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

1. Susceptibility of Pine Wood Nematode to Methyl Bromide, Sulfuryl Fluoride and Methyl Isothiocyanate

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Abstract: Susceptibility of pine wood nematode, *Bursaphelenchus zylophilus* (STEINER and BUHRER) NICKLEI infecting conifer wooden board (2cm thick × 15cm wide × 30cm long in size) and lumber (15cm thick × 15cm wide × 30cm long in size) was confirmed by fumigation of methyl bromide (MB), sulfuryl fluoride (SF) and methyl isothiocyanate (MITC) with conditions at doses of 20, 30, 40, 60 and 80g/m^3 of the fumigant for 24 and 48 hours at 15℃ with 25% (v/v) loading. The following fumigation schedules provided for complete mortality against the pine wood nematode infesting lumber; at 60g/m^3 for 24 hours, 40g/m^3 for 48 hours in MB fumigation and at 40g/m^3 for 24 hours, 20g/m^3 for 48 hours in MITC fumigation, respectively, while some survivors were confirmed on the sample fumigated with SF at 60g/m^3 for 48 hours.

Key words: fumigation, methyl bromide, sulfuryl fluoride, methyl isothiocyanate, *Bursaphelenchus xylophilus*, wooden packages, quarantine treatment

Introduction

Wooden materials, such as palettes, drums, crates, boxes are used for packages of agricultural and industrial commodities. Plant quarantine authorities in many countries, however, have import inspection these wooden materials at ports of entry to prevent of introduction of quarantine significant forest insect pests. Wooden products used for packages of export commodities from Japan have been disinfected with methyl bromide (MB) fumigation, vapor or dry heat treatments in accordance with official regulations of importing countries such as Australia (Australian government requirement Reference No.360/1/1; 1958), New Zealand (Forest Produce Import and Export Regulations, 1966), the USA (Code of federal regulations USDA § 319.40-7, 1984).

In 1999, 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 zylophilus* (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). Alternative technologies to hot air treatment has strongly demanded by exporters who have concern about the limitation of export of industrial commodities packaged by wooden board and lumber because of shortage of hot air treatment facilities.

Thereupon, disinfection tests for the pine wood nematode infecting the red pines were carried out with fumigants of MB, sulfuryl fluoride (SF) and methyl isothiocyanate (MITC) which have some advantages of highly effective against a wide scope of insects, easy applicable to a large volume of commodities and lower cost performance. These fumigants have shown highly efficacy against forest insect pests in the laboratory test at the Research Division of Yokohama Plant Protection Station, MAFF (SOMA *et al.*, 1996; 1999).

Here we report results of the susceptibility of the pine wood nematode to MB, SF and MITC in a series of the disinfection test.

Materials and Methods

Test Wooden Materials

Red pine, *Pinus densiflora* naturally infested with the pine wood nematode were collected in Ibaraki and Fukuoka Prefectures and sawn into board (2cm thick × 15cm wide × 30cm long in size) and lumber (15cm thick × 15cm wide × 30cm long in size), respectively. Five pieces of board and 3 pieces of lumber were tied up into a bundle by plastic band, respectively. Board and lumber of same size of test sample were also prepared as filler materials for achieving a certain fumigation loading. The filler was always placed outside of the bundle.

A number of the pine wood nematode in the test wooden material was always confirmed before fumigation. The wooden materials including more than 10,000 nematodes per 100g of the sample were used for the tests. The stage of the nematode in a series of the test accounted for preceding the dispersal 3rd-and 4th-stage larvae (MAMIYA, 1995) to more than 90% of the nematode in each sample.

Fumigation

The bundle of the test wooden material was placed in a 100*l* fiber-glass fumigation box (50cm × 40cm × 50cm) with 25% (v/v) loading as simulating warehouse fumigation and then stored for overnight at 15℃ of fumigation temperature. Fumigation was conducted at doses of 20, 40, 60 and 80g/m^3 of MB (purity; 99% or more) and at doses of 30 and 60g/m^3 of SF (purity; 99% or more) and at doses 20 and 40g/m^3 of MITC (30% MITC in CO_2) for 24 and 48 hours at 15℃ with 25% loading, respectively. For MB and SF prescribed doses were collected in a syringe and then introduced into the fumigation chamber. MITC was introduced directly from a cylinder by measuring of prescribed doses. Gas concentrations during fumigation were monitored periodically with gas chro-

matography (FID for MB, MITC, and TCD for SF; Shimadzu) and temperatures were also monitored with automatic temperature recorder (Hybrid recorder AH, Chino). 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.

