Research Article

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Preliminary trials of the ethanedinitrile fumigation of logs for eradication of *Bursaphelenchus xylophilus* and its vector insect *Monochamus alternatus*

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Abstract

BACKGROUND: The nematode *Bursaphelenchus xylophilus* and its insect vectors from the *Monochamus* genus are major global quarantine pests of timber products. Owing to the phase-out of methyl bromide for plant quarantine and preshipment treatments, an alternative fumigant is essential. Based on preliminary laboratory studies on the efficacy of ethanedinitrile (C_2N_2) to *B. xylophilus* and *Monochamus alternatus*, three quarantine trials were conducted at three dosages and three temperatures. Potential for inhalation exposure was assessed by monitoring atmospheric C_2N_2 in relation to the threshold limit value.

RESULTS: Concentration × time products (*Ct*) of 398.6, 547.2 and 595.9 g h m^{-3} were obtained for each trial. A 100% mortality of *B. xylophilus* and *M. alternatus* larvae at 23 ± 4 °C and 10 ± 4 °C occurred with a load factor of pine logs of 46% and at 3 ± 1 °C with a load factor of 30%. During all fumigations, atmospheric levels of C_2N_2 20 m downwind were below the TLV. During aeration, levels 10 and 5 m downwind were below the TLV after 0.4 and 1 h respectively.

CONCLUSION: For the purpose of quarantine or phytosanitary treatment, specific doses of C_2N_2 at the trial temperatures could control *B. xylophilus* and *M. alternatus* larvae without significant inhalation risk to workers.

Keywords: *Bursaphelenchus xylophilus*; ethanedinitrile; fumigant; quarantine fumigation; logs; *Monochamus alternatus*

1 INTRODUCTION

Newly emerging exotic insects in forest and agricultural systems are increasing owing to the global growth in trade of various perishable and durable commodities.[1] Quarantine pests such as the pinewood nematode, *Bursaphelenchus xylophilus*, originally a native of North America,[2] and its insect vectors in the genus *Monochamus*, which infest import and export timber, logs and other wood products, cause huge economic damage and destruction to pine forest systems.[3,4] *B. xylophilus* is a major threat to pine trees in East Asian countries, including Japan, China and Taiwan, and has been of increasing concern in Europe since its identification in Portugal in 1999 in association with the native vector species *Monochamus galloprovincialis*.[5]

Unfortunately, *B. xylophilus* has now become one of the most serious pests in pine forests in South Korea owing to the failure of quarantine eradication programmes at the domestic border.[3] Since the first reports of pine wilt disease,[6] the annual spread of *B. xylophilus* in Korea has been rapid, with the greatest increase in area reported to be 11 550 ha in 2013 and a further 9644 ha in 2014.[7] The Japanese pine sawyer, *Monochamus alternatus*, is the most important vector of pine wilt disease in South Korea,[8] and continuing incursions and the spread of these quarantine pests persist,[1] even though there has been strengthening of border quarantine procedures for imports and exports.

The use of fumigants is the most economical and practical method to control soil-borne pathogens and nematodes as well as quarantine pests in durable and perishable commodities.[9] Methyl bromide has been widely used as a major fumigant for these purposes.[10] However, the Montreal Protocol designated it as having significant ozone-depleting potential in 1992; methyl bromide was completely phased out for soil, grain and structural treatments in 2015, except for quarantine and preshipment (QPS) treatment and a few critical use exemptions for soil fumigations.[11] The QPS use of methyl bromide has not decreased and actually exceeded non-QPS use in 2009.[12] Unfortunately, there are few possible chemical options and limited physical options as alternatives to methyl bromide treatment for logs and wood products.[13] Current alternative fumigants under investigation for

