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

5. Mortality of Pine Wood Nematode and Fumigation Standards by Methyl Iodide

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Abstract: Mortality tests were conducted in a large-scale applied test with methyl iodide (MI) to confirm complete mortality and 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) infected with the pine wood nematode were fumigated at different doses of MI at different temperatures for 24 hours with 25 and 50% loading. No surviving nematode was observed on samples fumigated at 60 g/㎥ at 10℃, 40g/㎥ at 15 and 20℃ and at 30g/㎥ at 25℃, respectively. The CT product for complete mortality was estimated to be 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. Applied doses of MI were required for warehouse and tarpaulin fumigation, that is, at each of 84g/㎥ at 10℃, 60g/㎥ at 15℃, 48 g/㎥ at 20℃ and 36g/㎥ at 25℃ in warehouse fumigation and 112g/㎥ at 10 ℃, 80g/㎥ at 15℃, 64g/㎥ at 20℃ and 48g/㎥ at 25℃ in tarpaulin fumigation, respectively under conditions of 24-hour fumigation with 50% loading of wood packing materials.

Key words: quarantine treatment, fumigation, methyl iodide, *Bursaphelenchus xylophilus*, CT product, fumigation standards, wood packing material

Introduction

The Chinese plant quarantine authority has accepted hot air treatment as the only control measure against the pine wood nematode, *Bursaphelenchus xylophilus* (STEINER and BUHRER) NICKLEI, which has potential to infect conifer wood packing materials 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) and KAWAKAMI *et al.* (2004) proposed methyl bromide fumigation schedules based on mortality testing against the pine wood nematode infecting red pine boards and lumber for packing materials. Methyl bromide, however, as an ozone depleting substance may be limited for the use in the future, although use for quarantine treatment has been excepted from restriction of the Montreal Protocol.

Here we report the result of the mortality tests against the pine wood nematode by fumigation with methyl iodide (MI) which is one of halogen compounds as an alternative to methyl bromide.

Materials and Methods

Wood Materials infected with Pine Wood Nematode

Red pine, *Pinus densiflora* naturally infected with the pine wood nematode were collected in Ibaraki Prefecture in April 2002 and 2003. The red pine was sawed into lumber (15 cm thick × 15 cm wide × 30 cm and 40 cm long in size) and into boards (3 cm thick × 15 cm wide × 45 cm long in size). The red pine lumber with more than 10,000 nematodes per 100g of a sample with the 3rd stage larvae was used for the mortality test. The pine lumber and board were also used for gas absorption test.

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Fumigation

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

Several doses of MI liquid (purity 99% or more) collected by a gas-tight syringe were placed in a petri dish in the fumigation box. Fumigation tests were conducted at 10, 15, 20 and 25℃ for 24 hours with 25 and 50% (v/v) loading. Air-fumigant mixture was exhausted for one hour after fumigation. Fumigated wood materials were placed in netted bags and stored at ambient temperature until detection of the nematode by the Bermann funnel method.

Measurements of Gas Concentration, Temperature and Moisture Content

Gas concentrations during fumigation were monitored with gas chromatography (FID, Shimazu) and temperatures were also monitored with an automatic temperature recorder (Hybrid recorder AH, Chino). The wood pieces of lumber and boards were used to measure moisture content, which was determined by the difference in weight of a sample before and after drying at 120℃ for 24 hours. CT product (mg・h/ℓ) was calculated by the following formula; CT product $= C_1 + 2C_2 + 2C_4 + 10C_6 + 9C_{24}$ (where, Cx is gas concentration after X hours of fumigation).

Evaluation of Mortality

The number of the nematodes was confirmed on samples before the fumigation mentioned above and on those for evaluation of mortality in 6-7 days after fumigation. Wood pieces from a few places of fumigated or untreated control lumber were collected by sawing and then they were cut to size of 3 mm×3 mm×5 mm with scissors. A sample of 30-40g per place was detected by the Bermann funnel method for 48 hours at room temperature, and then the number of surviving nematodes was counted under a microscopes.

Results and Discussion

Gas Concentrations during Fumigation in Mortality Tests

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

Fig. 1 also shows progressive gas concentrations for attaining complete mortality for the pine

Table 1. Residual gas concentrations, ratios of residual gas and CT products for the red pine infected with the pine wood nematode fumigated with methyl iodide at 24 hours with 25% (v/v) loading.

