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

2. Mortality of Pine Wood Nematode by Methyl Bromide Tent Fumigation

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Abstract: Large-scale applied tests under tent fumigation with methyl bromide (MB) were conducted to confirm a complete mortality of pine wood nematode, *Bursaphelenchus xylophilus* (STEINER and BUHRER) NICKLEI infecting red pine lumber (15–20 cm thick×15–20 cm wide×40 cm long in size). No survivor of the nematode was observed on the samples fumigated at 48, 60 and 72 g/m^3 for 24 hours at 15℃ with 51.3% (v/v) loading, respectively. The standard of MB tent fumigation at 48 g/m^3 for 24 hours at 15℃ with 51.3% loading in the large-scale fumigation, which was 22.3 mg/*l* of the residual gas concentration and 764 mg·h/*l* of the CT product, could provide for a complete mortality of the pine wood nematode infecting wooden lumber.

Key words: fumigation, methyl bromide, polyethylene tent, *Bursaphelenchus xylophilus*, wooden packages, quarantine treatment

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) reported that very small survivors of the pine wood nematode were confirmed at 40 g/m^3 of methyl bromide (MB) for 24 hours at 15℃ with 25% loading when fumigated the pine wood nematode infecting red pine board and lumber in the small-scale test, while a complete mortality was confirmed at 60 g/m^3 for 24 hours at 15℃ with 25% loading.

Photo 1. Pine wood nematode detected from the sample of red pine wooden packages.

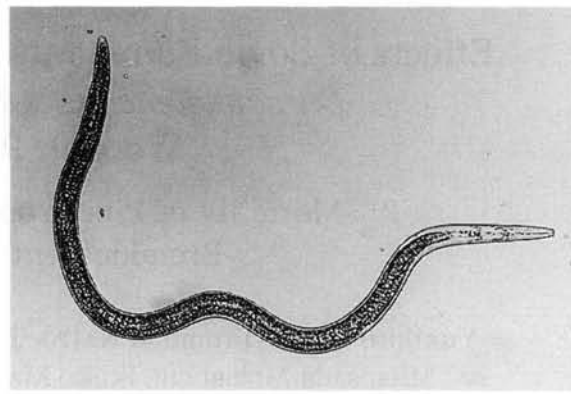

Photo 2. Pine wood nematode (dispersal 3rd stage larvae).

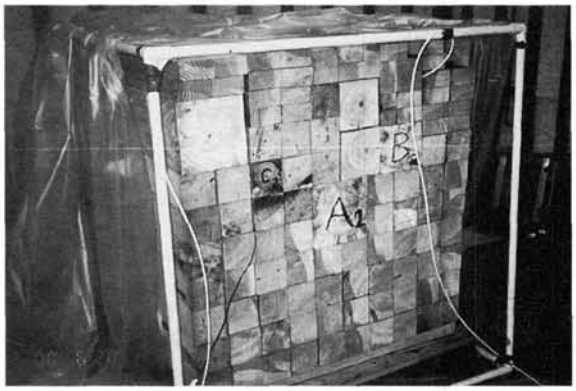

Photo 3. Test wooden bundle with pine wood nematode infecting red pine lumber and a gas dosing pipe, a gas sampling pipe and a temperature probe.

Photo 4. Polyethylene tent fumigation of the wooden bundle.

Here we report the results of infecting the large-scale tent fumigation with three fumigation schedules of MB conducted to confirm a complete mortality for the pine wood nematode infecting red pine lumber.

Materials and Methods

Test wooden materials infected with pine wood nematode

Red pine, *Pinus densiflora* naturally infecting with the pine wood nematode were collected in Ibaraki Prefectures in June and July 2002. The red pine was sawn into lumber (15–20 cm thick×15–20 cm wide×40 cm long in size). More than 10,000 nematodes per 100 g of the sample with more than 90% of the dispersal 3rd stage larvae (photos 1 and 2) were confirmed in the test lumber when detected the nematode by Bermann funnel method before fumigation.

Two to four of infecting lumbers were placed in the center of the bundle which was tied up by plastic band with 98–96 filler lumbers (10 cm thick×10 cm wide×100 cm long

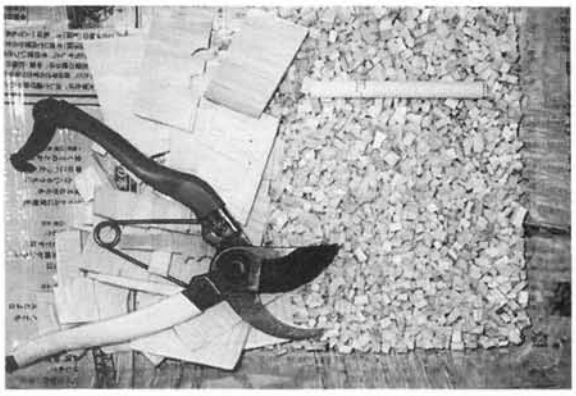

Photo 5. Wooden pieces (3 mm×3 mm×5mm) for detection of pine wood nematode.