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QPS purposes are sulphuryl fluoride, phosphine gas and ethanedinitrile (C_2N_2). These have been comprehensively reviewed.[14] Sulphuryl fluoride was originally developed as a structural fumigant for the purpose of controlling termites[15] and has since been registered in various countries for the control of stored-product insects and dry-wood-destroying pests. Although there have been extensive studies on the use of sulphuryl fluoride against various general timber pests,[16] there has been difficulty in achieving complete control of *B. xylophilus*, even at a high *Ct* product of 5866 g h m^{-3} at 10 °C, and it has been relatively ineffective at low temperatures against internal stages of the pest, particularly the egg stage.[17,18] However, sulphuryl fluoride has been considered effective under the International Standard for Phytosanitary Measures (ISPM) No. 15 against insect pests in wood packaging material,[5] and Bonifácio *et al.*[5] have controlled *B. xylophilus* at higher temperatures of 15 and 30 °C in pine tree boards at dosages ranging from 3169 to 4407 g h m^{-3} and from 1385 to 2141 g h m^{-3} respectively. Phosphine, which is broadly used on grains in silos and for structural fumigation, seems to be a better option than other compounds in terms of timber penetration properties.[19] It has also shown good efficacy when used to eliminate wood pests in logs.[20,21] However, compared with methyl bromide and the other alternatives, the fumigation time required is longer and efficacy is inconsistent when used at low temperature.[13]

A new alternative chemical, ethanedinitrile, which has been shown to be effective against stored-product insects[22] and to have promise as an effective substitute to methyl bromide for disinfesting soil pathogens, including plant-parasitic nematodes,[23] has also been investigated as a timber fumigant.[19,24–29] It offers fast penetration through timber along and across the grain, and fast action against insects and nematodes.[26] It has been registered in Australia for treatment of timber and is under registration in Korea for fumigation of import timber and logs. A recent limited field trial using 72 h exposures suggested that C_2N_2 could replace methyl bromide and metam sodium to control *B. xylophilus* and *M. alternatus* under low-temperature conditions, which could allow C_2N_2 to be used as a practical application for all seasons in Korea and other places of similar climate.[27]

Herein we report on the results of preliminary laboratory studies on the efficacy of C_2N_2 on *B. xylophilus* in two pine log moisture content ranges at a constant temperature, and *M. alternatus* at constant relative humidity at three different constant temperatures. Based on the lethal concentration × time L$(Ct)_{99}$ from these studies, three quarantine trials on logs covered with PVC tarpaulins were conducted at three different dosages, and the potential for worker exposure was assessed by comparing measured atmospheric levels of C_2N_2 to the threshold limited value (TLV) of 10 ppm, v/v,[30] the maximum level of a chemical compound to which ongoing exposure is considered possible without adverse effects.[31]

2 EXPERIMENTAL METHODS

2.1 Fumigant

Ethanedinitrile (99% C_2N_2 and 1% air) was supplied from BOC Australia and Draslovka Services Pty Ltd, Czech Republic.

2.2 Preparation of *B. xylophilus* and larvae of *M. alternatus* for laboratory fumigation experiments

The test samples were prepared using Korean red pine (*Pinus koraiensis*) naturally infested with *B. xylophilus*. The pinewood samples (5–8 cm diameter) were cut approximately 10 cm long. Moisture contents of five randomly sampled pieces of pinewood were determined by standard test methods.[32] Based on their moisture contents (dry basis), the samples were then divided into two sets for testing. The moisture content of the sets ranged from 15.6 to 24.9% and from 30.9 to 35.6%.

For *M. alternatus*, test samples were prepared using *P. koraiensis* naturally attacked by the insect. The larvae of *M. alternatus* comprising mixed-instar stages were removed from the wood, which had been cut along an axis parallel to the grain. An individual larva was put into a wide-necked glass jar (2 cm × 10 cm i.d.) containing 2–3 g of pinewood sawdust as a food source before fumigation.

2.3 Preparation of pine log samples for quarantine trials

The samples of *P. koraiensis* naturally infested with *M. alternatus* and *B. xylophilus* were 6.6–13.0 cm in diameter and cut approximately 80–90 cm long. The moisture content of the wood samples was measured before each trial by using a moisture meter (Huatec G.C. MC-7825P; Huatec Group Corporation, Beijing, China) and ranged from 25.1 to 30.5% (dry basis).

