Ethanedinitrile: potential methyl bromide alternative to control *Arhopalus ferus* (Mulsant) in New Zealand sawn timber exports

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Abstract The efficacy of ethanedinitrile (EDN, syn. cyanogen, EDN Fumigas®) for the control of burnt pine longhorn beetle (*Arhopalus ferus* (Mulsant)) adults was tested using a range of ethanedinitrile concentrations. The LD_{99} for adults after a 3 h exposure at 15°C was 12.6 g/m^3. The results demonstrate that ethanedinitrile is a potential phytosanitary alternative to methyl bromide for disinfesting burnt pine longhorn adults from sawn timber exported from New Zealand.

Keywords fumigation, burnt pine longhorn beetle, *Pinus radiata* D. Don, quarantine treatments.

INTRODUCTION

Methyl bromide is used in New Zealand for quarantine and pre-shipment treatments for both imported and export products (Glassey 2013), with a current national annual consumption of 565 tonnes for the year ending June 2013. The majority of methyl bromide is used for pre-shipment treatment of forest products to meet quarantine requirements (STIMBR 2014). Methyl bromide is an ozone-depleting compound and reduction of its use and recapture are strongly encouraged by governmental authorities (Glassey 2013). Capture and destruction of all remaining methyl bromide in the head space following fumigation will be mandatory in New Zealand in 2020 (New Zealand Environmental Protection Authority 2011a). Therefore, finding alternative treatment methods or fumigants to methyl bromide is important to maintain the export markets for New Zealand timber.

Ethanedinitrile (EDN; C_2N_2, molecular weight 52.04) is a colourless, pungent gas with a boiling point of -21°C (Matheson Tri-Gas 2008). Ethanedinitrile was proposed as an alternative fumigant to replace methyl bromide for quarantine treatment as an ozone-safe alternative (CSIRO 2005; BOC 2012) and earlier studies showed the potential of ethanedinitrile as a disinfestation treatment for logs, timber and wood packaging materials (CSIRO 1996). Recently, EDN Fumigas® was registered in Australia as a disinfestation treatment for logs and sawn timber (BOC 2012; Australian Pesticides and Veterinary Medicines Authority 2013) and ethanedinitrile became available for testing in New Zealand (New Zealand Environmental Protection Authority 2011b).

The burnt pine longhorn beetle (BPL), *Arhopalus ferus* (Mulsant) (Coleoptera:

Cerambycidae), is a key forest pest that may infest pine (*Pinus radiata* D. Don) logs exported from New Zealand. Additionally, BPL adults are considered the main pest in sawn timber exports because adults may use timber packets as a daytime refuge, which can result in the unwanted transport of adult beetles in exported timber packets (Pawson et al. 2009).

This study evaluated ethanedinitrile as a methyl bromide alternative fumigant for the control of BPL adults and developed an ethanedinitrile fumigation schedule for further testing as a phytosanitary treatment for export timber.

MATERIALS AND METHODS

Insects

BPL adults were hand-collected at night near floodlights at timber processing mills near Nelson (Eves Valley mill, Brightwater) and Napier (Pan Pac mill, Whirinaki) during February 2014. They were placed in 1-litre plastic containers with moist paper towels to enhance humidity, prevent damage to the insects during shipping and offer hiding places for the adults to reduce the potential for damage caused by aggressive behavior. Containers were placed in polystyrene boxes containing frozen gel packs and delivered to the laboratory in Palmerston North within 36 h of collection.

For each replicate, two vented plastic containers (1 litre) holding 25 BPL active adults each were prepared. Each plastic container held two moist paper towels (weighing approximately 3 g each). The containers of adult beetles were prepared and held in the lab at ambient temperature for up to 2 h before fumigation.

Insect fumigation with ethanedinitrile

Fumigation of BPL adults was carried out in 28-litre chambers (Labconco® desiccators, Kansas City, Missouri, USA) fitted with an internal fan to provide headspace mixing during the treatment period. Fumigation chambers were placed inside a temperature-controlled room at 15 ± 2°C and the temperature inside the room during the fumigation period measured using a data logger (Temprecord International Limited, model ILTE-953101C, Auckland, NZ). All fumigations were for 3 h.

Preliminary fumigations at 0, 2, 5, 10, 20, 30, 40 and 50 g/m^3 were done to identify efficacious dose ranges for further testing that would provide data points between 50% and 100% mortality to enable curve fitting. Eight ethanedinitrile concentrations were identified from the preliminary fumigations: 0, 2, 4, 6, 8, 10, 15 and 20 g/m^3. Three replicates of BPL adults were treated separately over 3 days. In total, 1200 adult insects were used in the experiments. The insects for replicate 1 were collected from Napier and the other two replicates were from Nelson. After the completion of each fumigation, the chamber was aerated for 5–10 min using a vacuum pump connected to a fumigant scrubbing system (Nordiko Quarantine Systems Pty Ltd, Sydney) designed to capture ethanedinitrile. After aeration, the containers with the fumigated adults were placed under a fume hood for 18 h.