Temperature (℃)	Dose (g/㎥)	Replicate	Residual gas concentration (mg/ℓ ± S.D.)	Ratio of residual gas * (%) 1 hr	Ratio of residual gas * (%) 24hrs	CT product (mg・h/ℓ ± S.D.)
10	40	3	9.9 ± 3.2	60.5	24.8	314 ± 84
	50	3	12.6 ± 2.8	61.2	25.2	391 ± 79
	60	3	14.4 ± 0.3	59.0	24.0	451 ± 19
15	20	2	7.0 ± 1.0	68.0	35.0	206 −
	30	3	11.5 ± 1.3	68.0	38.3	349 ± 3
	40	3	14.9 ± 1.0	73.3	37.3	476 ± 19
	50	1	16.0 −	63.0	32.0	490 −
20	30	3	9.6 ± 2.5	63.7	32.0	276 ± 72
	40	3	12.9 ± 3.1	66.0	32.3	379 ± 88
25	10	2	4.5 ± 0.2	77.0	45.0	129 −
	20	4	8.6 ± 2.2	77.5	43.0	245 ± 59
	30	3	11.3 ± 2.5	69.7	37.7	310 ± 67

* 100×gas concentration in 1 or 24hrs fumigation (mg/ℓ) /applied dose (g/㎥).

wood nematode by different temperatures and doses. The remaining gas ratios (gas concentration at the end of fumigation /applied dose ×100) ranged from 59.0-77.5% for one-hour fumigation , and from 24-45% for 24-hour fumigation. A lower remaining gas ratio was confirmed at low fumigation temperatures. The remaining gas concentrations at the end of 24-hour fumigation were ranged from 12 ～26%, lower compared with those for methyl bromide fumigation (Soma et al., 2003).

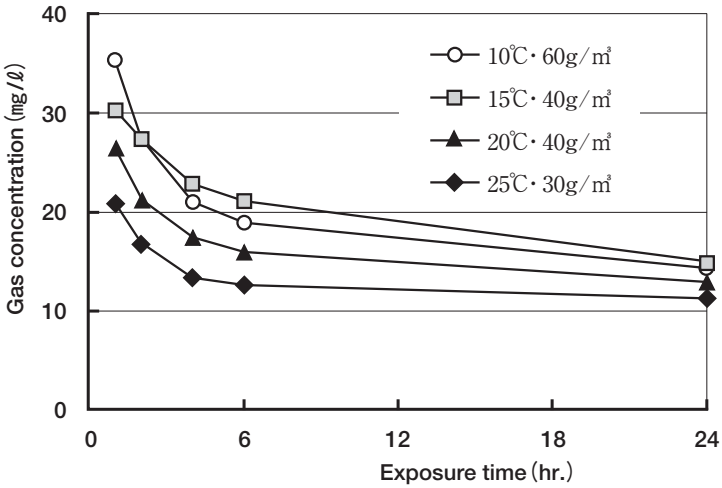

Fig. 1. Progressive gas concentrations for attaining complete mortality of pine wood nematode in methyl iodido fumigation with 25% loading of pine wood packing materials.

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

Temperature (℃)	Dose (g/m³)	Replicate	Before fumigation			After fumigation			
			Moisture content [2] (%)	Weight of board [3] (g)	No of nematode /100g	Moisture content [2] (%)	Weight of board [3] (g)	No of nematode /100g	Survivor (%)
10	40	3	33.9	256.1	22,400	15.9	383.7	12.5	0.06
	50	3	33.9	256.1	22,400	15.9	464.5	3.9	0.02
	60	3	15.9	162.8	10,800	14.9	541.5	0	0
	cont.	3	24.9	290.9	16,600	13.0	133.0	10,700	64.5
15	20	2	28.1	73.6	22,400	20.4	328.0	0.30	0.001
	30	3	32.3	224.6	19,200	19.4	460.8	1.52	0.008
	40	3	32.3	224.6	19,200	19.7	487.6	0	0
	50	1	24.9	36.7	14,300	20.2	157.2	0	0
	cont.	2	32.3	224.6	19,200	18.7	93.2	19,200	100.0
20	30	3	33.9	256.1	22,400	14.7	454.5	1.76	0.008
	40	3	33.9	256.1	22,400	15.3	448.7	0	0
	cont.	3	33.9	256.1	22,400	15.9	162.8	10,800	48.2
25	10	2	38.8	73.9	23,300	24.7	278.9	9.3	0.04
	20	4	33.5	150.4	15,700	17.3	610.7	139.5	0.89
	30	3	29.9	123.5	21,100	21.5	510.8	0	0
	cont.	3	31.1	160.2	21,500	14.7	133.8	31,000	144.2