2.4 Laboratory fumigation trials

The fumigation chambers were 8 L glass desiccators equipped with a ground glass stopper fitted with a septum (Cat. No. 15419; Alltech Associates, Australia). The volume of each desiccator was estimated by the weight of water it held at 25 °C. For the fumigation of *B. xylophilus*, three pinewood samples, each infested with about 150–210 individuals, were placed into individual unsealed desiccators and left overnight at 5 ± 1 °C and 70% relative humidity (RH) prior to treatment the next morning. For the fumigation of *M. alternatus* larvae, ten glass jars, each containing one larva to avoid cannibalism, and 2–3 g of sawdust to sustain feeding individuals, were placed in individual unsealed desiccators and left overnight at one of the following conditions: 5 ± 1 °C, 12 ± 1 °C or 20 ± 1 °C, each at 70% RH, prior to treatment the next morning.

The desiccators were closed with lids equipped with a sealed glass stopper containing a septum for injecting fumigant and for sampling gas. Calculated gas volumes (g m^{-3}) of C_2N_2 were injected into the desiccators via the septa and stirred for 10 min with a magnetic stirrer located at the bottom of each desiccator. The range of C_2N_2 concentrations was 4–40 g m^{-3} over a maximum of eight different dosage levels for both treatments of *B. xylophilus* and *M. alternatus*.

Treatments of *B. xylophilus* consisted of two replicates for each log moisture content set. The replicates comprised eight and six different doses, respectively, for the lower moisture content set, and seven doses each for the higher moisture content set. Treatments of *M. alternatus* consisted of three replicates at each temperature with each replicate consisting of eight doses. Thus, 3 × (150–210) *B. xylophilus* or ten *M. alternatus* were present at a given dose. Each experimental run consisted of four fumigated desiccators of different C_2N_2 concentrations and one unfumigated control desiccator.

The fumigations of *B. xylophilus* were conducted at 5 ± 1 °C, while the fumigations of *M. alternatus* larvae were conducted at three temperatures, 5 ± 1 °C, 12 ± 1 °C and 20 ± 1 °C, all for a 6 h exposure. The gas concentrations were measured over the exposure time at internals of 0.1, 0.5, 1, 2, 3, 4, 5 and 6 h by removing a gas sample at each interval with a 100 µL gastight

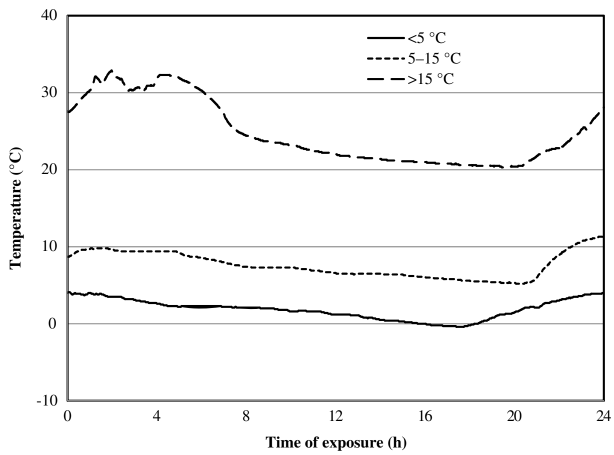

Figure 1. Temperatures during quarantine trials of C_2N_2 on naturally infested logs at Gunsan Port, South Korea (−1–3 °C, 20–21 January 2015; 6–12 °C, 17–18 November 2014; 21–33 °C, 23–24 September 2014). A temperature sensor was placed inside each PVC tarpaulin covering, and data were continuously collected with a TR-71U Thermo Recorder.

syringe via the septum for analysis. At the end of fumigation, the desiccators were aerated in a fume hood for 24 h.

