Effects of Some Fumigants on Mortality of the Pine Wood Nematode, *Bursaphelenchus xylophilus* Infesting Wooden Packages

6. Mortality of Pine Wood Nematode and Longhorn Beetles by Methyl Iodide Tarpaulin Fumigation

Yukihiro Soma, Hitoshi Komatsu,* Yutaka Abe,** Toru Itabashi,**
Yasuo Matsumoto* and Fusao Kawakami*

Research Division, Yokohama Plant Protection Station, 1–16–10, Shin-yamashita, Naka-ku, Yokohama 231–0801, Japan, *Research Laboratory, Japan Fumigation Technology Association, Tokyo and **MITC Research Group, Tokyo

Abstract: Mortality test was conducted with methyl iodide (MI 50%w/w, carbon dioxide 50%w/w) to confirm CT products for complete mortality of the pine wood nematode, *Bursaphelenchus xylophilus* infesting red pine lumber (10–15 cm thick×10–15 cm wide×50 cm long in size) and the Japanese pine sawyer, *Monochamus alternatus*, and the Far East rusty longicorn beetle, *Arhoalus rusticus* infesting red pine logs (10–20 cm diameter, 1 m long). The test and filler material were fumigated at different temperatures and doses for 24 hours with 51.2% loading under a tarpaulin sheet of 1.95 m^3. Complete mortality of the nematode was attained at each of 84 g/m^3 at 10℃, 60 g/m^3 at 15℃, 64 g/m^3 at 20℃ and 48 g/m^3 at 25℃, while some survivors of the pine wood nematode were confirmed at 36 g/m^3 at 25℃ in one of four replicate tests, because of delayed gas uniformity caused by breakdown of a gas circulation fan. Complete mortality of larvae and pupae of longhorn beetles was attained at each of 84 g/m^3 at 10℃, 60 g/m^3 at 15℃, and 36 g/m^3 at 25℃. MI fumigation schedules for the tarpaulin sheet fumigation are at 84 g/m^3 at 10–14.9℃, 60 g/m^3 at 15–19.9℃, 48 g/m^3 at 20–24.9℃ and 36 g/m^3 at 25℃ or above for 24 hours with 50% or below loading.

Key words: quarantine treatment, methyl iodide, fumigation, mortality, CT product, tarpaulin sheet, *Bursaphelenchus xylophilus, Monochamus alternatus, Arhoalus rusticus,* wood packing material

Introduction

Methyl bromide fumigation schedules for the pine wood nematode, *Bursaphelenchus xylophilus* infesting red pine packing material have already been proposed based on test data (Soma *et al.,* 2001, 2002, 2003; Kawakami *et al.,* 2004).

Further, Soma *et al.* (2005) conducted susceptibility test on the pine wood nematode, and gas sorption test of wood packing material using methyl iodide (MI) as an alternative fumigant of methyl bromide in a 29.5-liter fiberglass fumigation box. They proposed MI fumigation schedules for warehouse and tarpaulin sheet fumigation based on data that complete mortality of the pest was attained at 60 g/m^3 at 10℃, 40 g/m^3 at 15 and 20℃, and 30 g/m^3 at 25℃, and CT products for complete mortality were 450 mg・h/l at 10℃, 400 mg・h/l at 15℃, 350 mg・h/l at 20℃ and 300 mg・h/l at 25℃, respectively for 24 hours with 25% (v/v) loading.

Here we report the result of mortality confirmatory tests for the pine wood nematode, the Japanese pine sawyer, *Monochamus alternatus* and the Far East rusty longicorn beetle, *Arhoalus rusticus* that may be found in wood packing material.

Materials and Methods

Test Wood Packing Material

Pine Wood Nematode

Red pine, *Pinus densiflora* naturally infested with the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nicklei was collected in Ibaraki Prefecture in March to July

2004 and March to May 2005. A log was sawed into lumber (10–15 cm thick×10–15 cm wide×50 cm long). Lumber infested with more than 10,000 nematodes per 100 g of sample was used for the mortality test. The lumber was stored for two to four days at fumigation temperatures until testing.