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

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. Complete mortality was attained at each of 60g/㎥ at 10℃, 40g/㎥ and 50g/㎥ at 15℃, 40g/㎥ at 20℃ and 30g/㎥ at 25℃, respectively, although some survivors were confirmed at each of 50g/㎥ at 10℃, 30g/㎥ at 15℃, 30g/㎥ at 20℃ and 20g/㎥ at 25℃, respectively. These results showed that minimum doses for complete mortality were at each of 60g/㎥ at 10℃, 40g/㎥ at 15 and 20℃ and 30g/㎥ at 25 ℃, respectively.

Complete mortality was attained at the same dose of 40g/㎥ both at 15 and 20℃. The CT product from fumigation at 15℃ was higher compared with that at 20℃ (Table 1 and Fig. 1) because of the lower absorption rate of MI to wood materials at 15℃.

Relationship between Mortality and CT products

Fig. 2 shows the relationship between mortality of the pine wood nematode and the CT product in fumigation at different doses for 24 hours with 25% loading. Although CT products differed from applied doses and the absorption rate of MI to wood materials, complete mortality of the pine wood nematode was attained under certain CT products in all off the tests. The CT product showed a tendency to be high at low temperatures. The estimated CT products for complete mortality of the pine wood nematode 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 (Table 1 and Table 2).

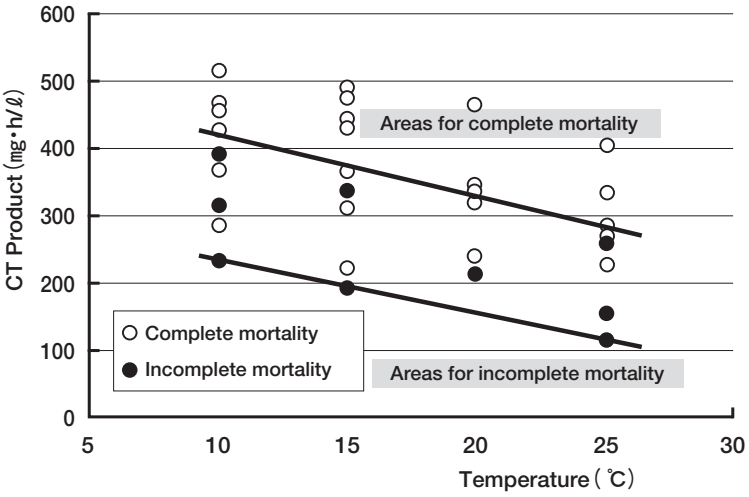

Fig. 2. Relationship between mortality efficacy and CT products for pine wood nematode infecting pine wood packing materials fumigated with methyl iodide for 24 hours with 25% loading.

Ratios of Residual Gas and CT Products in Gas Absorption Tests

Fig. 3 and Table 3 show progressive gas concentrations, ratios of residual gas at the end of fumigation and CT products, when the lumber and the boards were fumigated at 50g/㎥ with 50% loading at different temperatures. Lower gas concentrations were maintained till the end of fumigation at low fumigation temperatures. Ratios of residual gas were reduced in the range of 4-15% because of low moisture contents of 20.9-22.3% and a high load factor of 50% (v/v). Therefore, a higher dose than that in the mortality test would be required for fumigation with a low moisture content and with a high load factor of wood materials.

