Effect of dose and different fumigation procedure of ethanedinitrile (EDN) on *Ct* (concentration × time products) to control Japanese pine sawyer, *Monochamus alternatus*

Lee, B.H.#[1], Kim, B.S.[1], Park, M.G[2], Park, C.G.[3], Lee, S.M.[3], Ren, Y.L.*[4]
[1]Dongbu ARI, Dongbu Farm Hannong Co. Ltd., Nonsan, Republic of Korea
[2]Animal and Plant Quarantine Agency, Anyangsi, Gyeonggido, Republic of Korea
[3]Institute of Agriculture and Life Science, Gyeongsang National Univerity, Republic of Korea
[4]School of Veterinary and Life Sciences, Murdoch University, Australia

*Corresponding author, Email: y.ren@murdoch.edu.au
#Presenting author, Email: byungholee@dongbu.com

DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

Abstract

In Korea, methyl bromide (MB) is limited to use for quarantine pre-shipment (QPS) treatment of imported timber and logs. Metam sodium (MS) is only fumigant to fumigate Japanese pine sawyer (JPS, *Monochamus alternatus*), which is well known main vector of pine wood nematode (PWN) for the purpose of preventing the spread of PWN in Korea. However, MS fumigation requires high level dosage (1 L/m^3) to control PWN. It has led to occupational health safety and environmental (OHS&E) issue and there are problems to reuse fumigated fresh pine log. There is an urgent requirement for development of fumigant to control JPS. Ethanedinitrile (EDN) showed fast penetrations into the timber, good efficacy on various wood pests. Therefore, we evaluated EDN as alternative to replace MB and MS for control of JPS. In this study, we reported more than 10 practical applications of EDN with different fumigation conditions since 2007 until 2013. The *Ct* (Concentration × time products) depending on different dose and conditions including fumigation types (plastic films: LDPE, tarpaulin, PVC-tarpaulin and metal container), water content of logs (18.8- 68.2%), filling ratio (5-50%), average temperature (5-16°C). The result shows that EDN have great potential for treatment of fresh pine wood logs to manage the JPS and its vector of PWN.

Keywords: ethanedinitrile, Japanese pine sawyer, pine wilt nematode, *Monochamus alternates, Bursaphelenchus xylophilus*

1. Introduction

Pine wilt nematode (*Bursaphelenchus xylophilus*, PWN) causes serious damages in pine forests. PWN is vectored by a number of bark beetles and wood borers, and is most often associated with Japanese pine sawyer (*Monochamus alternatus*, JPS). Because of the presence of susceptible pine hosts and suitable insect vectors, many countries have restricted the importation of unseasoned wood from areas where the pine wood nematode is known to occur.

In Korea, methyl bromide (MB) is limited to use for Quarantine Pre-shipment (QPS) treatment of imported timber and logs. Metam sodium (MS) is only fumigant to fumigate JPS in Korea. However, MS fumigation requires high level dosage (1 L/m^3) to control PWN. It has led to occupational health safety and environmental (OHS&E) issue and there are problems to reuse fumigated fresh pine log. There is an urgent requirement for development of new fumigants to control JPS.

A new alternative chemical, ethandinitrile (C2N2) has been investigated as a timber fumigant (Vilijoen and Ren 2001, Ren et al., 2005, Ren et al., 2011). Unlike methyl bromide, ethandinitrile offers fast penetration through timber along and across grain, fast response to insects and nematodes and minimum of environmental problems (Ren et al., 2005, Park et al.,

2014). It has been registered in Australia for disinfestation treatment of timber. Therefore, we evaluated EDN as alternative to replace MB and MS for control of JPS. In this study, we reported more than 10 practical applications of EDN with different fumigation conditions.

2. Materials and Methods

2.1. Preparation of wood samples

Test samples were prepared from Korean red pine (Pinus koraiensis S. & Z.) naturally infested by *M. alternatus*. Moisture contents of randomly sampled 5 pine logs were determined by standard test methods.

2.2. Fumigants

Ethandinitrile (99% purity, balance air) was supplied from BOC Australia.

2.3. Medium scale field trials

Medium scale field trials were performed at Kosung, Jinju and Jeju. The fumigation was performed in a several fumigation chamber types (polyethylene (0.1 cm thick), PVC tarpaulin, tarpaulin, metal container). Each treated and untreated chambers were loaded with JPS infested wood logs by several filling ratios (5-50%) and water contents (18.8- 68.2%). During the fumigation, the headspace temperature in the fumigation chamber was automatically recorded with a Thermo Recoder (TR-71U). After 24 hours of fumigation, the chambers were opened and aerated for 1 day.