Japanese Pine Sawyer and Far East Rusty Longicorn Beetle

Red pine logs (10–15 cm diameter×100 cm long) naturally infested with larvae and pupae of the Japanese pine sawyer, *Monochamus alternatus* HOPE and the Far East rusty longicorn beetle, *Arhoalus rusticus* (LINNAEUS) were collected in Ibaraki Prefecture in March to May 2005, and then they were stored for two to four days at fumigation temperatures until testing.

Fumigation Schedules and Fumigation

The following fumigation schedules (SOMA *et al.,* 2005) were applied for fumigation.

Temperature (℃)	Exposure time (h)	Load factor (%)	Dose		CT product (mg・h/l)
			Warehouse (g/m^3)	Tarpaulin (g/m^3)	
10–14.9	24	50	84	112	450
15–19.9	24	50	60	80	400
20–24.9	24	50	48	64	350
25 or above	24	50	36	48	300

Fumigation was conducted under a tarpaulin sheet in an air-conditioned fumigation chamber. MI (MI 50%, CO_2 50%) filled in a high-pressurized gas cylinder was used for fumigation. This type of MI was registered as agricultural chemical for logs infested with forest insect pests in Japan in 2005.

Filler red pine lumber (15 cm thick×15 cm wide×100 cm long, 21 lumber; and 10 m thick×10 cm wide×100 cm long, 5 lumber) and board (3 cm thick×15 cm wide×100 cm long, 87 boards; and 2 cm thick×10 cm wide×100 cm long, 43 boards) were loaded in the size of 1m^3 (1 m×1 m×1 m). Test lumber infested with the nematode was placed in the center of the load. The red pine log infested with the Japanese pine sawyer and the Far East rusty longicorn beetle was also placed on top of the load. A plastic pipe frame (1.25 m×1.25 m×1.25 m) was placed over the load, and then the frame was covered with an EVOH sheet (Variastar®) of 0.1 mm thickness. Sand snakes were placed on the sheet skirt to prevent gas leakage. The capacity of the tarpaulin sheet was 1.95 m^3, and the load factor of wood material was 51.2% (v/v) in the test.

A gas dosing Teflon pipe and temperature sensors were fixed to upper and lower places in air space and between lumber of the load. A small-size air circulation fan was placed under the tarpaulin sheet. MI was introduced to the sheet by connecting a stainless steel pipe fixed to the top layer of the load. A gas circulation fan was operated for 30 minutes during dosing. Fumigation was conducted for 24 hours. The air-fumigant mixture was exhausted for one hour after fumigation. Fumigated material was placed in netted bags and was stored at ambient temperature until the evaluation of mortality.

Measurements of Temperature, Gas Concentration, and Moisture Content

Temperature was monitored with an automatic temperature recorder (Hybrid recorder AH, Chino). Gas concentration during fumigation was monitored at time intervals of 1, 2, 4 and 24 hours after dosing with gas chromatography (FID, Shimazu). The residual gas ratio was calculated with the following formula;

$$\text{Residual gas ratio (\%)} = \frac{100 \times \text{gas concentration after 24 hour fumigation (mg/}l\text{)}}{\text{applied dose (g/m}^3\text{)}}$$

CT product was calculated with the following formula;

$$\text{CT product (mg·h/}l) = 1.5C_1 + 1.5C_2 + 11C_4 + 10C_{24}$$
(where C_x is the gas concentration after x hour fumigation).

Moisture content was measured by the weight difference of samples of lumber and board pieces before and after drying at 115℃ for 18–24 hours. Moisture content of filler lumber and board was 13.6±3.4%.