Evaluation of Mortality

The number of the nematodes was confirmed on the samples before fumigation as mentioned above and on those for evaluation of mortality in 6-7 days and 20-21 days after fumigation.

Wooden pieces were taken from a few places in the fumigated and untreated samples by saw separately and then cut them into the size of 3mm × 3mm × 5mm with scissors.

The samples of 10-20g per place were left Bermann funnel for 24 hours at the room temperature and then survival nematodes were counted under microscopes.

Results and Discussion

Gas Concentrations, Ratios of Residual Gas and CT products

Figure 1 shows progressive gas concentrations during fumigation of MB, SF and MITC with 40g/m^3 at 15℃ for 48 hours with 25% loading. Corrected gas concentrations from 40g/m^3 from 60g/m^3 with the coefficient of 0.66 = 40g/60g were shown as gas concentrations in SF fumigation. Highest progressive gas concentrations were confirmed in SF fumigation, while lowest gas concentrations were observed in MITC fumigation. Gas concentrations in MITC decreased rapidly from the commencement of fumigation.

Table 1 shows ratios of residual gas [100 × gas concentration (mg/*l*) at the end of fumigation for 24 and 48 hours/applied dose (g/m^3)] and CT products [gas concentration (mg/*l*) × fumigation time (hr)] in three fumigants. The ratios of residual gas to initial doses of SF for 24 hour and 48 hour fumigation were more than 95%, while average ratios for 24 and 48 hours fumigation were 3.3% and 2.7% in MITC, and 58.1% and 54.6% in MB, respectively. These data indicated that MITC was absorbed to wooden materials with higher ratios and the residual gas concentration was extremely low at the end of fumigation when compared to other fumigants of SF and MB. CT products fumigated with 60g/m^3 at 15℃ with 25% (v/v) loading showed that 1,539mg·h/*l* (for 24 hours), 2,932mg·h/*l* (for 48 hours) in SF and 900mg·h/*l* (for 24 hours), 1,704mg·h/*l* (for 48 hours) in MB, respectively. In case of the MITC, CT products of 53mg·h/*l* (for 24 hours) and 78mg · h/*l* (for 48 hours) were confirmed on the fumigation with 40g/m^3 at 15℃ with25% (v/v) loading. These CT products would be indicators of the efficacy in practical warehouse and tent fumigation.

Mortality on Pine Wood Nematode

Nematodes in board

Table 2 shows the mortality of the nematode fumigated at 15℃ with 25% loading and evaluated in 6-7 days after fumigation. In MB fumigation, some survivors (0.01%) were

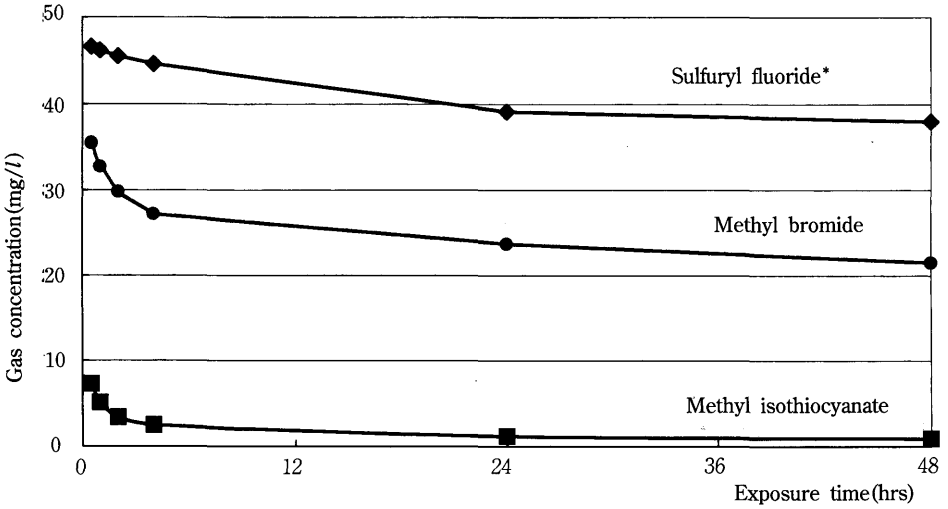

Fig. 1. Progressive gas concentrations during fumigation for wooden packages with three fumigants at 40g/m³ for 48 hours at 15℃ with 25%(v/v) loading. (*Gas concentrations were corrected with those from 60g/m³ of sulfuryl fluoride)

Table 1. Ratios of residual gas and CT products for the red pine infected with the pine wood nematode fumigated with methyl bromide, methyl isothiocyanate and sulfuryl fluoride.

