Comparison of Ethanedinitrile (C_2N_2) and Metam Sodium for Control of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchidae) and *Monochamus alternatus* (Coleoptera: Cerambycidae) in Naturally Infested Logs at Low Temperatures

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ABSTRACT The *Bursaphelenchus xylophilus*, commonly known as pinewood nematode in Japan, is a quarantine pest and is most often associated with beetles of the genus *Monochamus*, the pine sawyers, particularly *Monochamus alternatus*. Long-distance dispersal of the nematode and its vectors led to widespread losses in pine forests. Two fumigation trials were conducted for treatment of logs naturally infested with both *M. alternatus* and *B. xylophilus*. The logs were treated with ethanedinitrile or metam sodium at low temperature (−7–25.7°C and −3.7–23.1°C) for 3-d exposure in winter and early spring. Fumigation with ethanedinitrile at concentrations of 48, 68, 97 and 158 g/m^3 resulted in 34.6–58.3, 91.5–97.2, 100, and 100% mortality for *M. alternatus* and 88.4, 77.9, 96.4, and 98.0% mortality for *B. xylophilus*, respectively. With Metam sodium fumigation at a dose rate of 1162 g/m^3, 100% *M. alternatus* and 97.4% *B. xylophilus* were killed. These results suggest that 97 g/m^3 of ethanedinitrile is adequate for complete control of *M. alternatus* in pine wood and >158 g/m^3 is required for eradication of *B. xylophilus* at low temperature fumigation. These results suggest that 97 g/m^3 of ethanedinitrile offers complete control of *M. alternatus* in pine wood and control of >98% *B. xylophilus* in winter or spring fumigation at a dosage rate of 158 g/m^3. Therefore, ethanedinitrile has great potential for treatment of fresh pine wooden logs to manage the nematodes and the vector insects at low temperature.

KEY WORDS nematode, *Monochamus alternatus*, *Bursaphelenchus xylophilus*, fumigant, ethanedinitrile

Infestations of *Bursaphelenchus xylophilus*, known by the common name as the pinewood nematode or pine wilt nematode in Japan, cause widespread losses in pine forests. It has been most serious pest of pine forests in Japan since early 1900s (Mamiya and Enda 1972, Morimoto and Iwasaki 1972, Tomminen 1991, Braasch et al. 2007). Since 1973, the annual loss of pine timber in Japan frequently has exceeded 1 million m^3, with the greatest loss (2.4 million m^3) recorded in 1979 (Bergdahl 1988). The *B. xylophilus* is vectored by a number of bark beetles and wood borers, and is most often associated with beetles of the genus *Monochamus*, the pine sawyers, particularly *Monochamus alternatus.* Long-distance dispersal of the nematode and its vectors is related to the global commerce and transport of untreated round and sawn wood. Nematodes of the *Bursaphelenchus* genus are often detected in untreated wood packaging materials worldwide (Tomminen 1991, Braasch et al. 2007, Li et al. 2009). Because of the presence of susceptible pine hosts and suitable insect vectors, in many countries (e.g., United States, Sweden, and China) the importation of unseasoned wood from areas where the nematode is known to occur has been restricted. The pest was also one of the main reasons for the development and adoption of ISPM 15, an international phytosanitary standard for treatment of wooden packaging materials in trade.

Despite a variety of preventative measures, the nematode continues to be highly damaging. Pinewood nematode has recently become established in Portugal. In Japan, where the disease is much more devastating, insecticide and nematicide treatments, biological control, and induced resistance through use of less virulent strains of *Bursaphelenchus* have been investigated. Control of nematode is challenging for several reasons. Chemical control of the vector, *M. alternatus,* is difficult due to the insect's behavior and because of environmental and health hazards of available measures.