Evaluation of Mortality

The number of nematodes was confirmed using wood sample before fumigation and sample for evaluation of mortality at six to seven days after fumigation. Small pieces from a few places per fumigated or non-fumigated lumber and board were cut off at a still smaller size (3 mm×3 mm×5 mm) with a special designed cutting instrument and scissors. The nematode was detected by the Bermann funnel method. A 20–40 g of sample was placed in a funnel and then the sample was stored for 48 hours at room temperature. The number of surviving nematodes was counted under a microscope. The longhorn beetle was also evaluated by dissecting infesting logs at seven days after fumigation.

Results and Discussion

Progressive Gas Concentration of MI

Figure 1 shows average progressive MI concentrations, as a typical example, in four replicate tests fumigated at 80 g/m^3 at 15℃ with 51.2% loading. No difference was observed on gas concentrations between upper and lower places in the air space just after dosing. A 16–22% difference in gas concentration was confirmed between air space and the center of the load in the first 15 minutes to four hours after dosing, and then the difference became small gradually. The small difference of gas concentration would be caused by two conditions of both operation of a circulation fan for 30 minutes during dosing and rapid evaporation and diffusion of particulate MI dissolved to carbon dioxide.

Fig. 2 shows average progressive MI gas concentrations at the center of the load during fumigation for 24 hours with 51.2% loading. Gas concentrations at one to four hours during dosing decreased with the same rate at each applied dose at different temperatures. However, gas concentrations at the end of fumigation of 24 hours were low at lower temperatures. The gas reduction during fumigation was almost the same result of the test in the 100-liter fiberglass fumigation box (SOMA *et al.*, 2005).

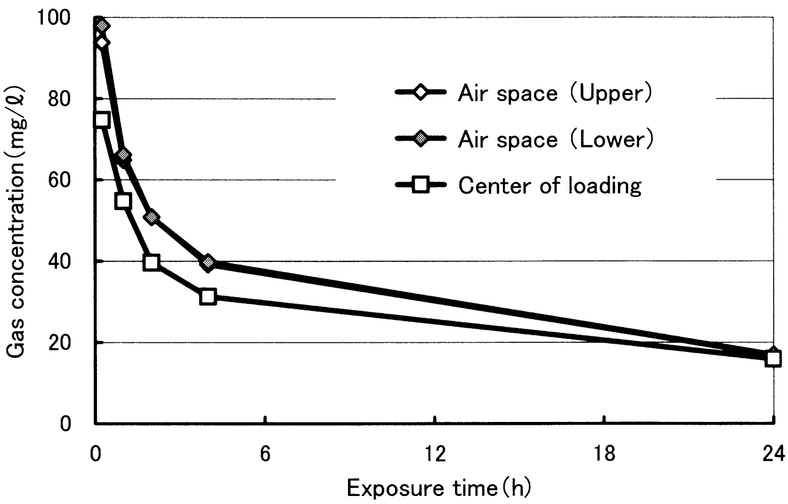

Fig. 1. Progressive gas concentrations for red pine packing material fumigated at 80 g/m^3 of methyl iodide at 15℃ with 51.2% loading (an air circulation fan was operated for 30 minutes during dosing).

