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ETHANDINITIRILE (C_2N_2): TIMBER AND LOG FUMIGATION UPDATE

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ABSTRACT

Ethanedinitrile (C_2N_2) is a potential fumigant to replace methyl bromide (MB) for timber and log treatment. It is highly toxic to wood destroying pests and timber related insect pests such as *Anaplophora glabripennis* (Asian Long-horned Beetle), *Monochamus alternatus* (Japanese Pine Sawyer), *Rhyzopertha dominica* (Lesser Grain Borer), *Reticulitermes speratus* (Japanese Termite), *Tomicus piniperda* (Pine Bark Beetle), *Hypanria cunea* (Fall Webworm) and *Bursaphlenchus xylophilus* (Pine Wilt Nematode). Several field trials have been reported at low temperatures in Korea. The trial reported here demonstrates control of natural infestations of PWN and of artificial inoculation of JPS larvae in pine logs at high moisture content. The $L(Ct)_{99}$ of C_2N_2 to larvae of JPS was 329.4 g h m^{-3} at low temperature (5±0.5°C).

Key words: Fumigant, Fumigation, Ethanedinitrile, Methyl bromide alternatives, Timber and log, Wood destroying pest

INTRODUCTION

In Korea, pine wilt disease (PWD) is the most serious cause of damage to pine forest systems (Kwon et al., 2005). It well known that the main vector of the Pine wilt nematode(PWN) is the Japanese pine sawyer (JPS). Under the Clean Air Act and Montreal protocol, the use of MB is not an option in Korea. Fumigation with metam sodium (MS) is the only one of registered practical option to prevent the spreading disease in Korea.

Since ethanedinitrile (C_2N_2) was developed in 1995, it has been demonstrated to be highly toxic to wood destroying pests and timber related insect pests such as *Anaplophora glabripennis* (Asian Long-horned Beetle), *Monochamus alternatus* (Japanes Pine Saywer), *Rhyzopertha dominica* (Lesser Grain Borer), *Reticulitermes speratus* (Japanese Termite), *Tomicus piniperda* (Pine Bark Beetle), *Hypanria cunea* (Fall Webworm) and *Bursaphlenchus xylophilus* (Pine Wilt Nematode) (Ren and Lee 2008). The penetration of C_2N_2 into timber blocks occurred with and across the grain of the timber (Desmarchelier and Ren, 1996). Among the MB alternatives, C_2N_2 has excellent penetration and is the fastest to achieve even concentration throughout the timber (Ren et al., 2011). Efficacy of C_2N_2 to JPS, PWN, JT and YMB(Yellow minute bark beetle, *Crypahlus fulvus*) in natural infestations at low temperature have been shown (Ren et al., 2005, Ren et al., 2006, Park et al., 2007, Ren and Lee 2008, Cho et al., 2011).

We report here trials of C_2N_2 against artificial inoculation of JPS larvae in logs infested with PWN.

MATERIALS AND METHODS

Artificial Inoculation of JPS Larvae into Logs

Pine wood was sawn from Korean red pine (*Pinus koraiensis*) that had been naturally infested with PWD. The Korean pine tree with 15-20 cm in diameter cut approximately 40-50 cm in length. Moisture content of pine trees averaged 18.8%. To investigate artificial inoculation, larvae of JFS were placed in pre-drilled holes in pine logs (Figure 1) and left for 1 day before fumigant was added. Larvae of JPS in these experiments were purchased in Kinsect Co. in Korea. Mortality of untreated pests was to be obtained same experimental conditions for 30 days.

Drilling hole in the pine logs infested PWN and then JPS larvae was put inside holes	Covering with sawdust gently	Covered aluminum foil wrap and incubated at 5°C

Fig. 1- Artificial inoculation process of Japanese pine sawyer larvae into the pine logs which infested with Pine wilt nematode

Fumigation trials under LDPE film

Field fumigation trials were conducted at the Kyungnam Province, Korea. Fumigation tents (100 × 100 × 100 cm) were constructed of wooden frame and covered with LDPE. Each tents had a load of similar sized dimensions of prepared woods. Wood loads per tent were approximately equal. Calculated amounts of EDN or MB were injected into the tents to give nominal concentrations (g m^{-3}). The amount of fumigant required was calculated from the ideal gas laws as outlined by Ren et al., 2011.

The air temperature in each chamber was recorded using Thermo Recorder (TR-71U). After 24hr fumigation, the fumigated tents were aerated for 24 hours with natural condition.

