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

3. Mortality and Fumigation Standards for Pine Wood Nematode by Methyl Bromide

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Abstract: Mortality tests were conducted in the large-scale applied tests by methyl bromide (MB) to confirm a complete mortality and their CT products for the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nicklei. Red pine lumber (15 cm thick×15 cm wide×30 cm long in size) infested with the pine wood nematode were fumigated with MB for 24 hours with 25% and 50% loading. No survivor of the nematode was observed on the samples fumigated at 70 g/m^3 at 10℃, 50 g/m^3 at 15℃, at 40 g/m^3 at 20℃ and 30 g/m^3 at 25℃, respectively, while some survivors were observed on the sample fumigated at as much as 100 g/m^3 at 5℃. Approximately CT products for complete moralities were 900 mg·h/*l* at 10℃, 700 mg·h/*l* at 15℃, 600 mg·h/*l* at 20℃ and 500 mg·h/*l* at 25℃, respectively. Gas absorption for lumber and board (15 cm thick×15 cm wide×30 cm long in size) fumigated at 50 g/m^3 for 24 hours at 15 and 25℃ showed that higher gas absorption was observed on fumigation conditions of low temperature, high loading, low moisture content, board with larger surface area.
Key words: quarantine treatment, fumigation, methyl bromide, *Bursaphelenchus xylophilus,* wooden packages, fumigation standards, CT product

Introduction

Chinese plant quarantine authority has accepted hot air treatment at 56℃ or above for 30 minutes as the only control measure against pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nicklei potentially infecting conifer wooden packages for export to China from Japan (Public Notice No. 32, Bureaus of Immigration and Quarantine, Republic of China, November 1, 1999).

Soma *et al.* (2001, 2002) reported that a complete mortality of the pine wood nematode was confirmed at 60 g/m^3 of methyl bromide (MB) for 24 hours at 15℃ with 25% loading,

and that a complete mortality was also confirmed at 48 g/m^3 for 24 hours at 15℃ with 50% loading when fumigated the pine wood nematode infecting red pine board and lumber in the small-scale test.

Here we report the results of further tests for the mortality of the pine wood nematode infecting red pine lumber at different fumigation temperatures of 5 and 25℃ and for gas absorption rate for different types of red pine wood and red pine lumber with different moisture contents. We also report MB fumigation conditions for the pine wood nematode based on data from a series of mortality tests.

Materials and Methods

Test wooden materials infected with pine wood nematode

Red pine, *Pinus densiflora* naturally infected with the pine wood nematode were collected in Ibaraki Prefectures in April 2002. The red pine was sawn into lumber (15 cm thick×15 cm wide×30 cm long in size) and into board (3 cm thick×15 cm wide×30 cm long in size). The red pine lumber with more than 10,000 nematodes per 100 g of the sample with the dispersal 3rd stage larvae when detected the nematode by Bermann funnel method before fumigation was used for the mortality test. The pine lumber and board with different moisture contents (10–19% and more than 20%) were used for gas absorption tests.

Fumigation

The test lumber and board were stored at fumigation temperatures for 1 to 3 days, and then put in a 29.5 liter fiber-glass fumigation box equipped with a circulation fan, ventilation apparatus, and ports for gas application and sampling, a manometer and temperature probes.

Several doses of MB (purity; 99% or more) were applied to determine the mortality in the test. MB was introduced with a gas-tight syringe and then fumigated for 24 hours at 5, 10, 15, 20 and 25℃ with 25% (v/v) loading. Air-fumigant mixture was exhausted for one hour after fumigation. Fumigated wooden materials were placed in netted bags and stored at ambient temperature until detection of the nematode by Bermann funnel method.

Measurements of gas concentration, temperature and moisture content

Gas concentrations during fumigation were monitored at time intervals of 1, 2, 4, 6 and 24 hours after the commencement of fumigation with gas chromatography (FID) and temperatures were also monitored with an automatic temperature recorder (Hybrid recorder AH, Chino). The following is a formula for calculating CT product; CT product (mg・h/l)$=C_1+2C_2+2C_4+10C_6+9C_{24}$ (where: C_x is gas concentration after x hours of fumigation).

Evaluation of mortality

The number of the nematodes was confirmed on the samples before fumigation mentioned above and on those for evaluation of mortality in 6–7 days after fumigation.