The use of fumigation is often an economic and practical method to control quarantine pests of timber and other durable products in trade (Annis and Waterford 1996). Methyl bromide fumigation is often

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used as a treatment for quarantine (biosecurity) purposes. However, this fumigant is a potent ozone-depletor and, with exemptions, it is being phased out of use under the Montreal Protocol (United Nations Environment Programme [UNEP] 2006). Methyl bromide used for quarantine and preshipment (QPS) purposes is exempted from phaseout, but it is urged that users adopt alternatives where technically and economically feasible.

Currently, sulfuryl fluoride (SF) with commercial brands Vikane, ProFume, and Zythor, is registered in various countries for the control of stored-product insects and wood-destroying pests. Previous studies (Soma et al. 2001, Dwinell et al. 2003, Flack et al. 2008, Luís et al. 2014) have demonstrated the toxicity of SF against *B. xylophilus* on wood of low moisture content at high dosage rates (>100 g/m^3) and temperature (>15°C) and for long-term exposure (>24 h). However, SF eradicates the egg stage of various insects at economic and registered dosages, e.g., *Lyctus brunneus* (Stephens) and the anobiid beetle *Euvrilletta peltate* (Harris) (Outram 1967, Su and Scheffrahn 1990, Williams and Sprenkel 1990). Fumigation with metam sodium as methyl bromide alternative effectively controls internal stage of *M. alternatus* and the pine wilt nematode itself (Kwon 2005). However, fumigation with metam sodium at very high label dosage of 1 liter/m^3 has led to occupational health safety and environmental issues (Hartley and Kidd 1983, Hellwig 1987).

There is an urgent requirement for the development of a fumigant that kills both nematodes and vectors quickly and at lower temperatures. A new alternative chemical ethanedinitrile (C_2N_2) has been investigated as a timber fumigant (O'Brien et al. 1995; Viljoen and Ren 2001; Ren et al. 2005, 2011). Unlike methyl bromide, ethanedinitrile offers fast penetration through timber along and cross grain, fast response to insects and nematodes, and minimum of environmental problems (Pruett et al. 2001, Hooper et al. 2003, Ren et al. 2005). It has been registered in Australia for disinfestation treatment of timber.

This article reports field trials of application of ethanedinitrile for control *M. alternatus* and *B. xylophilus* in naturally infested pine logs at low temperature in Korea. Fumigation with metam sodium was as a comparative treatment.

Materials and Methods

Preparation of Wood Samples. Test samples were prepared from Korean red pine (*Pinus koraiensis* Siebold & Zucc.) naturally infested by both *M. alternatus* and *B. xylophilus.* The wood samples were cut approximately to 80–90 cm in length, with natural variation in diameter. All samples were divided into three lots, based on diameter of logs (lot 1: 4–6.5 cm, lot 2: 6.6–13 cm, and lot 3: >13 cm in diameter). Moisture contents of five randomly sampled pine logs were determined by standard test methods (American Standard Test Methods 1983). The moisture content of the logs was on average 55.5% for the winter trials and 68.2% for the early spring trials. The total number of logs used in the experiment was 648.

Fumigants. Ethanedinitrile (99% purity, balance air) was supplied from BOC Australia. Metam sodium (Kilper, 25% SL) was purchased from Bayer Crop Science in Korea.

Fumigation of Pine Wood Logs. Two field fumigation trials (winter and spring) were conducted at the Geomjeongri Forest (34° 98′ N, 128° 24′ E), Sacheon, Kyungnam Province, Korea. The fumigation was performed in a fumigation chamber (100 by 100 by 100 cm^3) covered with polyethylene (0.1 cm in thickness). Each treated and control chamber were loaded with 30–37 wooden logs (depending on the size of logs), achieving ~50% filling ratio. The calculated dosage ranges of 48, 68, 97, and 158 g/m^3 for ethanedinitrile and 1000 ml/m^3 or 1162 g/m^3 for metam sodium were injected into each fumigation chamber, respectively. Each treatment was duplicated. During the fumigation, the headspace temperature in the fumigation chamber was automatically recorded with a Thermo Recoder (TR-71U). After 3-d fumigation, the chambers were opened and aerated for 1 d.