Measurement of EDN and MB concentration

Ethanedinitrile and MB were determined on a Agilent GC(7890A), equipped with a flame ionization detector (FID) after separation on a HP-5 Column (J &W Sci, 19091J-413). The oven temperature was 150°C. Injector and detector temperature were 200°C. During fumigation, gas samples in the fumigation tents were pumped into tedlar bags and analyzed by gas chromatography. The concentrations of gas were calculated from the relative response of fumigation samples to gas standards.

Bioassays of pine wilt nematode and pine sawyer larvae

PWN mortality was counted after 7 days fumigation. Five randomly sawn timber blocks (approximately 2 cm thicknesses from the 30 cm bottom of logs with diameter size over 13 cm) were taken for bioassay. Nematodes were then extracted using the modified Baermann funnel procedure (Southey, 1985). In the assay of JPR larvae, each fumigated piece of wood was carefully removed from sawdust. All larvae were kept under natural conditions for 72 hours and then counted. Non-fumigated logs with artificial inoculation of JPS larvae were used for control mortality.

Determination of concentration × time products (Ct)

Concentrations of fumigant were monitored at timed intervals over the exposure periods (24 hours) and were used to calculate the product *Ct* = Concentration × time. The *Ct* products were calculated from Eq. 1.

$$Ct = \sum (C_i + C_{i+1})(t_i - t_{i-1})/2 \qquad \textbf{Eq. 1.}$$

Where: **C** is fumigant concentration (g m^{-1})
t is time of exposure (hours)
i is the order of measurement
Ct is concentration × time products (g h m^{-1})

RESULTS

Survival rate of JPS larvae at different conditions in artificial inoculation assay

Survival rate of JPS larvae at different temperatures in artificial inoculation assay without fumigant treatment are shown in Table 1. Mortality was very low for 3 d exposure at low temperature, such as 5°C or 7.3°C (Table 1). However, there were no survived JPS larvae founded at 25°C. For fumigant bioassays, the conditions chosen to minimize mortality were assessed for 3 days inoculation at 5°C.

Table 1. Number of alive Japanese pine sawyer larvae at different temperatures and periods after 3, 15 and 30 days inoculation in artificial inoculation assay without fumigant treatment

Temperature (°C)	No. inoculated insect	No. Alive insects		
		3 days	15 days	30 days
25±1	30	10	0	0
5±0.5	30	29	22	10
7.2±1.3	30	30	30	28

Toxicity of EDN to pine wilt nematode and Japanese pine sawyer larvae

Ethanedinitrile gave 100% control of JPS larvae and PWN when the Ct product was 329.3g h m^{-3} (Tables 2 and 3). This product was obtained for an initial application of 34 g m^{-3} for 24 hours exposure at 5.0±0.5°C. The $L(Ct)_{99.0}$ of ethanedinitrile was estimated at 329.4 g h m^{-3} at 5.0±0.5°C. Also, MB gave good control of JPS larvae and PWN when applied 80 g m^{-3} for 24 hours exposure at 5.0±0.5°C; the Ct products of MB was 495.4 g h m^{-3}. This was lower than that reported by Barak et al (2005) who reported the Ct products was 1000 g h m^{-3} to *A. glabripennis* which applied in well sealed chambers.

Table 2. Efficacy of EDN and MB to larvae stage of Japanese pine sawyer larvae with artificial inoculation assay

Fumigant	Ct (g h m^{-3})	Time (hours)	Total insects	Alive insects	Dead insects	Mortality (%)	Temperature (°C)
Untreated	-	-	30	30	0	0.0	5.0±0.5
EDN	152.5	24	30	28	2	5.0	
EDN	231.7	24	30	6	24	80.0	
EDN	329.3	24	30	0	30	100.0	
MB	495.4	24	30	0	30	100.0	

Our trials demonstrated that EDN and MB can control PMN and JPS on cold and wet logs. As fumigation procedures were not ideal, it is possible that the dose can be further reduced. The novel bioassay procedure of artificial inoculation will provide a new tool for timber research. In summary, EDN has great potential for the treatment of pine wood logs to control insect pests and nematodes, particularly at low temperatures.

Table 3. Efficacy of EDN and MB to Pine wilt nematode

Fumigant	Ct (g h m^{-3})	Time (hours)	Mean No. of nematodes per 100g wood chips	Mortality (%)	Temperature (°C)
Untreated			8738	-	5.0±0.5
EDN	152.5	24	4	99.99	
EDN	231.7	24	0	100	
EDN	329.3	24	0	100	
MB	495.4	24	0	100	

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