Wooden pieces from a few places of fumigated or unfumigated lumbers were collected by sawing and then cut them to the size of 3 mm×3 mm×5 mm with scissors. The samples of 30 g per place were detected by Bermann funnel method for 48 hours at the room temperature and then the number of survival nematodes were counted under microscopes.

Results and Discussion

Gas concentrations record during fumigation

Table 1 shows means MB gas concentrations at the end of fumigation, ratios of residual gas and CT products fumigated with several doses at 5, 10, 15, 20 and 25℃ for 24 hours with 25% loading.

High concentrations were observed at just after dosing of MB and then reduced gradually during fumigation. The gas decline curve in each dose showed almost the same gas reduction pattern in each fumigation. Ratios of residual gas (gas concentration at the end of fumigation/applied dose×100) were ranging 62–86% in one hour fumigation and were ranging 40–63% in 24 hours fumigation. It was clarified that lower ratio of residual gas was confirmed at low fumigation temperatures and higher ratio of MB absorption would be done at low temperatures.

Mortality of pine wood nematode

Table 2 shows the mortality of the pine wood nematode fumigated at different temperatures and doses for 24 hours with 25% loading.

Some survivors were confirmed at as much as 90 and 100 g/m^3 at 5℃. At 10℃ no survivors was confirmed at 70 and 80 g/m^3, while some survivors was confirmed at 60 g/m^3. At 15℃ no survivors was confirmed at 50 g/m^3, while some survivors was confirmed

Table 1. Residual gas concentiations, Ratios of residual gas and CT products for the red pine infested with the pine wood nematode fumigated with methyl bromide at 24 hours with 25% (v/v) loading.

Temperature (℃)	Dose (g/m^3)	Replicate	Residual gas concentration	Ratio of residual gas* (%)		CT product
			mg/*l*±S.D.	1 hr	24 hrs	mg·h/*l*±S.D.
5	90	3	36.6±4.6	62.0	40.7	977± 73
	100	3	44.1±2.9	62.9	44.1	1,146± 30
10	60	2	27.9±5.1	76.8	46.5	803± 9
	70	3	31.7±5.9	74.7	45.3	909± 41
	80	3	36.7±4.6	73.3	45.9	1,041± 72
15	30	1	15.1 —	72.3	50.3	403 —
	50	3	24.1±1.0	83.5	48.2	703± 66
20	30	1	16.2 —	73.0	54.0	416 —
	40	3	23.2±1.9	81.3	58.0	585±100
25	20	1	11.5 —	85.5	57.5	329 —
	30	3	18.5±2.2	82.6	61.7	503± 36
	40	3	24.8±1.2	83.5	62.0	687± 57

* 100×gas concentration for 1 or 24 hrs fumigation (mg/*l*)/applied dose (g/m^3).

Table 2. Mortality data for the pine wood nematode infesting red pine board[1] fumigated with methyl bromide at 24 hours with 25% (v/v) loading.

Temperature (℃)	Dose (g/m^3)	Replicate	Before fumigation			After fumigation			Survivor (%)
			Moisture content[2] (%)	Weight of board[3] (g)	No. of nematode per 100 g	Moisture content[2] (%)	Weight of board[3] (g)	No. of nematode per 100 g	
5	70	1	28.6	15.2	21,710	18.6	120.1	242.3	1.12
	80	1	28.6	15.2	21,710	14.1	113.1	5.3	0.02
	90	3	16.9	93.2	15,100	13.8	420.2	3.1	0.02
	100	3	18.0	84.1	23,700	14.6	416.9	1.2	0.01
	cont.	2	17.5	59.1	19,400	14.2	89.2	8,500	43.8
10	60	2	16.0	30.7	17,900	12.8	152.2	5.3	0.03
	70	3	20.8	86.1	14,100	16.5	432.9	0	0
	80	3	26.9	85.5	16,300	15.9	434.3	0	0
	cont.	2	29.2	58.0	21,600	16.6	79.0	15,900	73.6
15	30	1	24.9	36.7	14,300	17.2	156.1	3.2	0.02
	50	3	23.7	91.9	17,800	17.6	492.9	0	0
	cont.	2	23.2	55.4	19,500	18.0	62.9	15,700	80.5
20	30	1	16.0	30.7	17,900	12.8	88.9	1.1	0.01
	40	3	24.8	107.7	13,000	19.2	456.6	0	0
	cont.	2	29.3	78.0	17,700	15.2	76.6	20,300	114.7
25	20	1	28.6	30.7	18,800	17.1	135.7	3.7	0.02
	30	3	21.2	89.8	19,200	15.5	412.9	0	0
	40	3	21.2	89.8	19,200	15.1	420.6	0	0
	cont.	2	17.5	59.1	19,400	13.0	81.2	21,300	109.8

[1] Size of board; 15 cm thick×15 cm wide×30 cm long.
[2] Average moisture content in test wooden board.
[3] Weight of wooden samples used for the detection of nematodes.

at 30 g/m^3. At 20℃ no survivors was confirmed at 40 g/m^3, while some survivors was confirmed at 30 g/m^3. At 25℃ no survivors was confirmed at 30 g/m^3 and 40 g/m^3, while some survivors was confirmed at 20 g/m^3.