2.5 Quarantine trials on pinewood log samples covered with PVC tarpaulin

Three separate quarantine trials were conducted during the autumn of 2014 (23–24 September and 17–18 November) and the winter of 2015 (20–21 January) at Gunsan Port, Jeonbuk Province, South Korea. Each trial was conducted in one of the following natural ambient temperature ranges: 21–33 °C, 6–12 °C or −1–3 °C, and was not replicated (Fig. 1). The sizes of the fumigation chambers, which were enclosed with PVC tarpaulin, were 107, 50 and 108 m^{-3} respectively for the above temperature ranges. A commercial load factor (volume of logs over total volume × 100) of 46% was used for treatments at 21–33 °C and 6–12 °C, while for the low-temperature trial (−1–3 °C) it was 30%. The fumigant was applied at 100, 120 and 150 g m^{-3} at 21–33 °C, 6–12 °C and −1–3 °C respectively. The decision to use these relatively high doses was due to the relatively high moisture content of the timber logs and the low treatment temperatures. During the fumigation, the temperature inside each fumigation chamber was automatically recorded with a TR-71U/TR-72U Thermo Recorder (Technox Inc., Korea).

During the quarantine field trials, to monitor the concentration of C_2N_2 in the fumigation chamber, a gas sample was withdrawn with an electric pump at timed intervals of 0.5, 1, 2, 4, 8, 16 and 24 h and stored in a 1 L Tedlar® gas sampling bag until analysis, which was usually within 1 h of sampling. The cumulative concentration × time (*Ct*) products of C_2N_2 were estimated for a 24 h exposure time. The *Ct* products were calculated from the equation

$$Ct = \sum \left(C_i + C_{i+1}\right)\left(t_{i+1} - t_i\right)/2 \quad (1)$$

where C is the fumigant concentration (g m^{-3}), t is the time of exposure (h), i is the order of measurement and Ct is the concentration × time product (g h m^{-3}).

After 24 h of fumigation, the tarpaulin was opened and the logs were aerated with natural wind for 24 h. The average wind speed was 2–3 m s^{-1} for the trial at 10 ± 4 °C and 3–5 m s^{-1} for the other two trials.

Table 1. Toxicity of C_2N_2 to *B. xylophilus*, based on *Ct* products in two ranges of moisture contents of infested logs at 5 ± 1 °C for 6 h exposure. Values of $L(Ct)_{50}$ could not be calculated owing to the limited low mortality data and substantial variation

Moisture content of pine logs (%)	$L(Ct)_{99}$ (g h m^{-3}) (95% CL)	Slope ± SE	df[a]	χ^2 [b]
<25 (15.6–25.0)	31.29 (23.38–52.73)	1.00(±0.11)	14	6.93
>30 (30.9–35.6)	430.04 (180.10–856.88)	0.95(±0.18)	20	343.09

[a] Degrees of freedom.
[b] χ^2 based on pooling of data with low expectation.

To monitor environmental levels of C_2N_2 during the chemical applications, exposure periods and aerations of each trial, environmental air samples were collected with a 1 L syringe (Syringe Super GASTIGHT S01000; Capital Analytical, UK) at distances downwind 1.8 m from the ground (head height) and 5, 10 and 20 m from the trial area. The samples were stored in Tedlar® gas sampling bags (1 L) (SKC Inc.) and analysed to evaluate worker safety in relation to the TLV of C_2N_2.