Measurement of Ethanedinitrile and Metam Sodium. The ethanedinitrile and methylisothiocyanate, which is the active compound of metam sodium, gas samples were drawn with an electric pump at timed interval and stored in Tedlar gas sampling bags (1 liter; SKC Inc., Sydney, Australia) until analysis, usually within 1 h of sampling. The concentration of ethanedinitrile was determined using a Agilent Technology 7890N gas chromatography (GC) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m by 0.32 mm i.d. HP-5 (0.25 μm film thickness)-fused silica capillary column (Restek Co. Ltd.). The GC oven, injector, and detector temperature was 150, 200, and 200°C, respectively. Helium was used as the carrier gas at a rate of 2 ml/min. The peak areas were calibrated periodically using a standard (inject the known volume of ethanedinitrile in 1-liter Tedlar gas sampling bags), and the data presented are the mean of duplicate samples. For calculation of the dosage or volume of ethanedinitrile and metam sodium at the experimental ambient temperature and pressure, the following equation (Ren et al. 2006) was used:

$$V_f = \left(1 + \frac{T}{273}\right)\left(\frac{1.7 \times 10^6 \times C \times V}{P \times M \times N}\right) \qquad [1]$$

where: V, volume of fumigation chamber (liter); P, atmospheric pressure (mmHg); T, temperature (°C); C, intended concentration (mg/liter); V_f, dose volume of fumigant (ml); M, molecular weight of fumigant; N, purity of fumigant (%).

Bioassays of *B. xylophilus* and *M. alternatus* Larvae. The mortality of *B. xylophilus* was determined after fumigation. The fumigated wood samples (10 logs) from each chamber were randomly selected, and wood samples (≈2 cm in thickness) were cut with axis parallel to the grain and cut at least 30 cm from the end of the piece of logs. Nematodes were extracted using

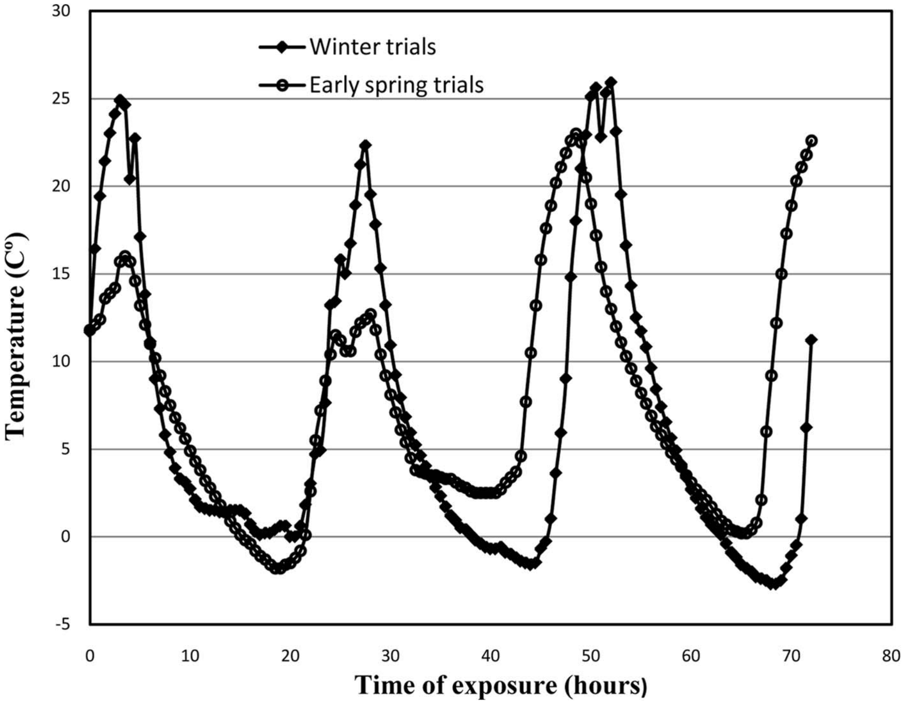

Fig. 1. Temperature of the fumigation chamber during the 3 d of winter (*o*) and 3 d of spring (◆) fumigation. The temperature sensors were placed in the headspace of fumigation chamber, and data were automatically collected with Thermo Recoder (TR-71U).