Photo 6. Detection of pine wood nematode by Bermann funnel method.

per lumber in size) and was 1 m^3 in capacity (100 cm thick×100 cm wide×100 cm long in size).

Fumigation and measurement of gas concentration and temperature

A gas dosing teflon pipe was fixed to the top layer of the bundle. A temperature probe and a gas sampling teflon pipe were also fixed between lumbers of the bundle (photo 3). The plastic pipe frame (125 cm ×125 cm ×125 cm in size, 1.95 m^3 in capacity) was placed over the test bundle and covered the frame with polyethylene sheet of 0.015 mm in thickness. Pressing of the tent hems were done by laying sand bags to protect gas leakage (photo 4). The load factor of the test bundle was calculated a 51.3% (1.0 m^3/1.95 m^3×100).

MB (purity; 99% or more) doses of 48, 60 and 72 g/m^3 were collected in a graduated dispenser from a cylinder and then introduced into the tent by connecting dosing pipe fixed to the top layer of the bundle. Fumigation was conducted for 24 hours at 15℃ with 51.3% (v/v) loading. Gas concentrations during fumigation were monitored periodically with an automatic infrared gas measurement device (Komyo rikagaku kogyo) and temperatures were also monitored with an 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 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 (photo 5). The samples of 10–20 g per place were detected by Bermann funnel method for 24 hours at the room temperature (photo 6) and then the number of survival nematodes were counted under microscopes.

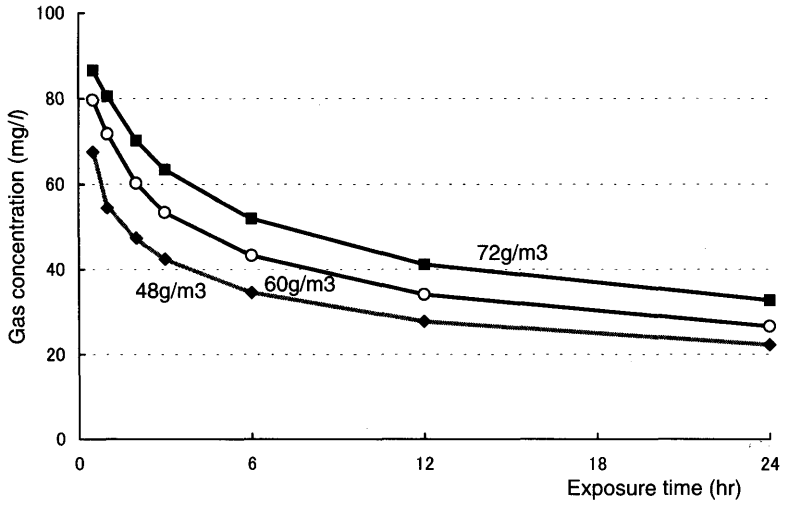

Fig. 1. Progressive gas concentrations during fumigation for wooden packages at 48, 60 and 72 g/m^3 of methyl bromide for 24 hours at 15℃ with 51.3% (v/v) loading in the polyethylene tent fumigation.

Table 1. Residual gas concentrations and CT products for red pine wood 15–20 cm square[1] infecting with the pine wood nematode fumigated with methyl bromide at 48, 60 and 72 g/m^3 for 2 hours at 15℃ with 51.3% loading[2].

Dose (g/m^3)	Replicate	Temperature (℃)	Residual gas concentration		CT product	
			mg/*l*	Mean	mg·h/*l*	Mean
48	1	14.9–15.1	24.5	22.3	776	764
	2	14.5–14.9	20.1		751	
60	1	15.7–14.6	26.4	26.7	944	942
	2	15.9–15.3	26.9		940	
72	1	15.2–14.8	34.0	32.8	1,164	1,124
	2	15.2–15.3	31.5		1,083	

[1] Size of lumber: 15–20 cm thick×15–20 cm wide×40 cm long.
[2] Loading: capacity of wooden bundle (1 m^3)/capacity of polyethylene sheet (1.95 m^3).