2.6 Measurement of C_2N_2 concentration during the laboratory and quarantine field trials

The concentration of C_2N_2 was determined using a Agilent Technology 7890 N gas chromatograph (GC) equipped with a flame ionisation 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, USA). The GC oven, injector and detector temperatures were 150, 200 and 200 °C respectively. Helium was used as the carrier gas at a rate of 2 mL min^{-1}. The peak areas were calibrated periodically using two spiking standards (a known volume of C_2N_2 injected into a 1 L Tedlar® gas sampling bag). For calculation of the dose or volume of C_2N_2 at an experimental ambient temperature and pressure, the following equation was used:[33]

$$V_f = \left(1 + \frac{T}{273}\right)\left(\frac{1.7 \times 10^6 \times C \times V}{P \times M \times N}\right) \quad (2)$$

where V is the volume of the fumigation chamber (or enclosed space) (L), P is atmospheric pressure (mmHg), T is temperature (°C), C is the intended concentration (mg L^{-1}), V_f is the dose volume of fumigant (mL), M is the molecular weight of fumigant and N is the purity of the fumigant (%).

2.7 Bioassays of *B. xylophilus* nematodes and *M. alternatus* larvae

2.7.1 *Laboratory bioassays*

The mortalities of *B. xylophilus* and *M. alternatus*, initially based on lack of movement, were determined when the 24 h aeration period was completed after a 6 h fumigation. For *B. xylophilus*, three fumigated pinewood samples from each desiccator were cut along an axis parallel to the grain to produce strips approximately 2 cm thick. The nematodes (150–210 individuals per log) were then extracted using a modified Baermann funnel procedure.[34] To assay *M. alternatus*, the larvae (ten per desiccator) were removed and kept in an incubator at 25 ± 2 °C and 75% RH with 2–3 g of fresh wood sawdust. For both species, endpoint assessments of mortality occurred 72 h later, at which stage

Table 2. Toxicity of C_2N_2 to *M. alternatus* larvae, based on *Ct* products at three different temperature conditions for 6 h exposure

Temperature (°C)	$L(Ct)_{50}$ (g h m^{-3}) (95% CL)	$L(Ct)_{99}$ (g h m^{-3}) (95% CL)	Slope ± SE	df[a]	χ^2 [b]
5 ± 1	109.77 (61.93–155.18)	312.63 (224.86–504.46)	5.11(±0.46)	11	8.32
12 ± 1	189.76 (167.82–202.58)	246.19 (235.06–262.78)	20.58(±3.11)	26	30.1
20 ± 1	102.19 (83.98–141.84)	230.31 (160.62–429.73)	6.59(±0.82)	50	146.48

[a] Degrees of freedom.
[b] χ^2 based on pooling of data with low expectation.

the immobile individuals were tending to darken. Unfumigated samples of similar size to the treated ones were used as controls (natural control mortality was less than 2% for both *B. xylophilus* and *M. alternatus*).

2.7.2 Quarantine field trials

The mortalities of *B. xylophilus* and *M. alternatus* were initially determined when the 24 h aeration period was completed after a 24 h fumigation. For the *B. xylophilus* assay, ten fumigated pinewood log samples from each trial were randomly selected and cut along an axis parallel to the grain to form strips approximately 2 cm in thickness. The nematodes were then extracted using the modified Baermann funnel procedure.[34] The mean number of individuals recovered in 100 g of wood were 1500, 2100 and 1700 for each trial at 21–33 °C, 6–12 °C and −1–3 °C respectively (Table 3). For the *M. alternatus* assay, larvae were collected by splitting the pine logs. The larvae were collected, counted and placed on fresh wood sawdust before being held in an incubator at 25 ± 2 °C and 75% RH prior to assessment. The numbers recovered were 801, 563 and 583 respectively for each trial (Table 3). Endpoint mortality of both species was assessed 72 h later using the same criteria described above. Individuals from unfumigated pinewood were used as controls to calculate mortality (natural control mortality less than 1% for both *B. xylophilus* and *M. alternatus*).

2.8 Statistical analysis

The toxicological dose response to C_2N_2 by *B. xylophilus* and larvae of *M. alternatus* was calculated by probit analysis using a computer program produced by P C Annis of the Commonwealth Scientific and Industrial Research Organisation Entomology, Australia, based on Finney.[35] The indices of toxicity measurement derived from this analysis were $L(Ct)_{50} = 50\%$ and $L(Ct)_{99} = 99\%$ lethal concentration × time, or the doses required to cause 50 and 99% mortality response in tested subjects. The variations (standard deviation) of fumigant concentrations for duplicate treatments were analysed using Microsoft Excel 2007.