2.4. Measurement of ethanedinitrile

EDN was drawn with an electric pump at timed intervals and stored in Tedlar's gas sampling bags (1 liter) (SKC Inc.) until analysis, usually within 1 hour of sampling. The concentration of EDN was determined using an Agilent Technology 7890N gas chromatography (GC) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m × 0.32 mm I.D. HP-5 (0.25 μm film)-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 ethandinitrile in 1 L Tedlar's gas sampling bags).

2.5. Bioassays of Japanese Pine Sawyer Larvae

The mortality of Japanese pine sawyer (JPS) was determined after fumigation. After each treatment, JPS larvae were collected by splitting naturally infested fresh Korean red pine (*Pinus koraiensis*) logs. The larva were collected and counted, then cultured in incubator for 72 hours at 25±2°C. Unfumigated pine logs were used as control for calculation of mortality.

3. Results and Discussion

3.1. Medium scale field trial results

Medium scale field trial at a low temperature condition (<5°C) was performed during Winter. JPS larvae was completely controlled at 711.2 g h m^{-3} Ct product of EDN at low temperature conditions (Table 1). Ct product of EDN was lower with LDPE fumigation than with PVC tarpaulin fumigation. Medium scale field trial at a medium temperature condition (5~15°C) was performed Autumn. JPS larvae was completely controlled at 230.9 g h m^{-3} Ct product of EDN at medium temperature condition (Table 2). Ct product of EDN was lower at tarpaulin fumigation than LDPE fumigation. Medium scale field trial at a room temperature condition (>15°C) was performed during Spring. JPS larvae were completely controlled at 154.6 g h m^{-3} Ct product of EDN at room temperature condition (Table 3). Ct product of EDN was lower at

tarpaulin fumigation than metal container fumigation. These results show that the efficacy of EDN can be affected by temperature conditions. When treated at low temperature, high dosage of EDN is required to control JPS larvae. And Ct product of EDN can be affected by fumigation chamber. Metal container and PVC tarpaulin fumigation increased the Ct product.

Table 1 Efficacy of ethanedinitrile to Japanese pine sawyer, *Monochamus alternatus* larvae at low temperature condition (< 5°C) with different fumigation type (LDPE and PVC tarpaulin), moisture contents (18.8~68.2%) and filling ratio of log (40~50%).

Fumigant	Time (hr)	Temp. (°C)	Filling ratio (%)	Film type	CT (gh/m^3)	m.c. of logs (%)	Mortality (%) (No. Dead /No.Tested) *M. alternatus* Larvae
EDN	24	5.0	50	LDPE	54.2	18.8	6.1 (4/66)
EDN	24	5.0	50	LDPE	78.4	18.8	33.3 (32/96)
EDN	24	5.0	50	LDPE	104.5	18.8	34.0 (34/100)
EDN	24	4.4	40	PVC	317.1	55.4	59.5 (44/74)
EDN	24	3.1	40	PVC	348.8	68.2	60.4 (110/182)
EDN	24	3.1	40	PVC	642.9	68.2	96.4 (106/110)
EDN	24	3.0	40	PVC	711.2	68.2	100 (98/98)
EDN	24	3.1	40	PVC	1074.1	68.2	100 (118/118)
EDN	24	3.1	40	PVC	1262.8	68.2	100 (120/120)

Table 2 Efficacy of ethanedinitrile to Japanese pine sawyer, *Monochamus alternatus* larvae at medium temperature condition (5~15℃) with different fumigation type (LDPE and tarpaulin), moisture contents (25.3~33.5%) and filling ratio of log (5~50%).

Fumigant	Time (hr)	Temp (°C)	Filling ratio (%)	Film type	CT (gh/m^3)	m.c. of logs (%)	Mortality (%) (No. Dead /No.Tested) *M. alternatus* Larvae
EDN	6	10.5	50	Tarp	48.4	25.3	16.7 (6/36)
EDN	6	10.5	50	Tarp	84.2	25.3	62.5 (25/40)
EDN	24	10.9	5	LDPE	105.7	33.5	75.0 (45/60)
EDN	6	10.5	50	Tarp	107.3	25.3	73.1 (19/26)
EDN	6	10.9	5	LDPE	145.7	33.5	95.0 (57/60)
EDN	24	10.5	50	LDPE	230.9	25.3	100 (29/29)
EDN	24	10.9	5	LDPE	241.9	33.5	100 (62/62)
EDN	24	10.5	50	LDPE	251.9	25.3	100 (40/40)

Table 3 (Con.).