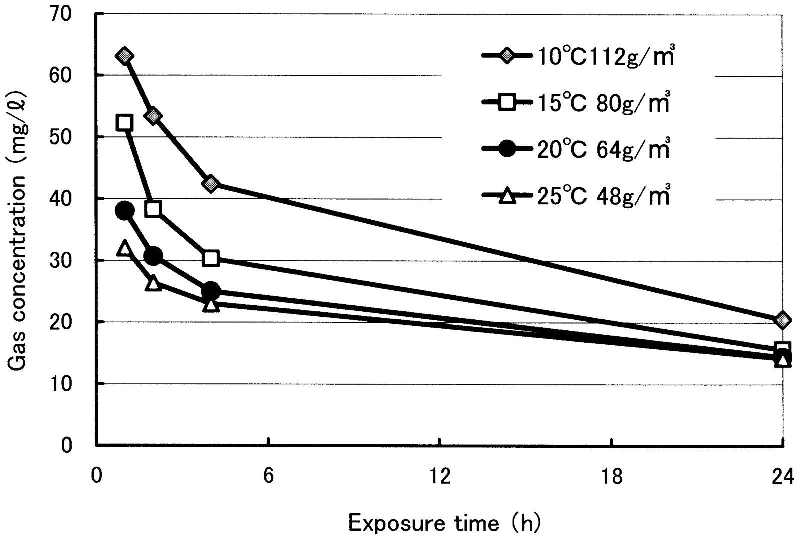

Fig. 2. Progressive gas concentrations for red pine packing materials fumigated with methyl iodide with 51.2% loading for 24 hours under a tarpaulin sheet.

Mortality of Pine Wood Nematode, Residual Gas Ratio and CT Product

Tables 1 and 2 show mortality of the pine wood nematode and gas concentration at the end of fumigation, residual gas ratio and CT products for the pine wood nematode fumigated at different temperatures and doses for 24 hours with 51.2% loading.

Residual gas ratios of MI were 14.8–29.6%, which were lower than the 24.5–45.0% from the

Table 1. Mortality data for the pine wood nematode infesting red pine packing material fumigated with methyl iodide for 24 hours with 51.2% (v/v) loading under a tarpaulin sheet.

Temperature (℃)	Dose (g/m^3)	Replicate	No. of test lumber[1]	Before fumigation: Moisture content[2] (%)	Before fumigation: Weight of sample[3] (g)	Before fumigation: No. of nematode[4] per 100 g	After fumigation: Moisture content[2] (%)	After fumigation: Weight of sample[3] (g)	After fumigation: No. of nematode[4] per 100 g	Survivor (%)
10	84	2	6	29.8	186	23,600	24.4	1,189	0	0
	96	1	3	19.4	58	72,100	18.8	602	0	0
	112	1	3	19.4	58	72,100	19.6	603	0	0
	Cont.	—	7	25.3	181	41,300	16.0	134	15,600	37.8
15	60	2	8	28.8	183	23,700	16.5	1,655	0	0
	80	4	12	20.3	550	42,200	20.8	2,453	0	0
	Cont.	—	12	23.7	385	41,100	18.5	288	40,700	99.0
20	64	2	6	31.8	122	20,400	23.3	1,001	0	0
	Cont.	—	4	31.2	81	21,100	21.8	142	21,700	102.8
25	36	1[5]	3	30.4	92	23,400	22.0	587	1.2	0.005
		3	9	31.0	276	23,900	22.0	1,793	0	0
	48	2	6	18.6	123	168,200	17.1	1,196	0	0
	Cont.	—	9	26.2	184	86,900	18.6	243	70,300	80.9

[1] Size of lumber: 10–15 cm thick×10–15 cm wide×50 cm long.
[2] Average moisture content in test lumber.
[3] Weight of wood samples used to detect nematode.
[4] Number of survivors of the pine wood nematode.
[5] No circulation fan during fumigation.

Table 2. Residual gas concentration, residual gas ratio and CT product for the red pine infested with the pine wood nematode fumigated with methyl iodide at 24 hours with 51.2% (v/v) loading under a tarpaulin sheet1).

Temperature (℃)	Dose (g/m³)	Replicate	Residual gas concentiation[2)]		Ratio of residual gas[3)]	CT product[4)]	
			Average (mg/l)	Minimum (mg/l)		Average (mg·h/l)	Minimum (mg·h/l)
10	84	2	12.5	12.3	14.8	521	518
	96	1	15.6	—	16.3	605	—
	112	1	20.5	—	18.3	846	—
15	60	2	11.4	11.1	19.0	472	467
	80	4	15.9	13.4	19.9	645	548
20	64	2	14.1	13.5	22.0	519	481
25	36	1[5)]	7.5	—	20.8	265	—
		3	8.6	7.2	23.9	322	273
	48	2	14.2	13.2	29.6	483	444