3 RESULTS AND DISCUSSION

3.1 Effects of C_2N_2 on *B. xylophilus* and *M. alternatus* larvae in preliminary laboratory tests

The toxicity of C_2N_2 to *B. xylophilus* at the least suitable temperature of 5 ± 1 °C and two different moisture conditions is shown in Table 1. The results appear to show that C_2N_2 is considerably more effective against this species in pinewood at the lower moisture conditions [$L(Ct)_{99}$ of 31.3 g h m^{-3}] than at the higher ones [$L(Ct)_{99}$ of 430.0 g h m^{-3}], although there is a large degree of variation in the data ($P < 0.05$). Higher moisture also appears to favour a greater abundance of the nematode by enhancing conditions for population growth, as well as its ability to transfer between timber products.[2] However, Park *et al.*[27] reported that C_2N_2 was highly effective at 97 g m^{-3} even when applied to high-moisture-content logs, while Pranamornkith *et al.*[36] showed that there was little impact on the sorption of C_2N_2 owing to timber moisture content. Further investigation of the impact of moisture content on the efficacy of C_2N_2 to *B. xylophilus* is therefore necessary. In this preliminary study, experiments were not conducted on *B. xylophilus* at various temperature conditions, so further research in this area is also required. This would allow greater comparison with other possible methyl bromide alternatives such as hydrogen cyanide, where recent data obtained at 24 °C and moisture contents of 21.7–32.3% have shown efficacy comparable with sulphuryl fluoride.[37] The toxicity of C_2N_2 to larvae of *M. alternatus* at three different temperature conditions (5 ± 1 °C, 12 ± 1 °C and 20 ± 1 °C) is shown in Table 2. Based on the cumulative *Ct* products of C_2N_2 estimated for a 6 h exposure time, doses of 312.6, 246.2 and 230.3 g h m^{-3} of C_2N_2 can be expected to achieve 99% mortality at 5 ± 1 °C, 12 ± 1 °C and 20 ± 1 °C respectively. While the 95% confidence intervals for $L(Ct)$ values at 12 ± 1 °C are fairly close, confidence intervals for values at 5 ± 1 °C and 20 ± 1 °C show large variation in the data, and although C_2N_2 was found to be most toxic to *M. alternatus* larvae at the highest temperature and least toxic at the lowest temperature, the $L(Ct)$ values were not significantly different. According to Ren *et al.*,[33] the $L(Ct)_{99.5}$ value of C_2N_2 for *Anoplophora glabripennis* larvae was 353.3, 221.8 and 65.6 g h m^{-3} at 4.4, 10.0 and 21.1 °C respectively.[33] It therefore appears that C_2N_2 could have similar toxicity against the larvae of both genera, *Monochamus* and *Anoplophora*, in terms of $L(Ct)_{99.5}$ values (349.7 at 5 ± 1 °C and 253.1 at 12 ± 1 °C) except at higher temperature conditions.

It also appears that C_2N_2 shows higher toxicity to *Monochamus* and *Anoplophora* genera than methyl bromide, because methyl bromide required a dose of 1196 g h m^{-3} at 4.4 °C for 24 h exposure to achieve a value of $L(Ct)_{99}$ against both genera.[38] Najar-Rodriguez *et al.*[28] also observed that lower doses of C_2N_2 (Ct_{99}) were required to achieve mortality in eggs, larvae and adults of burnt pine longhorn beetles, *Arhopalus ferus*, at 10 and 20 °C relative to methyl bromide. For these two temperatures, Ct_{99} values of 22.0 and 19.5 g h m^{-3}, respectively, were recorded. The disparity in results between the two studies may be due to differences in species and experimental conditions.