Results and Discussion

Gas concentrations and CT products

Figure 1 shows means MB gas concentrations in two replicated fumigation with doses of 48, 60 and 72 g/m^3 for 24 hours at 15℃ with 51.3% (v/v) loading. The gas decline curve in each dose showed almost the same gas reduction pattern in MB fumigation of wooden package, that is, higher gas concentrations than those from applied doses at just after dosing and high rate of MB reduction in 1 to 6 hours after dosing, and gas reduction less than 50% of applied doses in 12 hours after dosing. This is because of extremely high load factor of wooden packages.

Table 2. Mortality data for the pine wood nematode infecting red pine lumber 15–20 cm square[1] fumigated with methyl bromide at 48, 60 and 72 g/m^3 for 24 hours at 15℃ with 51.3% loading.

Dose (g/m^3)	Replicate	Before fumigation				6–7 days after fumigation			
		No. of lumber	Moisture content[2] (%)	Weight of lumber (g)	No. of nematode[3]	Moisture content[2] (%)	Weight of lumber (g)	No. of nematode[3]	Survivor (%)
48	1	4	24.8	76.8	23,100	13.2	357.3	0	0
48	2	4	24.8	76.8	23,100	14.3	379.8	0	0
cont.	–	4	24.8	76.8	23,100	13.2	110.6	22,300	96.5
60	1	3	30.0	174.3	14,900	20.4	443.7	0	0
72	1	3	30.0	174.3	14,900	23.3	431.4	0	0
cont.	–	3	30.0	174.3	14,900	20.9	274.3	18,100	121.5
60	2	3	17.7	97.6	17,800	14.6	204.6	0	0
72	2	3	17.7	97.6	17,800	14.6	206.4	0	0
cont.	–	3	17.7	97.6	17,800	17.0	144.0	15,600	87.6

[1] Size of lumber: 15–20 cm thick×15–20 cm wide×40 cm long.
[2] Average moisture content in test wooden lumber.
[3] A 100 g of wooden sample was used for the detection of nematode.

Table 1 shows residual gas concentrations (mg/*l*) and CT products (gas concentration, mg/*l*×fumigation time, hour) at three doses in the large-scale tent fumigation. A 22.3 mg/*l* of the residual gas concentration and 764 mg·h/*l* of the CT product were confirmed under conditions at 48 g/m^3 for 24 hours at 15℃ with 51.3% loading at the minimum dose used for the test.

Mortality of pine wood nematode

Table 2 shows the mortality of the pine wood nematode fumigated at 15℃ with 51.3% loading and evaluated in 6–7 days after fumigation. No survivor of the nematode was confirmed on the sample fumigated at 48, 60 and 70 g/m^3 for 24 hours, respectively, although very small survivors (99.99% of mortality) were observed on the same size of wooden samples fumigated at 40 g/m^3 for 24 hours at 15℃ with 25% loading (with 23.7 mg/*l* of the residual gas concentration and 631 mg·h/*l* of the CT product) in the gas tight fiber-glass fumigation box (SOMA *et al.*, 2001).

Therefore, the large-scale tent fumigation under the schedule at 48 g/m^3 for 24 hours at 15℃ with 51.3% (v/v) loading (with 22.3 mg/*l* of the residual gas concentration and 764 mg·h/*l* of the CT product) could provide for a complete mortality of the pine wood nematode in lumber (15–20 cm thick×15–20 cm wide×40 cm long in size) and the schedules could provide for sufficient quarantine security in the commercial tent 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

2. 臭化メチル天幕くん蒸における殺線虫効果

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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（厚さ 15～20 cm×幅 15～20 cm×長さ 40 cm）に製材し，ベルマン法により木片 100 g から 10,000 頭以上が検出されたものを供試材として，臭化メチルを用いた天幕くん蒸による殺線虫試験を実施した。供試材は正立方体状（100 cm×100 cm×100 cm：容積 1 m^3）に配置して，厚さ 0.15 mm のポリエチレンシートで覆い，15℃, 24時間，収容率 51.3% (v/v)，臭化メチル 48, 60 及び 72 g/m^3 の条件でくん蒸を実施した。くん蒸後は供試材から木片（1 供試材当たり 3 カ所以上，約 100 g）を採取し，ベルマン法により殺線虫効果を調査した。その結果，いずれの薬量区においても生存線虫は認められなかった。したがって，薬量 48 g/m^3, 15℃ 以上，24 時間の臭化メチル天幕くん蒸で，梱包材に寄生したマツノザイセンチュウは完全殺線虫されるものと考える。