Comparison of Ethanedinitrile (C$_2$N$_2$) 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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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
used as a treatment for quarantine (biosecurity) purposes. However, this fumigant is a potent ozone-depleter 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, Luõ et al. 2001, Dwinell et al. 2003, Flack et al. 2008, Soma et al. 2014) have demonstrated the toxicity of SF against *B. xylophilus* on wood of low moisture content at high dosage rates (>100 g/m³) 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., *Lyticus brunneus* (Stephens) and the anobid beetle *Eucrilellata peltata* (Harris) (Outram 1967, Su and Scheffrahn 1990, Williams and Sprekel 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 dosage levels of 1 liter/m³ 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 (C₂N₂) 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° 9.5′ N, 128° 24′ E), Sacheon, Kyungnam Province, Korea. The fumigation was performed in a fumigation chamber (100 by 100 by 100 cm³) 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/m³ for ethanedinitrile and 1000 ml/m³ or 1162 g/m³ 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 Recorder (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 an 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:

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

where: V, volume of fumigation chamber (liter); P, atmospheric pressure (mmHg); T, temperature (°C); C, intended concentration (mg/liter); Vₜ, 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
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°C. Un-fumigated 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 Ethanedinitrile 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 CO₂ (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/m³. The cumulative Ct products of ethanedinitrile were esti-
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/m³ 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 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.

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

where: C is fumigant concentration (g/m³), t is time of exposure (h), i is the order of measurement, Ct is concentration × time products (g h/m³).

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/m³ 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 Arthrodes rusticus larva on dry wood can be completely killed by methyl iodide (MI) at 84 g/m³ 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 affected 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>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/m³)</th>
<th>Log index</th>
<th>Logs no.</th>
<th>No. M. alternatus</th>
<th>Mortality (%)</th>
<th>Avg. mortality ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Alive</td>
<td>Dead</td>
</tr>
<tr>
<td>C₂N₂</td>
<td>48</td>
<td>1</td>
<td>16</td>
<td>19</td>
<td>13</td>
<td>6</td>
</tr>
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<td></td>
<td></td>
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<td>16</td>
<td>19</td>
<td>6</td>
<td>13</td>
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<td>3</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
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<td>C₂N₂</td>
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<td>1</td>
<td>9</td>
<td>16</td>
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<td>15</td>
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<td>6</td>
<td>15</td>
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<td>14</td>
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<td>MS</td>
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<td>1</td>
<td>20</td>
<td>23</td>
<td>0</td>
<td>23</td>
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<td>5</td>
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</table>

*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/m³. There are no statistically significant differences of mortality (P > 0.05) at applied dose of 48 and 68 g/m³. The highest dose of ethanedinitrile at 158 g/m³ 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/m³ and 10°C in 48 h and 97–169.5 g/m³ for 24 h at 20°C (Laws et al. 2014). However, for complete mortality, 112 g/m³ 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.

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.

### Acknowledgments

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### References Cited


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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/m³)</th>
<th>Log index</th>
<th>Logs no.</th>
<th>No. <em>M. alternatus</em></th>
<th>Mortality (%)</th>
<th>Avg mortality ± SD (%)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Alive</td>
<td>Dead</td>
</tr>
<tr>
<td>C2N2</td>
<td>48</td>
<td>1</td>
<td>17</td>
<td>20</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>2</td>
<td>13</td>
<td>26</td>
<td>15</td>
<td>11</td>
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<tr>
<td></td>
<td>97</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>C2N2</td>
<td>158</td>
<td>1</td>
<td>10</td>
<td>18</td>
<td>0</td>
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<tr>
<td>Untreated</td>
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<td>2</td>
<td>18</td>
<td>31</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>C2N2</td>
<td>250</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>4</td>
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</table>

* 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 3. Efficacy of ethanedinitrile (C2N2) and metam sodium (MS) to *B. xylophilus*

<table>
<thead>
<tr>
<th>Fumigant</th>
<th>Dose (g/m³)</th>
<th>Logs</th>
<th>Mean no. nematode</th>
<th>Mortality ± SD (%)</th>
<th>95.0% CI</th>
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<tr>
<td>C2N2</td>
<td>48</td>
<td>10</td>
<td>190.5</td>
<td>88.43 ± 4.96</td>
<td>131.9–249.1</td>
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<td></td>
<td>68</td>
<td>10</td>
<td>363.2</td>
<td>77.94 ± 16.14</td>
<td>173.2–533.2</td>
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<td></td>
<td>97</td>
<td>25</td>
<td>359.9</td>
<td>96.36 ± 2.77</td>
<td>41.9–78.7</td>
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<tr>
<td></td>
<td>158</td>
<td>25</td>
<td>323.6</td>
<td>98.02 ± 1.43</td>
<td>22.9–42.3</td>
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<tr>
<td>MS</td>
<td>1162</td>
<td>25</td>
<td>338.8</td>
<td>97.43 ± 2.68</td>
<td>20.6–57.1</td>
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<tr>
<td>Untreated</td>
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<td>60</td>
<td>1646</td>
<td>-</td>
<td>1,133–2,159</td>
</tr>
</tbody>
</table>

* a: Mean number of nematode per 100 g of wood samples.
* b: Calculated based on untreated control samples.


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