1) Wood packing material was loaded in the size of 1 m³.
2) Gas concentration at the center of loading.
3) 100×gas concentration after 24 hours fumigation (mg/l)/applied dose (g/m³).
4) $1.5C_1+1.5C_2+11C_3+10C_{24}$ (C_x: gas concentration after x hours).
5) No circulation fan during dosing.

test in a 100-liter box with 25% loading (SOMA *et al.*, 2005), because of a high load factor of 51.2% and low air tightness of the tarpaulin sheet. Residual gas ratios were low at low fumigation temperatures, which tendency was almost the same as the result from a 100-liter box. A higher gas sorption ratio would be caused at lower fumigation temperatures.

Complete mortality was attained at each of 112 g/m³ at 10℃, 80 g/m³ at 15℃, 64 g/m³ at 20℃ and 48 g/m³ at 25℃ in the schedule for tarpaulin sheet fumigation, and at each of 84 g/m³ at 10℃ and 60 g/m³ at 15℃ in the schedule for warehouse fumigation, respectively, while some survivors (0.005%) of the pine wood nematode were confirmed at 36 g/m³ at 25℃ in one of four replicate tests, because of delayed gas uniformity caused by breakdown of a gas circulation fan.

Further, some survivors (0.33–9.0 nematodes per 100 g of wood sample) of free-living nematode (*Rhabditidae Gen. et* sp. and *Plectidae Gen. et* sp.) were confirmed in all of the fumigation schedules that attained complete mortality of the pine wood nematodes. These surviving free-living nematodes could be disregarded in the mortality test, as they are non-quarantine pests.

CT products were 846 mg·h/l at 112 g/m³ at 10℃, 548 mg·h/l at 80 g/m³ at 15℃, 481 mg·h/l at 64 g/m³ at 20℃ and 444 mg·h/l at 48 g/m³ at 25℃ by the schedule for tarpaulin sheet fumigation, and 518 mg·h/l at 84 g/m³ at 10℃ and 467 mg·h/l at 80g/m³ at 15℃ by the schedule for warehouse fumigation, respectively. These CT products were higher than those (450 mg·h/l at 10℃, 400 mg·h/l at 15℃, 350 at 10℃, and 300 mg·h/l at 25℃) from the schedule for a tarpaulin fumigation proposed by SOMA *et al.* (2005).

In fumigation at 36 g/m³ at 25℃, CT products from two replicates in four replicate tests were lower (265 and 273 mg·h/l) than that (300 mg·h/l) for complete mortality (SOMA *et al.*, 2005). Some survivors of the pine wood nematode were observed on fumigation with CT product of 265 mg·h/l (Table 1) and the low CT product was caused by delayed gas uniformity at the beginning of fumigation, because of breakdown of a gas circulation fan. Therefore, it would be essential to operate a gas circulation fan for more than 30 minutes at the beginning of fumigation.

Mortality of Longhorn Beetles

Table 3 shows the mortality of the Japanese pine sawyer and the Far East rusty longicorn beetle fumigated at different temperatures and doses for 24 hours with 51.2% loading. Complete mortality of larvae and pupae of the pest in xylem was attained at each of 84 g/m³ at 10℃, 60 g/

Table 3. Mortality data for two species of longhorn beetle infesting red pine packing material fumigated with methyl iodide at 24 hours with 51.2% (v/v) loading under a tarpaulin sheet.

Temperature ℃	Replicate	Dose (g/m^3)	No. of test log[1]	*Monochamus alternatus*[1] Larva			*Monochamus alternatus*[1] Pupa			*Arhoalus rusticus*[1] Larva		
				n	Survivor	Mortality (%)	*n*	Survivor	Mortality (%)	*n*	Survivor	Mortality (%)
10	2	84	12	71	0	100	22	0	100	55	0	100
15	2	60	12	70	0	100	4	0	100	32	0	100
20	2	64	16	106	0	100	1	0	100	—	—	—
25	3	36	14	96	0	100	9	0	100	87	0	100
Cont.	3	—	8	65	59	9	5	5	0	23	23	0

[1] Number of survivors of the longhorn beetle was confirmed by dissecting red pine log.

m^3 at 15℃, 64 g/m^3 at 20℃ and 36 g/m^3 at 25℃, respectively.