Fumigant	Time (hr)	Temp (°C)	Filling ratio (%)	Film type	CT (gh/m^3)	m.c. of logs (%)	Mortality (%) (No. Dead /No.Tested) *M. alternatus* Larvae
EDN	24	10.5	50	LDPE	269.5	25.3	100 (32/32)
EDN	24	10.9	5	LDPE	297.4	33.5	100 (55/55)
EDN	24	10.5	50	LDPE	319.7	25.3	100 (23/23)
EDN	24	10.5	50	LDPE	350.7	25.3	100 (33/33)
EDN	24	10.5	50	LDPE	587.9	25.3	100 (18/18)

Table 4 Efficacy of ethanedinitrile to Japanese pine sawyer, *Monochamus alternatus* larvae at room temperature condition (> 15℃) with different fumigation type (tarpaulin and container), moisture contents (24.1~43.5%) and filling ratio of log (10~50%).

Fumigant	Time (hr)	Temp (℃)	Filling ratio (%)	Film type	CT (gh/m^3)	m.c. of logs (%)	Mortality (%) (No. Dead /No.Tested) *M. alternatus* Larvae
EDN	24	15.0	50	Tarp	93.0	43.5	60.6 (20/33)
EDN	24	15.1	50	Tarp	124.5	24.1	71.4 (20/28)
EDN	24	15.1	50	Tarp	138.0	24.1	82.4 (28/34)
EDN	24	15.1	50	Tarp	152.5	24.1	89.3 (25/28)
EDN	24	15.0	50	Tarp	154.6	43.5	100 (33/33)
EDN	24	15.0	50	Tarp	173.9	43.5	100 (35/35)
EDN	24	15.0	50	Tarp	191.7	43.5	100 (24/24)
EDN	24	15.0	50	Tarp	245.1	43.5	100 (48/48)
EDN	24	15.0	50	Tarp	255.2	43.5	100 (37/37)
EDN	24	15.0	50	Tarp	360.5	43.5	100 (45/45)
EDN	24	16.0	10	Container	594.8	42.1	100 (72/72)
EDN	24	16.0	10	Container	606.7	42.1	100 (99/99)

4. Conclusions

Based on our results, efficacy of EDN on JPS larvae can be affected by temperature. At low temperatures efficacy on JPS larvae was lower than at higher temperatures. LDPE, PVC-tarpaulin and metal container were more suitable to maintain EDN concentration during the fumigation than tarpaulin fumigation. EDN shows potential for the treatment of fresh pine wood logs infested with Japanese pine sawyer.

Acknowledgements

We thank to Korea Forest Service and Korea Animal and Plant Quarantine Agency to support this national project of controlling pine wilt disease.

References

Park, C.G., Son, J.K., Lee, B.H., Cho, J.H., Ren, Y.L., 2014. Comparison of ethanedinitrile (C_2N_2) and metam sodium for control of pine wood nematode and Japanese pine sawyer in naturally infested logs at low temperature. (Accepted in Journal of Economic Entomology)

Ren, Y.L., Dowsett, H.A., Wang, Y.J., Wang, X., Barak, A.V., 2005. Toxicity of ethandinitrile (C_2N_2) to timber or wood related insect pests, In: Proceedings of International Research Conference on Methyl bromide Alternatives and Emissions Reductions, ed by Obenauf GL, Oct.31-Nov. 3. 2005. San Diego.USA, pp86 1-3.

Ren, Y.L., Lee, B.H., Padovan, B., 2011. Penetration of Methyl Bromide, Phosphine, Sulfuryl Fluoride and Ethanedinitrile through a Timber Block. Journal of Stored Product Research. 47, 63-68.

Vilijoen, J.H., Ren, Y.L., 2001. Cyanogen and carbonyl sulfide as potential quarantine fumigants for timber. In: Proceedings of 2001 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 5-9, 2001. San Diego, California, USA, pp 114, 1-2.