the modified Baermann funnel procedure (Southey 1985). For *M. alternatus* larvae assay, after each treatment, *M. alternatus* larvae were collected by splitting naturally infested fresh Korean red pine (*Pinus koraiensis*) logs. The larvae were collected and counted, then cultured in incubator for 72 h at 25 ± 2°C. Unfumigated pine logs were used as control for calculation of mortality.

Statistical Analysis. Differences in mortality of *M. alternatus* larvae and *B. xylophilus* in different size and treatment (treated and untreated) samples with different fumigants were subjected to one-way analysis of variance (ANOVA). The variations (standard deviation) of fumigant concentrations and duplicate treatments were analyzed by Microsoft Excel 2007.

Results and Discussions

Fumigation Temperature. During 3-d winter and early spring fumigation trials, the treated and untreated (control) wooden logs were exposed at ambient air temperature, and the temperature varied between −7.0 (night time) to 25.7°C (day time) for the winter trials and −3.7 to 23.1°C for the early spring trials (Fig. 1). The 3-day average temperature was 4.4 and 6.1°C, respectively. The trials were conducted at the worst scenario aimed to control emerging adult from internal stage of *M. alternatus* at low temperature in winter and early spring in Korea.

Sorption of Ethanedinitrle and Metam Sodium on Pine Wooden Logs. Concentrations of ethanedinitrile and methylisothiocyanate in the fumigation chamber were measured at timed intervals during 3-d winter and early fumigation trials. The variations in the fumigant concentrations are shown in Fig. 2. The concentrations of ethanedinitrile and methylisothiocyanate declined rapidly to one third at 6 h of exposure and one eighth for ethanedinitrile and one fifth for methylisothiocyanate at 24 h in comparison with initial applied dose. After 3-d fumigation, 90–95% and 85% applied ethanedinitrile and methylisothiocyanate were absorbed by wooden logs (Fig. 2). During 3-d fumigation, hydrogen cyanide was not found. This indicated that ethanedinitrile was not converted to hydrogen cyanide in high moisture content pine wooden logs at low temperature. That is, ethanedinitrile was mainly absorbed by the logs. This result is consistent with previous trials and laboratory studies where it was demonstrated that ethanedinitrile can significantly penetrate into high moisture content logs and its toxicity to target pest is increased with increasing both relative humidity (RH) and CO_2.(Viljoen and Ren 2001; Ren et al. 2011, 2006).

Toxicity of Ethanedinitrile to *M. alternatus* Larvae. The ethanedinitrile was found highly toxic to *M. alternatus* larvae at very low temperature (average 4.4 and 6.1°C) conditions (Table 1). Ethanedinitrile can completely kill *M. alternatus* larvae at 97 g/m^3. The cumulative Ct products of ethanedinitrile were esti-

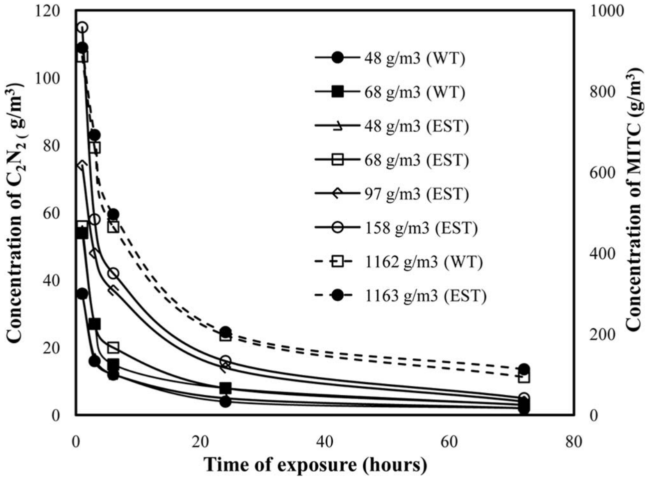

Fig. 2. Concentrations of ethanedinitrile (solid lines) and methylisothiocyanate (break lines) in 1 m^3 PVC tarpaulin fumigation chamber during 3-d winter (WT) and early spring (EST) fumigation trials.