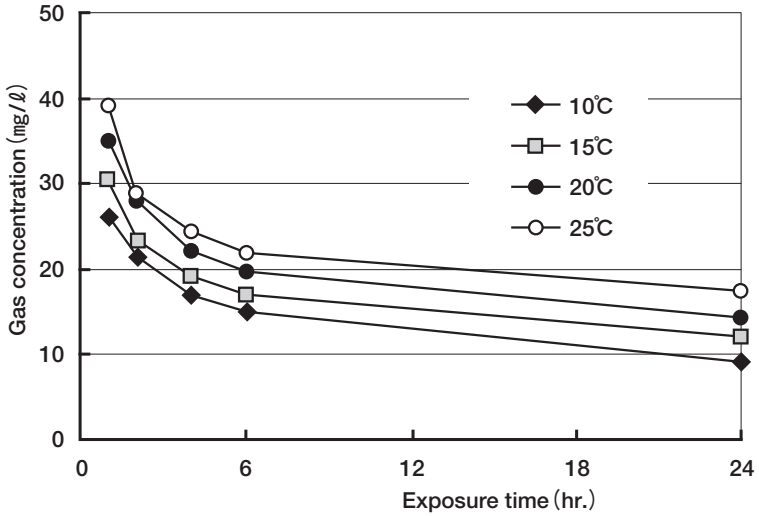

Fig. 3. Progressive gas concentrations in methyl iodide fumigation at 50g/㎥ at different temperatures with 50% loading of pine wood lumber and board.

Table 3. Ratios of residual gas and CT products for the red pine wood materials (lumber [1] and board [2]) fumigated with methyl iodide at 50g/㎥ for 24 hours with 50% loading.

Temperature (℃)	Moisture content [3] (%)	Ratio of residual gas [4] (%)	CT product (mg・h/ℓ)
10	22.3	18.0	326
15	21.8	23.6	410
20	20.9	27.8	477
25	21.5	34.2	540

[1] 10cm thick×10cm wide×40cm long.
[2] 2cm thick×10cm wide×45cm long.
[3] Average moisture content in test wood materials.
[4] 100×gas concentration in 24hr fumigation (mg/ℓ) /applied dose (g/㎥).

MI Fumigation Standards for Wood Packing Materials

Table 4 shows MI fumigation standard for wood materials infected with the pine wood nematode. Applied doses were used to estimate minimum CT products in Fig. 2 (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℃ for complete mortality of the pine wood nematode fumigated for 24-hour fumigation with 25% loading) and CT products obtained from the test in Table 3 (326 mg・h/ℓ at 10℃, 410 mg・h/ℓ at 15℃, 477 mg・h/ℓ at 20℃ and 540g・h/ℓ at 25℃ fumigated for 24-hour fumigation with a low moisture content and a higher load factor of 50% of lumber and boards).

Table 4. Methyl iodide fumigation standards for wood packing materials infected with the pine wood nematode.

Temperature (℃)	Time (hr)	Load factor (%)	Dose*		Minimum gas concentration			Minimum CT product (mg・h/ℓ)
			Warehouse (g/㎥)	Tarpaulin (g/㎥)	1 hr (mg/ℓ)	4 hr (mg/ℓ)	24 hrs (mg/ℓ)	
10-14.9	24	50	84	112	48	29	16	450
15-19.9	24	50	60	80	40	26	14	400
20-24.9	24	50	48	64	34	23	12	350
25 or above	24	50	36	48	28	20	10	300

* Gas circulation fan is operated more than 1 hour after dosing.

The applied doses for warehouse fumigation were supplemented with another 20% of the dose (dose equivalent in the amount of absorption to wood materials) and also another 60% of the dose (dose equivalent in the amount of gas leakage) was added to the above mentioned doses for the tarpaulin fumigation. This is the same calculation method as the methyl bromide fumigation standard for the pine wood nematode (Soma et al., 2003). The Calculation method for applied doses at 10℃ is as follows;

CT product obtained from absorption test with 50g/㎥ at 10℃: 326 mg・h/ℓ
CT product for a complete mortality at 10℃ (Fig. 2) : 450 mg・h/ℓ
Dose required for complete mortality at 10℃ : 50 g/㎥×450/326 = 69.0 g/㎥
Applied dose in the warehouse fumigation: 69.0×1.2 = 82.8 g/㎥
Applied dose in the tarpaulin fumigation: 69.0×1.6 = 110.4 g/㎥

The following doses of MI under conditions of 24-hour fumigation with 50% loading of wood packing materials were determined for the warehouse and tarpaulin fumigation, namely, at each of 84g/㎥ (warehouse) and 112g/㎥ (tarpaulin) at 10 ℃, 60g/㎥ (warehouse) and 80g/㎥ (tarpaulin) at 15℃, 48 g/㎥ (warehouse) and 64g/㎥ (tarpaulin) at 20℃ and 36g/㎥ (warehouse) and 48g/㎥ (tarpaulin) at 25℃, respectively as shown in Table 4.