Fumigant	Dose (g/m³)	Temp (℃)	Load (%)	Ratio of residual gas[1] (%)		CT product[2] (mg·h/l)	
				24 hr	48 hr	24 hr	48 hr
Methyl bromide	20	15	25	58.0	-	313	-
	40	15	25	59.3	53.8	631	1,174
	60	15	25	57.3	54.3	900	1,704
	80	15	25	57.7	55.8	1,188	2,277
Sulfuryl fluoride	30	15	25	97.8	-	765	-
	60	15	25	98.5	95.0	1,539	2,932
MITC[3]	20	15	25	3.5	3.0	32	47
	40	15	25	3.0	2.3	53	78

[1] 100×Gas concentration at the end of fumigation (mg/l)/applied dose (g/m³).
[2] Exposure time (hr)×gas concentration (mg/l).
[3] 30% methyl isothiocyanate in CO_2 was used for fumigation.

confirmed on the sample fumigated at 40g/m³ for 24 hours, while complete mortality was observed on the samples fumigated at 60g and 80g/m³ for 24 hours and 40g, 60g and 80 g/m³ for 48 hours, respectively. As for SF fumigation, higher ratios of survivors of 13.3% and 6.0% were observed on the sample fumigated at 60g/m³ for 24 hours and at 60g/m³ for 48 hours, respectively. No survivor was observed on the samples fumigated with 20 and 40g/m³ of MITC for 24 and 48 hours.

These results indicated that MB and MITC will have more significant effect to the pine wood nematode, and that SF will no be used for a practical fumigation because of

Table 2. Mortality data for the pine wood nematode infecting red pine board 2cm thick[1] fumigated with methyl bromide, sulfuryl fluoride and methyl isothiocyanate at 15℃ with 25% (v/v) loading (Mortality was assessed on samples in 6-7 days after fumigation).

Fumigant	Dose (g/m^3)	Exposure (time)	No. of board	Before fumigation			After fumigation			
				Moisture content[2] (%)	Weight of board[3] (g)	No. of nematode (per 100g)	Moisture content[2] (%)	Weight of lumber[3] (g)	No. of nematode (per 100g)	Survivor (%)
Methyl bromide	20	24	3	28.2	63.4	69,900	23.1	159.9	1,522	2.2
	40	24	6	25.1	151.8	36,500	22.4	276.8	4.7	0.01
	40	48	3	31.5	60.4	57,800	22.9	275.0	0	0
	60	24	5	30.6	95.3	53,600	22.7	321.5	0	0
	60	48	4	27.8	77.6	62,200	21.3	217.3	0	0
	80	24	5	29.4	135.5	41,500	24.8	203.8	0	0
	80	48	2	33.2	39.6	33,300	24.4	120.3	0	0
Sulfuryl fluoride	60	24	2	27.3	50.0	20,400	10.5	48.0	2,704	13.3
	60	48	2	27.3	50.0	20,400	12.7	48.0	1,227	6.0
MITC[3]	20	24	3	27.2	66.2	57,200	23.0	141.4	0	0
	20	48	3	26.1	59.0	68,000	22.9	169.7	0	0
	40	24	6	26.4	157.3	45,200	23.5	238.9	0	0
	40	48	6	27.2	148.5	45,500	22.3	243.3	0	0
Control	0	48	10	25.8	172.3	39,000	22.3	207.6	33,300	85.4

[1] Size of board; 2cm thick × 15cm wide × 30cm long.
[2] Average moisture content in test wooden board.
[3] Weight of wooden samples used for the detection of nematodes.
[4] 30% methyl isothiocyanate in CO_2 was used for fumigation.

low effect to the nematode.