mated in 72-h exposure time. The Ct products were calculated from equation 2. $L(Ct)_{99.0}$ values were between 657 and 1074 g h/m^3 for two trials. Toxicity of ethanedinitrile was not affected by the diameters of treated logs in this experiment ($P > 0.05$; Tables 1 and 2), which means ethanedinitrile penetrates through different tested size pine logs with high moisture content. This result is consistent with O'Brien et al. (1995) and Ren et al. (2011) who reported that ethanedinitrile can significantly penetrate into high moisture content logs.

$$Ct = \Sigma(C_i + C_{i+1})(t_{i+1} - t_i)/2 \qquad [2]$$

where: C is fumigant concentration (g/m^3), t is time of exposure (h), i is the order of measurement, Ct is concentration × time products (g h/m^3).

Barak et al. (2001) systematically evaluated fumigation with methyl bromide at different temperatures and doses against *Anoplophora glabripennis* larvae. The insect larvae showed 100% mortality at 80 g/m^3 methyl bromide and 4.4°C for 24-h exposure. While observing comparative toxicity between methyl bromide and ethanedinitrile against termites, *Reticulitermes speratus*, for 6 h at 21 ± 2°C, ethanedinitrile showed several times higher efficacy than methyl bromide (Ren et al. 2006). Soma et al. (2006) reported that *M. alternatus* and *Arhoalus rusticus* larva on dry wood can be completely killed by methyl iodide (MI) at 84 g/m^3 and 10°C for 24-h fumigation. For fumigation of high moisture content pine logs at low temperature, the dosage of methyl iodide will be significantly increased. Therefore, it is very important that ethanedinitrile offered higher efficacy to control *M. alternatus* in green timber logs and at low temperature.

Toxicity of Ethanedinitrile to *B. xylophilus*. Toxicity of ethanedinitrile was not effected with diameters of treated logs in this experiments ($P > 0.05$), which means ethanedinitrile is able to penetrate through high moisture content different tested size pine logs. This result is consistent with Ren et al. (2006 and 2011), who reported that ethanedinitrile can significantly penetrate into high moisture content logs

Table 1. **Efficacy of ethanedinitrile (C_2N_2) and metam sodium (MS) to *M. alternatus* larvae (winter trials)**

Treatments	Dose (g/m^3)	Log index	Logs no.[a]	No. *M. alternatus*[b]			Mortality (%)	Avg. mortality ± SD (%)
				Total	Alive	Dead		
C_2N_2	48	1	16	19	13	6	31.6	58.3 ± 23.4
		2	16	19	6	13	68.4	
		3	4	4	1	3	75.0	
C_2N_2	68	1	9	16	1	15	93.8	91.5 ± 3.4
		2	20	32	4	28	87.5	
		3	6	15	1	14	93.3	
MS	1162	1	20	23	0	23	100.0	100 ± 0.0
		2	16	23	0	23	100.0	
		3	4	3	0	3	100.0	
Untreated	0	1	10	10	10	0	0.00	0 ± 0.0
		2	15	4	4	0	0.00	
		3	5	5	5	0	0.00	

[a] 1: 4–6.5 cm, 2: 6.6–13 cm, and 3: >13 cm diameter.

[b] Total, alive, and dead insects in different sizes (diameter) of treated and untreated logs do not differ significantly ($P > 0.05$) in ANOVA test.