Minimum gas concentrations during fumigation were estimated by both data from progressive gas concentrations in the test for a complete mortality by different temperatures (Fig. 1) and from the CT product required for complete mortality. In particular, the minimum gas concentration at the end of 24-hour fumigation was indispensable concentrations for attaining adequate efficacy of the mortality for the pine wood nematode. The minimum gas concentration in 1 and 4 hours after dosing are indicators for the accurate fumigation. If the gas concentrations in 1 and 4 hours are lower than the minimum gas concentration, the fumigation would fail because of a shortage of gas concentration at the end of fumigation. In practical fumigation, a gas-tight fumigation chamber should be used for fumigation and care should be taken against gas leakage from tarpaulin. If minimum gas concentrations in 1 and 4 hours were insufficient suitable action with use of an additional dose or extension of the fumigation time should be taken for fumigation.

References Cited

KAWAKAMI, F., Y.SOMA, H.KOMATSU and Y.MATSUMOTO (2004) Effects of Some Fumigants on Pine Wood Nematode, *Bursaphelenchus xylophilus* Infecting Wooden Packages. 4. Mortality and CT product in Methyl Bromide Fumigation with High Loading of Wood Packing Materials. *Res.Bull.Pl.Prot.Japan* 40:7-12.

SOMA, Y., H. NAITO, T. MISUMI, M. MIZOBUCHI, Y. TSUCHIYA, I.MATSUOKA, F.KAWAKAMI and K.HIRATA (2001) 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. *Res.Bull.Pl.Prot.Japan* 37:19-26.

SOMA, Y., H. NAITO, T. MISUMI, M. MIZOBUCHI, Y. TSUCHIYA, I. MATSUOKA, F.KAWAKAMI and K.HIRATA (2002) Effects of Some Fumigants on Pine Wood Nematode, *Bursaphelenchus xylophilus* Infecting Wooden Packages 2. Mortality of Pine Wood Nematode to Methyl Bromide Tent Fumigation. *Res.Bull.Pl.Prot.Japan* 38:13-19.

SOMA, Y., M.GOTO, N.OGAWA, F.KAWAKAMI, K.HIRATA, H.KOMATSU and Y.MATSUMOTO (2003) Effects of Some Fumigants on Pine Wood Nematode, *Bursaphelenchus xylophilus* Infecting Wooden Packages 3. Mortality of Fumigation Standards for Pine Wood Nematode to Methyl Bromide. *Res.Bull.Pl.Prot.Japan* 39:7-14.

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

5．ヨウ化メチルによる温度別殺線虫効果とくん蒸基準の検討

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マツノザイセンチュウが100g当たり１万頭以上寄生したアカマツの梱包材（角材：15cm×15cm×30cm）を用い、収容率25%、24時間の条件で薬量別、温度別にヨウ化メチルくん蒸を行い、殺虫効果とＣＴ値を調査した。その結果、10℃では60g/㎥、15℃及び20℃では40g/㎥、25℃では30g/㎥の薬量でマツノザイセンチュウは完全殺虫された。試験で得られたＣＴ値（mg・h/ℓ）により、完全殺虫に必要な最低ＣＴ値を温度別に推定した結果、10℃では450mg・h/ℓ、15℃では400mg・h/ℓ、20℃では350mg・h/ℓ、25℃では300mg・h/ℓであった。また、乾燥した梱包材を収容率50%で24時間くん蒸したときのＣＴ値に基づき、倉庫及び天幕くん蒸の必要薬量を温度別に計算した結果、10℃が84g/㎥（倉庫）及び112g/㎥（天幕）、15℃が60g/㎥（倉庫）及び80g/㎥（天幕）、20℃が48g/㎥（倉庫）及び64g/㎥（天幕）、25℃が36g/㎥（倉庫）及び48g/㎥（天幕）であった。