)	46.63 (1.24)	39.0 (1.40)	33.28 (1.21)	27.1 (0.76)	826.6 (24.57)	15.58 ± 0.04
10.0°C (10.27 ± 0.042°C)	96	73.05 (3.12)	61.13 (1.84)	53.68 (2.26)	45.2 (2.14)	38.13 (1.82)	29.7 (1.32)	930.0 (40.97)	10.3 ± 0.04
4.4°C (4.84 ± 0.064°C)	112	93.05 (2.8)	78.2 (2.36)	66.68 (2.12)	55.0 (1.43)	47.4 (1.04)	37.55 (0.59)	1162.6 (25.48)	4.8 ± 0.06

There were four replicates at each concentration. Fumigations were conducted during Jan.-Feb. and May 2008 in Shenzhen, China. The CxT was calculated from the concentrations below.

Table 3. Periodic methyl bromide concentrations obtained at four doses at 15.6°C during 24-h fumigations of bamboo stakes in 403.2-liter glass chambers with an 82% load factor

Fumigation parameters		MeBr concentration at time from start loading factor about 82% vol./vol.						Concentration time product (g-h/m^3)	Bamboo temperature (°C) [mean (SEM)]
Temperature [mean (SEM)]	Applied dose (g/m^3)	0.5 h	1 h	2 h	4 h	8 h	24 h		
15.78°C (0.048)	64	59.9	49.8	41.2	33.8	27.9	20.7	688.5	15.7 (0.15)
	80	74.9	59.4	48.7	39.9	33.3	25.4	829.8	15.7 (0.06)
	96	72.4	63.2	53.5	45.3	39.3	31.3	960.5	16.0 (0.06)
	112	102.4	85.6	70.6	58.0	48.3	37.2	1198.8	15.7 (0.02)

There was one replicate at each concentration. Fumigations were conducted during Jan.-Feb. 2008 in Shenzhen, China.

Results and Discussion

Initial tests, intended to prove the efficacy a MeBr fumigation schedule proposed for inclusion into International Plant Protection Convention (IPPC) ISPM Rule 15 for SWPM developed for Asian longhorned beetle, were successful at the dose of 48 g/m^3 at 21.1°C, but at a dose of 64 g/m^3 at 15.6°C, there were 3/537 larval survivors, which, as a mortality rate of 99.44%, is unacceptable for quarantine consideration. For this reason, the entire series of initial doses needed to be increased a significant amount. This was not unexpected, because the previous SWPM fumigation for Asian longhorned beetle (Barak et al. 2005) involved sawn wood with multiple boring holes and coarse grain and therefore potentially greater penetration of the wood compared with the smooth, hard epidermal surface of bamboo poles. Figure 2 compares the lowest intended initial doses, the current T404-d schedule, and the intermediate schedule we decided to validate. The subsequent doses were increased by 16–32 g/m^3 from 21.1 to 4.4°C, which were still less than the T404-d schedule of 8–32 g/m^3 from the high to lower temperatures.

The fumigation parameters for doses chosen for the next tests are shown in Table 1. Temperatures were maintained, and weights were consistent throughout the tests. Moisture content of 24 representative sections of bamboo poles was 10.18% wet basis (±0.102; range, 9.5–11.62%). The rapid sorption of MeBr by bamboo is shown in Fig. 3a. The sorption curve was similar at the five temperatures tested. Sorption took place rapidly over the first 4–8 h. Readings were taken after 5 min, but presumably, differences in stack tightness, sample lead placement, and fan placement made a 5-min reading variable, and thus these were only used to confirm successful gas introduction. MeBr concentrations at sample times starting at 0.5 h are shown in Table 2, along with the calculated CxT values and internal bamboo temperatures.

Figure 3b shows a similar sorption curve at 82% chamber loading compared with 52%. Periodic MeBr concentrations were initially higher, presumably because of less free headspace, but after 24 h, the final concentrations were similar to those in the chambers with 52% loading. A comparison of CxT products obtained with load factors of 52 and 82% (Table 3) indicated that the sorption as a proportion of dose is predictable across temperatures and similar in slope (Fig. 3c) and that the higher load factor resulted in slightly higher CxT products most likely caused by less free air volume.

The PPQ Schedule T404-d minimum concentrations were compared with the treatment dose, and this proportion (concentration as a percent of applied dose) was used to calculate the CxT that would be obtained at the applied concentrations we used. These data are presented in Fig. 3d. The CxT data from our tests was similarly also plotted, and the result shows that similar sorption curves exist when the CxT derived from T404-d is compared with our data from bamboo. These data support the validity of specified

Table 4. Results of methyl bromide fumigation of bamboo garden stakes at five temperatures and doses for 24 h