Table 2. Efficacy of ethanedinitrile to *M. alternatus* larvae (early spring trials)

Treatments	Dose (g/m^3)	Log index[a]	Logs no.	No. *M. alternatus*[b]			Mortality (%)	Avg mortality ± SD (%)
				Total	Alive	Dead		
C_2N_2	48	1	17	20	11	9	45.0	34.6 ± 15.6
		2	13	26	15	11	42.3	
		3	7	6	5	1	16.7	
C_2N_2	68	1	10	18	0	18	100.0	97.2 ± 4.8
		2	24	24	2	22	91.7	
		3	4	3	0	3	100.0	
C_2N_2	97	1	4	4	0	4	100.0	100 ± 0.0
		2	24	48	0	48	100.0	
		3	3	5	0	5	100.0	
C_2N_2	158	1	11	16	0	16	100.0	100 ± 0.0
		2	18	31	0	31	100.0	
		3	4	3	0	3	100.0	
Untreated	0	1	22	17	17	0	0	0 ± 0.0
		2	18	21	21	0	0	
		3	4	1	1	0	0	

[a] 1: 4–6.5 cm, 2: 6.6–13 cm, and 3: >13 cm diameter.

[b] Total, alive, and dead insects in different sizes (diameter) of treated and untreated logs do not differ significantly ($P > 0.05$) in ANOVA test.

with and cross grain. Table 3 showed that ethanedinitrile was highly effective (>95%) to *B. xylophilus* at 97 g/m^3. There are no statistically significant differences of mortality ($P > 0.05$) at applied dose of 48 and 68 g/m^3. The highest dose of ethanedinitrile at 158 g/m^3 in the trials showed the highest nematocidal activity (97.43% kill), but did not achieve 100% mortality. In comparison with toxicity of ethanedinitrile to insect pests, *B. xylophilus* is more tolerant to ethanedinitrile, particularly at low temperature. Dwinell et al. (2005) reported that it was difficult to completely control *B. xylophilus* with fumigation of *B. xylophilus* with SF in field chamber, even at high Ct product of 5866 g h/m^3 and 10°C. Some survivors were confirmed on the dry wood samples fumigated with SF at 60 g/m^3 for 48 h and 97–168.5 g/m^3 for 24 h at 20°C (Luís et al. 2014). However, for complete mortality, 112 g/m^3 of methyl bromide was required at 10°C for dry wood treatment (Soma et al. 2006).

In this field trial, we did not make optimal fumigation condition in terms of temperature (lower temperature), but practical fumigation trials to control *B. xylophilus* and its vector *M. alternatus* in Korea should schedule in winter and spring season because period of emerging adult from internal stage of *M. alternatus* start in mid spring in Korea. Based on this first field application of ethanedinitrile, we need to use more delicate practical applications for different exposure times and to schedule dose with real practical temperature as methyl bromide alternatives.

Table 3. Efficacy of ethanedinitrile (C_2N_2) and metam sodium (MS) to *B. xylophilus*

Fumigant	Dose (g/m^3)	Logs	Mean no. nematode[a]	Mortality ± SD (%)[b]	95.0% CI
C_2N_2	48	10	190.5	88.43 ± 4.96	131.9–249.1
	68	10	363.2	77.94 ± 16.14	173.2–553.2
	97	25	359.9	96.36 ± 2.77	41.9–78.7
	158	25	232.6	98.02 ± 1.43	22.9–42.3
MS	1162	25	338.8	97.43 ± 2.68	20.6–57.1
Untreated	–	60	1646	–	1,133–2,159

[a] Mean number of nematode per 100 g of wood samples.

[b] Calculated based on untreated control samples.

In conclusion, based on field trial results, such as sorption of ethanedinitrile and toxicity of ethanedinitrile to *M. alternatus* and *B. xylophilus,* ethanedinitrile would provide greater efficacy when used for the treatment of fresh pine wood logs to control insect pests and nematodes at low temperature.

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