3.2 Effects of C_2N_2 on *B. xylophilus* and *M. alternatus* larvae in quarantine trials on naturally infested logs

The results of the three quarantine trials on infested logs covered with PVC tarpaulin at dosages of 100, 120 and 150 g m^{-3} of C_2N_2 for a 24 h exposure in the temperature ranges 21–33 °C, 6–12 °C and −1–3 °C, respectively, are shown in Table 3. The

Table 3. Toxicity of C_2N_2 to *B. xylophilus* nematodes and larvae of *M. alternatus* in naturally infested *P. koraiensis* logs in three different temperature ranges

Temperature (°C)	Dose ($g\ m^{-3}$)	Load factor (%)	Volume of chamber (m^3)	Infested pine logs	*Ct* products ($g\ h\ m^{-3}$)	*M. alternatus* larvae		*B. xylophilus* nematodes[a]	
						Dead/total	Mortality (%)	Dead/total	Mortality (%)
21–33	100	46	107	95	398.68	801/801	100.0	1500/1500	100.0
6–12	120	46	50	57	547.22	563/563	100.0	2100/2100	100.0
−1–3	150	30	108	73	595.95	583/583	100.0	1700/1700	100.0

[a] Mean number of nematodes per 100 g of wood sample.

temperature conditions over the 24 h fumigation period of each trial are shown in Fig. 1. The cumulative *Ct* products of C_2N_2 for a 24 h exposure time were 398.6, 547.2 and 595.9 $g\ h\ m^{-3}$ at 21–33 °C, 6–12 °C and −1–3 °C respectively. These *Ct* products were higher than expectations derived from previous laboratory studies on *A. glabripennis*.[33] This is also because experimental conditions were different for temperature, log moisture content and load factor. In contrast, using sulphuryl fluoride, Bonifácio *et al.*[5] obtained 100% mortality of *B. xylophilus* in pine boards with 25–31% moisture content at 15 and 30 °C with doses of 3169–4407 $g\ h\ m^{-3}$ and 1385–2141 $g\ h\ m^{-3}$ for 24 h, respectively. The load factor in this case was 16%.

The *Ct* products in the present trials were enough to achieve higher levels of mortality of *M. alternatus* than the $L(Ct)_{99}$ values (230.3, 246.1 and 312.6 $g\ h\ m^{-3}$) obtained in the preliminary laboratory tests at roughly similar temperatures, and of *B. xylophilus* (31.29 and 430.04 $g\ h\ m^{-3}$ at 15.6–24.9% and 30.9–35.6% moisture content) at 5 ± 1 °C. These higher *Ct* product values achieved 100% mortality for both species at 21–33 °C and 6–12 °C with a 46% load factor of logs and at −1–3 °C with a 30% load factor (Table 3). In a comparative field trial, Park *et al.*[27] obtained mortality levels for *M. alternatus* of between 34.6 and 100% and for *B. xylophilus* of between 88.4 and 98% at −7–25.7 °C and −3.7–23.1 °C at C_2N_2 concentrations of between 48 and 158 $g\ m^{-3}$ over 72 h with a 50% load factor.

One of the reasons why higher than expected *Ct* product values of C_2N_2 were gained at all three of the current quarantine trials could be the variation in the sizes of logs and hence surface area, which could affect the sorption of C_2N_2 given the same moisture content (unpublished data). However, an attempt was made to ensure that each trial had similar numbers of logs of similar sizes. While the results of the limited number of quarantine trials undertaken illustrate unforeseen outcomes associated with conducting field research based on initial enquiry under controlled conditions, they do show that the amount of C_2N_2 plays a more important role than time, which is of considerable practical importance for quarantine treatments. Further research can now focus on reducing treatment times while successfully fumigating these major pest species.