Nematodes in lumber

Table 3-1 shows that the mortality of the nematode fumigated at 15℃ with 25% loading and evaluated in 6-7 days after fumigation. In MB fumigation, some survivors (0.07%) were confirmed on the sample fumigated at $40g/m^3$ for 24 hours, while complete mortality was observed on the samples fumigated at 60g and $80g/m^3$ for 24 hours and 40g, 60g and $80g/m^3$ for 48 hours, respectively. As for SF fumigation, higher ratios of survivors of 10.6%, 8.4% and 1.9% were observed on the samples fumigated at 30g and $60g/m^3$ for 24 hours, and at $60g/m^3$ for 48hours, respectively. In MITC fumigation, some survivors (0.006%) were confirmed on the samples fumigated at $20g/m^3$ for 24 hours, while no survivor was observed on the samples fumigated at $20g/m^3$ for 48 hours and $40g/m^3$ for 24 and 48 hours. These were almost the same results in the test with board mentioned above, i.e. MB and MITC will have more effective fumigant than SF to the pine wood nematode.

Table 3-2 shows the mortality of the nematode fumigated at 15℃ evaluated in 20-21 days after fumigation. The criteria for the mortality were same as the test samples evaluated in 6-7 days after fumigation. The results in MB and SF fumigation were almost the

Table 3-1. Mortality data for the pine wood nematode infecting red pine lumber 15cm square[1] fumigated with methyl bromide, sulfuryl fluoride and methyl isothiocyanate at 15℃ with 25% (v/v) loading (Mortality was assessed on samples in 6-7 days after fumigation).

Fumigant	Dose (g/m^3)	Exposure (time)	No. of lumber	Before fumigation			After fumigation			
				Moisture content[2] (%)	Weight of lumber[3] (g)	No. of nematode (per 100g)	Moisture content[2] (%)	Weight of lumber[3] (g)	No. of nematode (per 100g)	Survivor (%)
Methyl bromide	20	24	2	45.5	103.6	23,000	30.2	127.9	1,124	4.9
	40	24	4	33.0	167.0	42,900	27.0	218.5	29	0.07
	40	48	2	46.1	104.7	40,700	34.5	143.8	0	0
	60	24	3	38.0	159.0	51,200	26.8	202.6	0	0
	60	48	2	42.4	113.3	58,200	32.6	116.2	0	0
	80	24	3	39.7	134.8	27,300	29.0	210.1	0	0
	80	48	3	36.3	110.1	46,200	29.7	165.5	0	0
Sulfuryl fluoride	30	24	2	27.7	70.3	20,500	26.4	109.0	2,183	10.6
	60	24	2	20.1	94.5	22,700	14.2	48.0	1,906	8.4
	60	48	2	20.1	94.5	22,700	14.2	47.7	426	1.9
MITC[3]	20	24	2	33.2	76.1	42,300	23.3	121.0	2.5	0.006
	20	48	2	46.1	124.8	25,900	39.6	175.3	0	0
	40	24	4	31.4	167.2	39,600	26.1	210.9	0	0
	40	48	4	32.3	177.0	57,300	28.6	273.0	0	0
Control	0	48	5	31.1	175.3	38,600	23.0	216.2	39,600	102.6

[1] Size of lumber; 15cm thick × 15cm wide × 30cm long.
[2] Average moisture content in test wooden lumber.
[3] Weight of wooden samples used for the detection of nematodes.
[4] 30% methyl isothiocyanate in CO_2 was used for fumigation.

Table 3-2. Mortality data for the pine wood nematode infecting red pine lumber 15cm square[1] fumigated with methyl bromide, sulfuryl fluoride and methyl isothiocyanate at 15℃ with 25% (v/v) loading (Mortality was assessed on samples in 20-21 days after fumigation).

Fumigant	Dose (g/m^3)	Exposure (time)	No. of lumber	Before fumigation			After fumigation			
				Moisture content[2] (%)	Weight of lumber[3] (g)	No. of nematode (per 100g)	Moisture content[2] (%)	Weight of lumber[3] (g)	No. of nematode (per 100g)	Survivor (%)
Methyl bromide	20	24	1	57.2	63.0	18,400	27.4	40.9	1,584	8.6
	40	24	1	30.5	55.4	79,400	13.7	58.3	142.4	0.18
	60	24	1	30.5	55.4	79,400	13.7	38.5	0	0
Sulfuryl fluoride	30	24	2	27.7	70.3	20,500	21.7	92.3	3,819	18.6
	60	24	2	20.1	94.5	22,700	11.7	63.7	453.7	2.0
	60	48	2	20.1	94.5	22,700	11.7	64.2	77.8	0.34
MITC[3]	20	24	2	33.2	76.1	42,300	14.2	86.0	0	0
	20	48	2	46.1	124.8	25,900	25.7	68.9	0	0
Control	0	48	3	33.4	105.3	35,500	12.2	68.1	53,100	149.6