These results showed that a complete mortality of the pine wood nematode would be achieved at 70 g/m^3 at 10℃, 50 g/m^3 at 15℃, 40 g/m^3 at 20℃ and 30 g/m^3 at 25℃, respectively. Furthermore approximately, CT products for complete mortalities were 900 mg·h/*l* (applied dose of 70 g/m^3) at 10℃, 700 mg·h/*l* (applied dose of 50 g/m^3) at 15℃, 600 mg·h/*l* (applied dose of 40 g/m^3) at 20℃ and 500 mg·h/*l* (applied dose of 30 g/m^3) at 25℃, respectively.

Relationship between gas absorption and load factor, moisture content of wooden packages

Table 3 shows gas concentrations at the end of fumigation and CT products for wooden packages with different moisture contents and load factor at 50 g/m^3 for 24 hours at 15 and 25℃. Figure 1 also shows progressive gas concentration for lumber and board with MB at 50 g/m^3 for 24 hours at 15℃ with 25 and 50% loading.

Gas concentrations in each fumigation were reduced rapidly in 1 to 3 hours after

Table 3. Gas concentrations at the end of fumigation and CT products for the red pine wooden packages fumigated with methyl bromide.

	Size (cm)	Dose (g/m^3)	Exposure time (hr)	Temperature (℃)	Load factor (%)	Moisture content* (%)	Residual gas concentration (mg/*l*)	CT product mg·h/*l*
Lumber	15×15×30	50	24	15	25	27.8	27.0	809
						15.9	21.5	707
				25	25	24.9	30.4	902
						15.9	24.9	755
Board	3×15×30	50	24	15	25	27.1	22.2	661
						11.1	16.8	554
					50	27.1	14.7	455
						11.1	8.6	340
				25	25	27.1	26.8	757
						10.6	19.2	590
					50	27.1	19.2	555
						10.6	11.0	364

* Average moisture content in test wooden board.

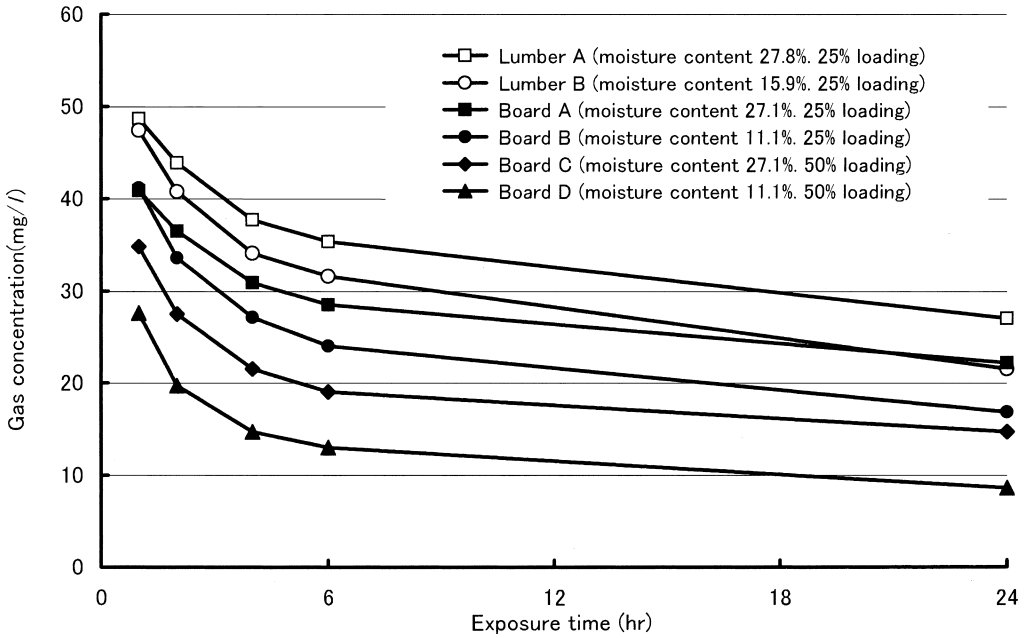

Fig. 1. Progressive gas concentrations for wooden packages fumigated with methyl bromide at 50 g/m^3 for 24 hours at 15℃.