Fumigation parameters		*Chlorophorus annularis* larval counts				CxT (g-h/m^3)	Pupae (adults) (alive/dead)
Target temerature (°C) (mean ± SEM)	Applied dose (g/m^3)	Larvae (mean ± SEM)	Total in replicates	Survivors	Inserted larvae survivors/live (mean ± SEM)		
21.1°C		128 (single rep.)					0/0
4.4°C	0 (controls)	177 (single rep.)	397	397	16/16 10.5 ± 5.5	0	0/0
27.6°C		92 (single rep.)			—		31/0(27/0)
26.7°C (26.33 ± 0.121°C)	48	126.5 ± 19.9	506	0	NA	503.1	0/140
21.1 (20.94 ± 0.054°C)	64	176.75 ± 8.93	671	0	0/59 14.75 ± 4.85	653.9	0/0
15.6 (15.58 ± 0.043°C)	80	147.25 ± 17.0	589	0	0/88 22.0 ± 1.87	826.6	0/0
10.0 (10.27 ± 0.042)	96	123.0 ± 10.6	492	0	0/28 7.0 ± 1.0	930.0	0/0
4.4 (4.84 ± 0.064°C)	112	147.25 ± 11.7	589	0	0/25 6.25 ± 0.85	1162.6	0/0
Sum, all temperatures (less controls)			2,847 (not controls)	0 (not controls)	0/164 (no controls)	NA	31/0 controls (0/140) test

NA, not applicable.

Table 5. Measured concentrations and calculated CxT exposure of methyl bromide during 24-h fumigations of bamboo poles in a 6.1-m container at five doses

Applied dose (g/m^3)	Methyl bromide concentration at time indicated							Accumulated CxT ($g\text{-}h/m^3$)
	5 min	0.5 h	1 h	2 h	4 h	8 h	24 h	
48	66.4	53.4	47.8	42.1	35.8	29.8	20.1	702.3
64	79.8	69.1	60.5	53.6	45.7	38.2	27.6	912.1
80	94.5	84.1	76.5	67.4	58.0	50.5	38.4	1,201.5
96	105.2	92.8	87.6	76.9	66.1	55.7	41.3	1,330.0
112	127.4	111.0	100.9	87.0	74.3	63.2	53.6	1,565.5

The container was loaded to 80% of capacity, 117.47 kg/m^3 bamboo load factor. All values and CxT are the average of three sample lines.

Table 6. Recommended methyl bromide doses at four temperatures and periodic min. concentrations, for quarantine level control of *C. annularis* F. in bamboo garden stakes

Fumigation parameters		Minimum concentrations (g/m^3) at				MeBr exposure (calculated CxT) ($g\text{-}h/m^3$)
Fumigation temperature (°C)	Applied dose (g/m^3)	0.5 h	2 h	4 h	24 h	
26.7	48	39	29	25	16	537
21.1	64	50	36	31	22	690
15.6	80	69	47	39	27	870
10.0	96	73	54	45	30	985
4.4	112	93	67	55	38	1,223

Observed concentrations were rounded up to nearest whole gram per cubic meter at the selected times. This CxT was calculated from these values, using the applied dose as beginning time.

Schedule T404d minimum concentrations compared with our current data.

The mortality data from our series of fumigations are shown in Table 4. Although the controls had high numbers of live larvae dissected from untreated bamboo batches, all treatments had no survivors from a total of 2,847 larvae, 140 pupae, and 122 test insects. Three control replicates had high numbers of live larvae, pupae, or adults (397, 31, and 27, respectively) with little or no natural mortality. Two other beetle species, both adults and larvae, were sometimes found in bamboo samples. All were dead. These were the cerambycid *Purpuricenus temminckii* (Guerin-Meneville) and the bostrichid *Bostrychopsis parallela* (Lesne).

The results of the fumigations of a 33.2-m^3 cargo container filled with commercial-sized load of bamboo poles (Figs. 1c, d, and 4) at the five tested doses are shown in Table 5. All gas sample leads within a container indicated essentially equal concentrations, presumably indicating proper fan operation and gas circulation. In all cases, the periodic concentrations and total CxT product exposure exceeded that of the small chamber tests. This was in part because of the higher load factor (4.5% greater) in the shipping container compared with the experimental chambers, which restricted the volume of free headspace. Because of this success, we propose a treatment schedule with required minimum concentrations at intervals of 0.5, 1, 2, 4, and 24 h, and the resultant CxT product, suitable for bamboo borer in bamboo poles, as described in Table 6. The proposed schedule has significantly lower applied doses of MeBr than the current T404-d and will result in large reductions in the use of MeBr. Reduction of MeBr use would amount to >584 kg/1,000 fumigations of 6.1-m-long standard height containers, provided the fumigations were distributed across all five doses equally.

The probit-9 standard for quarantine efficacy (99.99683%) was developed for tropical fruits with heavy infestations of fruit flies (Follett and McQuate, 2001). They also propose that these standards may be too high for situations of lower risk and less abundant populations. The bamboo borer, as well as possibly other wood-boring Cerambycids, fit this situation, because it was difficult to obtain large numbers of test subjects, and hand-selected sticks of bamboo with high numbers needed to be selected and inserted into the commercially purchased bamboo used here to obtain meaningful numbers. They describe several situations where population and risk parameters would require control levels of 99.532 with as few as 639 insects per test. The numbers of test insects needed to confirm probit-9 or extreme high control is not practical with species such as *C. annularis.* In our test, with 520 confirmed dead insects, the failure to find one survivor could indicate a control level of 99.81%, with the highest count replicate at 99.86%. In total, we had 3,011 recovered dead stages, with no survivors. The goal of ISPM Rule 15 is to "reduce the risk of introduction and/or spread of quarantine pests" and to describe measures that "significantly reduce the risk of pest spread." We believe this recommended schedule meets that requirement.

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