3.3 Workspace and environmental levels of C_2N_2

During all fumigant applications and 24 h exposure periods, atmospheric levels of C_2N_2 downwind at 5, 10 and 20 m from the PVC tarpaulin were below the TLV of 10 ppm, v/v. This may in part mean that all fumigations in these trials were well sealed. When the tarpaulin for the high-temperature (23 ± 4 °C) trial was opened at an edge and the chamber aerated with natural wind (average wind speed 3–5 $m\ s^{-1}$), atmospheric levels of C_2N_2 downwind at 5 and 10 m were safe at 1 and 0.05 h aeration, respectively, having decreased to below TLV regulations (Table 4). When the tarpaulins were fully opened and the chambers aerated with natural wind (average wind speed 2–3 $m\ s^{-1}$ at 10 ± 4 °C and 3–5 $m\ s^{-1}$

Table 4. Atmospheric levels of C_2N_2 (ppm, v/v) during two quarantine fumigation trials with different methods of aeration; tarpaulin opened at an edge and tarpaulin fully opened (trials at 23 ± 4 °C and 3 ± 1 °C respectively, both at wind speeds of 3–5 $m\ s^{-1}$)

	Atmospheric levels of C_2N_2 (ppm, v/v)																	
	Tarpaulin opened at an edge									Tarpaulin fully opened								
	Application			Exposure			Aeration			Application			Exposure			Aeration		
Time (h)	5 m	10 m	20 m	5 m	10 m	20 m	5 m	10 m	20 m	5 m	10 m	20 m	5 m	10 m	20 m	5 m	10 m	20 m
0.05	<0.05*	<0.05	–	–	–	–	35.3	8.7	0.9	<0.05	<0.05	–				92.4	25.8	6.2
0.1	<0.05	<0.05	–	<0.05	<0.05	–	50.6	9.3	1.4	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	46.9	21.1	5.8
0.2	<0.05	<0.05	<0.05	–	–	–	48.1	8.2	1.2	<0.05	–	–	–	–	–	44.1	18.6	1.7
0.4	<0.05	<0.05	–	<0.05	<0.05	<0.05	25.0	6.9	0.8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	25.7	9.7	0.5
0.5	<0.05	<0.05	<0.05	–	–	–	11.9	4.2	0.8	<0.05	<0.05	<0.05	–	–	–	13.4	5.1	0.4
1				<0.05	<0.05	<0.05	8.2	2.1	0.5				<0.05	<0.05	<0.05	3.5	0.4	<0.05
2				<0.05	–	–	7.5	0.8	<0.05				<0.05	–	–	1.1	<0.05	<0.05
4				<0.05	<0.05	<0.05	6.3	0.5	<0.05				<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
8				<0.05	<0.05	–	0.5	<0.05	<0.05				<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
18				<0.05	–	–	<0.05	<0.05	<0.05				<0.05	<0.05	<0.05			

at 3 ± 1 °C), atmospheric levels of C_2N_2 downwind of the fumigation sites at 5 and 10 m met with worker inhalation safety requirements (<10 ppm, v/v) at 1 and 0.4 h (Table 4). During aeration using both methods, atmospheric levels of C_2N_2 at 20 m downwind were always below the TLV (Table 4). The exclusion zone needed for current quarantine fumigations of import logs with methyl bromide is normally >500 m^3 in Korea, while in these trials it was only approximately 100 m^3. Further research is needed regarding worker exposure to C_2N_2 when applied to larger fumigation volumes (500–1000 m^3). Given that the trials were conducted only at wind speeds of 2–5 m s^{-1} and a specific set of experimental conditions, these preliminary results suggest that different doses of C_2N_2, which depend on particular temperature conditions, may offer complete control of *B. xylophilus* and *M. alternatus* larvae without presenting significant risk to workers in terms of inhalation. Furthermore, the dosages required to control these pests are considerably below flammability levels.[30]

In conclusion, C_2N_2 should be further pursued as an alternative to methyl bromide with suitable quarantine guidelines in Korea when it is necessary to control unexpected or emergent quarantine pests in timber and logs. Issues such as expense and other hazards associated with use and level of toxicity to other pests also require consideration.

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