[1] Size of lumber; 15cm thick × 15cm wide × 30cm long.
[2] Average moisture content in test wooden lumber.
[3] Weight of wooden samples used for the detection of nematodes.
[4] 30% methyl isothiocyanate in CO_2 was used for fumigation.

same as Table 3-1 (at 20g and 40g/m^3 for 24 hours in MB and 30g and 60g/m^3 for 24 hours and 60g/m^3 for 48 hours in SF). The results would also indicate that reduction of moisture content of wooden materials from 33.2% to 16.9% during storage after fumigation have no effect or little effect on the mortality of the pine wood nematode. Residual gas effect against the nematode, however, was confirmed in MITC fumigation because no survivor was observed on the sample evaluated in 20-21 days after fumigation. Reduction of moisture content of wooden materials from 33.2% to 16.9% during storage after fumigation have no or little effect on the mortality of the pine wood nematode.

Fumigation Schedules for Attaining Complete Mortality

From results of the susceptibility tests, the following MB and MITC fumigation schedules would be provided for complete mortality of the pine wood nematode in lumber (15cm thick × 15cm wide × 30cm long in size). SF would not be used alone for controlling of the pine wood nematode because of lower efficacy.

Warehouse fumigation

MB fumugation

a) at 60g/m^3 for 24 hours at 15℃ or above with 25% or below loading

b) at 40g/m^3 for 48 hours at 15℃ or above with 25% or below loading

MITC fumugation

a) at 40g/m^3 for 24 hours at 15℃ or above with 25% or below loading

b) at 20g/m^3 for 48 hours at 15℃ or above with 25% or below loading

These schedules could provide for sufficient guarantee quarantine security for fumigation in commercial warehouses maintained with highly air tightness, while the confirmation of complete mortality would be needed in a practical tent fumigation because of higher loading of wooden materials and lower air tightness.

References

MAMIYA, Y. (1975) The life history of the pine wood nematode, *Bursaphelenchus lignicolus*. Jap. J. Nematol. **5**: 16-25 (in Japanese).

SOMA, Y., S. YABUTA, M. MIZOBUCHI, H. KISHINO, I. MATSUOKA, M. GOTO, T. AKAWAGA, T. IKEDA and F. KAWAKAMI (1996) Susceptibility of Forest Insect Pests to Sulfuryl Fluoride. 1. WOOD BORERS and BARK BEETLES. *Res. Bull. Pl. Prot. Japan* **32**: 69-76.

SOMA, Y., H. NAITO, T. MISUMI and F. KAWAKAMI (1999) Effects of Gas Mixtures of Sulfuryl Fluoride and Methyl Bromide on Forest Insect Pests. *Res. Bull. Pl. Prot. Japan* **35**: 15-19 (in Japanese).

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

1. 臭化メチル，フッ化スルフリル及びメチルイソチオシアネートに対する感受性

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マツノザイセンチュウが寄生した赤松材を板材（厚さ2cm×幅15cm×長さ30cm）及び角材（厚さ15cm×幅15cm×長さ30cm）に製材し，3種類のくん蒸剤を用い15℃，収容率25%の条件でくん蒸した。くん蒸後は，供試材から木片を採取し，ベルマン法により殺虫効果を調査した。その結果，板材及び角材とも臭化メチルくん蒸では，60,80 g/m³，24時間及び40,60,80g/m³，48時間，メチルイソチオシアネートくん蒸では40g/m³，24時間及び20,40g/m³，48時間で生存虫は認められなかった。しかし，フッ化スルフリルくん蒸では，60 g/m³，24時間及び48時間のいずれのくん蒸でも生存虫が認められた。これらの結果から，臭化メチル及びメチルイソチオシアネートくん蒸は，次の条件により梱包材に寄生したマツノザイセンチュウの完全殺虫が可能であると考えるが，フッ化スルフリルくん蒸では殺虫効果が低く，実用くん蒸では使用が困難であると考える。

臭化メチル倉庫くん蒸
1．薬量60g/m³以上，15℃以上，収容率25%以下，24時間
2．薬量40g/m³以上，15℃以上，収容率25%以下，48時間

メチルイソチオシアネート倉庫くん蒸
1．薬量40g/m³以上，15℃以上，収容率25%以下，24時間
2．薬量20g/m³以上，15℃以上，収容率25%以下，48時間