dosing and then reduced gradually. Both residual gas concentrations and CT products in lumber and board with low moisture contents (10.6–15.9%) were lower than those with high moisture contents (24.9–27.8%) in all fumigation. The CT products in lumber and board with high loading (50%) were also lower than that with low loading (25%). The CT

Table 4. Methyl bromide fumgation standards for wooden package infested with the pine wood nematode.

Temperature (℃)	Exposure time (hr)	Dose		Minimum gas concentration		CT product
		Warehouse (g/m^3)	Tarpaulin (g/m^3)	1 hr (mg/l)	4 hrs (mg/l)	$mg \cdot h/l$
10–14.9	24	84	112	56	44	900
15–19.9	24	60	80	44	34	700
20–24.9	24	48	64	36	30	600
25 or above	24	36	48	28	24	500

products of both lumber and board fumigated at 15℃ were also lower than those of both lumber and board at 25℃ and the CT product of board was lower than that of lumber. Fumigation of wooden packages with conditions of low moisurte content and high loading at low temperature.

These data showed that MB gas would be much absorbed in fumigation with some factors of at low temperature, high loading, low moisture content of lumber and board and board with larger surface.

Methyl bromide fumigation standards for wooden packages

Table 4 shows methyl bromide fumigation standard for wooden packages. The standards were established with previous test data (SOMA *et al.,* 2001; Soma *et al.,* 2002). These standards would be applied for wooden packages (20 cm×20 cm×20 cm in size) infested with the pine wood nematode.

Applied doses in warehouse fumigation were added another 20% of dose (dose equivalent in amount of absorption to wooden packages) to 70 g/m^3 (10℃), 50 g/m^3 (15℃), 40 g/m^3 (20℃) and 30 g/m^3 (25℃) in the mortality test) and also another 60% of dose (dose equivalent in amount of gas leakage) was added to above mentioned doses in tarpaulin fumigation. Further, both minimum gas concentrations in 1 hour and 4 hours after dosing and minimum CT products are provided for each fumigation standard. The minimum CT product would be fulfilled for wooden packages when obtained minimum gas concentrations in 1 hour and 4 hours during fumigation.

SOMA *et al.* (2001, 2002) reported that gas concentration at the end of fumigation or in 24 hours was a little changed when gas concentration in 1 hour after dosing was greatly changed and was a little change in 4 hours, while a great change was observed on gas concentration in 24 hours when gas concentration was a little changed in 1 hour and was greatly changed in 4 hours. In the case of dried wooden board, gas concentrations may also reduce greatly because of high ratio of gas sorption. Such tendencies of gas reduction patterns should be considered in a practical fumigation.

If minimum gas concentrations in 1 hour and 4 hours were not provided for fumigation, suitable actions of use of additional dose or extension fumigation time should be taken in a practical fumigation.

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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

3. 臭化メチルによる殺線虫効果とくん蒸基準

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マツノザイセンチュウが寄生した赤松材を角材 (15 cm×15 cm×30 cm) に製材して収容率 25%，24 時間の条件で臭化メチルくん蒸し，殺虫効果と CT 値を温度別に調査した。その結果，10℃では 70 g/m^3，15℃では 50 g/m^3，20℃では 40 g/m^3，25℃では 30 g/m^3 の薬量で完全殺虫が得られたが，5℃では 100 g/m^3 でも完全殺虫されなかった。完全殺虫されたときの平均 CT 値 (mg･h/*l*) は，10℃で約 900, 15℃で約 700, 20℃で約 600, 25℃で約 500 であった。

また，同様に製材した角材及び板材 (5 cm×15 cm×30 cm) を用い，50 g/m^3，収容率 25%及び 50%，15℃及び 25℃，24 時間の条件で臭化メチルくん蒸し，梱包材によるガス収着性を調査した。その結果，角材よりも板材の方が，温度は低い方が，収容率は高い方が，含水率は低い方がガスの収着性が高く，特に収容率が 50%で含水率が低い板材では，収容率 25%で含水率が高い角材に比較して CT 値は半分以下であった。