Delivery, monitoring and analysis of ethanedinitrile

To achieve ethanedinitrile at selected concentrations a calculated volume of pure (100%) ethanedinitrile gas was injected into each chamber using a plastic gas syringe to obtain the desired g/m^3 concentration. Before injecting the ethanedinitrile into each chamber, an identical amount of air was removed so that the amount of ethanedinitrile injected would equal the partial vacuum created and return the chamber to ambient pressure. Chamber samples were collected at 5 min and 165 min after each fumigation was initiated. Internal fans were operated for the duration of the fumigation period. The ethanedinitrile injection procedures and the analysis of ethanedinitrile concentrations from chamber samples were those described by Pranamornkith et al. (2014a).

Mortality assessments

Fumigated adults were assessed as either dead (no movement) or alive (normal movement) about 18 h after treatment. Any adults that exhibited abnormal movement (e.g. the legs moved in an uncoordinated way, the beetle was incapable of walking, the beetle could not maintain an upright position) were kept for another 48 h to allow a more accurate assessment of their survival status. At this time the same assessment criteria was used to determine mortality.

Statistical analysis
The adult mortality data set was fitted into a binomial generalized linear model to relate percentage mortality to ethanedinitrile dose using the PROBITANALYSIS procedure in GenStat (version 16, 2013, VSNI Ltd, Hemel Hempstead, UK). The complementary log-log link was used to calculate the lethal dose required to produce a mortality of 99% (LD_{99}). The possibility of additional control mortality was tested, but was found not to be significant.

RESULTS AND DISCUSSION
Ethanedinitrile concentrations after fumigation
Ethanedinitrile concentrations measured 5 min after fumigant introduction were close to expected concentrations, e.g. 1.5 ± 0.5 and 20.3 ± 0.3 g/m^3 for the target doses of 2 and 20 g/m^3, respectively. During the 3 h treatment duration, the ethanedinitrile loss in the chambers was approximately 25% of the initial dose. The reduction in chamber concentration may be caused by the moist paper towels in the containers and the significant sorption characteristics of ethanedinitrile (Pranamornkith et al. 2014a).

Insect mortality
Figure 1 shows the mortality response of adults to ethanedinitrile. The mortality curve for each replicate may represent variability occurring when using insects from different sources and of different ages. Data have been presented for individual replicates to illustrate variability of this type of work when using insects collected from wild sources. BPL adults required an ethanedinitrile concentration of 9.6, 12.3 and 14.7 g/m^3 (Replicates 1, 2 and 3 respectively; arithmetic mean of 12.6 g/m^3 and 95% confidence interval of 6.1–19.1 g/m^3) for 3 h to achieve LD_{99}. The LD_{99} of 12.6 g/m^3 is much lower than that obtained by Ren et al. (2006) for larvae of another longhorn beetle, *Anoplophora glabripennis* (Motschulsky). They reported the LD_{99} of 43.5 g/m^3 at 15.6°C after 3 h exposure.

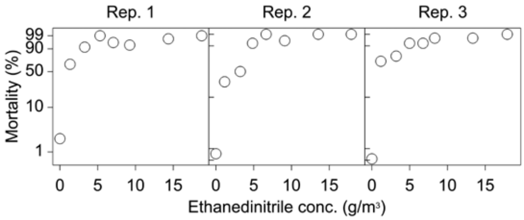

Figure 1 Mortality (%) of *Arhopalus ferus* (burnt pine longhorn beetle) adults fumigated with ethanedinitrile for 3 h at 15°C for the three individual replicates. Measured mean concentrations are shown for the dose rates of 0, 2, 4, 6, 8, 10, 15 and 20 g/m^3 of ethanedinitrile.

The present experiment using ethanedinitrile to control BPL adults compares well with the LD_{99} values obtained using methyl bromide (Pranamornkith et al. 2014b). They calculated a LD_{99} of 10.9 g/m^3 at 10°C and 6.7 g/m^3 at 20°C. The results suggest that ethanedinitrile is just as effective as methyl bromide for controlling BPL and indicate ethanedinitrile has potential as a methyl bromide replacement for the disinfestation of sawn timber and other forest products.

The approved Australian pesticide use label for EDN Fumigas® recommends fumigation with 50 g/m^3 for 10 h to control insect pests on logs and timber (BOC 2014; Australian Pesticides and Veterinary Medicines Authority 2013). The present data suggest that a lower ethanedinitrile concentration, a reduced treatment duration, or both, may be possible for New Zealand sawn timber exports where BPL adults are the only insect of quarantine concern (Pawson et al. 2009). The ethanedinitrile efficacy data reported herein and the ethanedinitrile sorption data reported by Pranamornkith et al. (2014a) can be used to develop an ethanedinitrile fumigation schedule for testing ethanedinitrile on sawn timber for export under commercial conditions.