Proposed MI Fumigation Schedule and Fumigation Method

The pine wood nematode, the Japanese pine sawyer and the Far East rusty longicorn beetle were killed completely by a proposed MI fumigation schedule as shown in Table 4, that is, at 84 g/m^3 at 10–14.9℃, 60 g/m^3 at 15–19.9℃, 48 g/m^3 at 20–24.9℃ and 36 g/m^3 at 25℃ or above for 24 hours with 50% or below loading. In tarpaulin sheet fumigation, a gas circulation fan for gas uniformity should be operated to obtain a minimum gas concentration at 1, 4 and 24 hours, and CT product for complete mortality of the target pest.

Table 4. Proposed methyl iodide fumigation schedule for wood packing material infested with the pine wood nematode under a tarpaulin sheet.

Temperature (℃)	Time (h)	Load factor (%)	Dose (g/m^3)	Minimum gas concentration 1 h (mg/*l*)	Minimum gas concentration 4 h (mg/*l*)	Minimum gas concentration 24 h (mg/*l*)	Minimum CT product (mg･h/*l*)
10–14.9	24	50 or below	84	42	20	14	450
15–19.9			60	36	18	12	400
20–24.9			48	30	16	10	350
25 or above			36	24	14	8	300

- The longhorn beetle is also killed completely by the fumigation standard.
- An air circulation fan is operated for more than 30 minutes for gas uniformity.

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和 文 摘 要

マツノザイセンチュウ *Bursaphelenchus xylophilus* が寄生した梱包材のくん蒸剤による消毒試験

6. ヨウ化メチルを用いたマツノザイセンチュウ及びカミキリムシの天幕くん蒸試験

相馬幸博・小松　仁*・阿部　豊**・板橋　享**・松本安生*・川上房男*
横浜植物防疫所調査研究部・*社団法人 日本くん蒸技術協会・**MITC 研究会

ヨウ化メチルくん蒸剤（ヨウ化メチル 50%、炭酸ガス 50%; w/w）を用い、マツノザイセンチュウが寄生した赤松梱包材 (10～15 cm×10～15 cm×50 cm) 並びにマツノマダラカミキリ及びサビカミキリが寄生した赤松材（直径 10～20 cm×1 m）を収容率 51.2% で 24 時間天幕くん蒸（内容積 1.95 m^3）を行い、くん蒸中のガス濃度（CT 値）及び殺虫効果を調査した。その結果、マツノザイセンチュウは 10℃ 84 g/m^3、15℃ 60 g/m^3、20℃ 64 g/m^3 及び 25℃ 48 g/m^3 で完全殺虫された。しかし、25℃ 36 g/m^3 では 4 反復試験のうち 1 反復において生存虫が認められた（生存率 0.005%）。生存虫が認められたのは、くん蒸中にかくはん機が稼働しなかったためガスの拡散が遅れ、ガス濃度の均一化が遅れたのが原因であった。マツノマダラカミキリ及びサビカミキリの幼虫及び蛹は 10℃ 84 g/m^3、15℃ 60 g/m^3 及び 25℃ 36 g/m^3 で完全に殺虫された。これらの結果から、梱包材に寄生するマツノザイセンチュウ、カミキリムシ類は、10～14.9℃ で 84 g/m^3、15～19.9℃ で 60 g/m^3、20～24.9℃ で 48 g/m^3 及び 25℃ 以上で 36 g/m^3、収容率 50% 以下、24 時間くん蒸により完全殺虫されるものと考える。