Initial results indicate that ethanedinitrile may also have potential as a methyl bromide alternative disinfestation treatment for logs exported from New Zealand. To determine the potential use for logs, ethanedinitrile efficacy at the LD_{99} level is needed for life stages of other insects, such as goldenhaired bark beetle (*Hylurgus ligniperda* (F.)) and black pine bark beetle (*Hylastes ater* (F.)) (both Coleoptera: Scolytidae) found on or in logs. In addition and concurrently, ethanedinitrile sorption by logs and penetration into logs must be elucidated.

It is concluded that the ethanedinitrile concentration required to achieve control of burnt pine longhorn beetle adults at the LD_{99} level is 12.6 g/m^3 when fumigated for 3 h at 15°C. The present research indicates that ethanedinitrile has the potential to replace methyl bromide as a phytosanitary disinfestation treatment of sawn timber exported from New Zealand.

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REFERENCES

Australian Pesticides and Veterinary Medicines Authority 2013. Public release summary on the evaluation of the new active constituent ethanedinitrile in the product Sterigas 1000 fumigant. 31 p. http://www.apvma.gov.au/registration/ assessment/docs/prs_ethane_dinitrile.pdf (accessed 1 May 2014).

BOC 2012. BOC trial report, ethanedinitrile fumigant (EDN) worker exposure field trial, timber application under tarp. BOC Australia, New South Wales, Australia. 41 p.

BOC 2014. EDN Fumigas label, safety directions before opening or using of fumigant, active constituent: 1000 g/kg ethanedinitrile (EDN), for the control of insect pests and fungi infesting timber and logs, New South Wales, BOC Australia. 3 p. http://www.infopest.com.au/extra/asp/infopest/nra/labels.asp?prodcode =60096 (accessed 28 March 2014).

CSIRO (Commonwealth Scientific and Industrial Research Organization), O'Brien IG, Desmarchelier FJM, Ren YL 1996. Cyanogen fumigants and methods of fumigation using cyanogen, PCT/AU 95/00409. International Patent No. WO 96/01051. World Intellectual Property Organization, International Bureau, Geneva, Switzerland. 171 p. Note: this reference is cited by Ren et al. (2006) as O'Brien IG, Desmarchelier, JM, Ren, YL 1995. Cyanogen fumigants and methods of fumigation using cyanogen, International Patent Application PCT/AU95/00409. The latter citation references the year the patent application was submitted.

CSIRO 2005. BOC licenses ozone-safe fumigant. www.csiro.au/Portals/Partner/ Invest-or-technology-transfer/BOC-licenses-ozone-safe-fumigant.aspx#a2 (accessed 6 November 2013).

Glassey K 2013. Official use of methyl bromide. Ministry for Primary Industries, New Zealand. 3 p. www.biosecurity.govt.nz/files/regs/treat/official-use-of-methyl-bromide.pdf (accessed 6 November 2013).

Matheson Tri-Gas. 2008. Material safety data sheet – Cyanogen. Matheson Tri-Gas, Inc., Basking Ridge, New Jersey, USA. 7 p.

New Zealand Environmental Protection Authority 2011a. Methyl Bromide: a reassessment of methyl bromide and its formulations. Decision – Amended Version (17 June 2011) (pdf). New Zealand Environmental Protection Authority, Wellington. http://www.epa.govt.nz/search-databases/Pages/applicationsdetails.aspx?appID=HRC 08002# (accessed 21 May 2013).

New Zealand Environmental Protection Authority 2011b. Decision on ERMA200981. http://www.epa.govt.nz/search-databases/Pages/applications-details.aspx?appID=ERMA200981 (accessed 14 May 2014).

Pawson SM, Watt MS, Brockerhoff EG 2009. Using differential responses to light spectra as a monitoring and control tool for *Arhopalus ferus* (Coleoptera: Cerambycidae) and other exotic wood-boring pests. Journal of Economic Entomology 102(1): 79-85.

Pranamornkith T, Hall MKD, Adlam AR, Somerfield KG, Page BBC, Hall AJ, Brash DW 2014a. Effect of fumigant dose, timber moisture content, end-grain sealing, and chamber load factor on sorption by sawn timber fumigated with ethanedinitrile. New Zealand Plant Protection 67: 66-74.

Pranamornkith T, Hall MKD, Adlam AR, Page BBC, Connolly P, Somerfield KG, Brash DW 2014b. Relative methyl bromide tolerances of *Arhopalus ferus* (Mulsant), *Hylurgus ligniperda* (F.) and *Hylastes ater* (Paykull) adults. New Zealand Plant Protection 67: 80-85.

Ren YL, Wang YJ, Barak AV, Wang X, Liu Y, Dowsett HA 2006. Toxicity of ethanedinitrile to *Anoplophora glabripennis* (Coleoptera: Cerambycidae) larvae. Journal of Economic Entomology 99: 308-312.

STIMBR 2014. http://www.stimbr.org.nz/our-resources.html (accessed 6 May 2014).