# Developing new fumigation schedules for the phytosanitary treatment of New Zealand export logs: comparative toxicity of two fumigants to the burnt pine longhorn beetle, *Arhopalus ferus*

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**Abstract** Ethanedinitrile (EDN) has been identified as a possible alternative fumigant to methyl bromide (MB) as a phytosanitary treatment for New Zealand export logs. A review of MB phytosanitary schedules has indicated that the treatment rates used in New Zealand may be able to be significantly reduced. The toxicity of EDN was compared in the laboratory to that of reduced rates of MB, using different life stages of the burnt pine longhorn beetle, *Arhopalus ferus*. Naked insects were fumigated with MB at 10°C and 20°C for 4 h or with EDN at the same temperatures for 3 h. The mortalities achieved and the CT products calculated indicate that (1) a reduction in MB usage may be possible for the treatment of logs exported from New Zealand and that (2) EDN has potential as a phytosanitary alternative to MB for the treatment of logs.

**Keywords** ethanedinitrile, methyl bromide, LD99, toxicity, mortality.

## INTRODUCTION

The burnt pine longhorn beetle (BPL), *Arhopalus ferus* (Mulsant) (Coleoptera: Cerambycidae), is an invasive forest insect introduced to New Zealand that is considered a key quarantine pest of export pine (*Pinus radiata* D. Don) logs and sawn timber by importing countries such as China and Australia (Wang et al. 2003; Ministry for Primary Industries 2013). BPL adults show a particular preference for recently burned trees, but they also attack logs, stumps and dead or dying pine *Pinus* spp. trees (Hosking & Bain 1977; van Epenhuijsen et al. 2012).

To control forest insects, including BPL, on or in New Zealand export logs, current phytosanitary certification requires that logs be fumigated with either methyl bromide (MB) or phosphine or be debarked. However, reduction of the use of MB, which is a toxic, ozone-depleting gas, is strongly encouraged by government authorities, particularly by the Ministry for Primary Industries (MPI) (Glassey 2013). Furthermore, MB recapture will become mandatory in New Zealand in 2020 (New Zealand Environmental Protection Authority 2011a).

Ethanedinitrile (EDN=$C_2N_2$, molecular weight=52.04), a colourless, pungent gas with a boiling point of -21°C (BOC 2012), was recently recommended as a potential alternative fumigant

to MB for use as a quarantine treatment for New Zealand export pine logs (Armstrong et al. 2014). EDN was recently registered in Australia as a disinfestation treatment for logs and sawn timber (BOC 2012), and became available for testing in New Zealand in 2011(New Zealand Environmental Protection Authority 2011b).

Robust information is needed if EDN is to be used as a quarantine treatment for New Zealand log exports. This will require knowledge of insect toxicity, fumigant penetration into logs and sorption characteristics. These datasets can then be used to develop potential EDN fumigation schedules for New Zealand export logs. The toxicity of EDN to BPL adults was recently tested using a range of EDN concentrations at 15°C (Pranamornkith et al. 2014c). The results demonstrated that EDN is highly toxic to the adult life stage of this species. When combined with EDN sorption data for sawn timber and logs (Pranamornkith et al. 2014b; Hall et al. 2015), the data indicate that EDN may be a promising phytosanitary alternative to MB.

Based on historical data and statistical analyses of the available MB efficacy information for New Zealand forest pests, Armstrong et al. (2011) recommended that the current MB rates could be reduced. Recent research has shown that reduced rates of MB were effective at controlling BPL adults and larvae, and the adults of the golden haired bark beetle, *Hylurgus ligniperda* (F.) (Coleoptera: Curculionidae) (Somerfield et al. 2013; Pranamornkith et al. 2014a). Consequently, the toxicity of EDN in the laboratory was compared at two contrasting temperatures, 10°C and 20°C, using eggs, larvae and adults of the BPL, and EDN toxicity compared with that of MB.

## MATERIALS AND METHODS

### Insects

BPL adults of mixed ages were hand-collected at night at timber processing mills near Nelson and Napier, from December 2014 to February 2015. Insects were placed in 1-litre plastic containers with wax paper to prevent damage to the insects during transport, to reduce aggressive encounters and to provide an oviposition substrate for the females. Containers were placed in polystyrene boxes each containing two frozen gel packs and were delivered to the Entomology Laboratory (Plant & Food Research) in Palmerston North within 24 h of collection. For fumigation, BPL adults were placed individually in plastic 8 cm × 3 cm × 1 cm vented queen bee cages (supplied by Waireka Honey, Palmerston North) (Figure 1). The adults were held in the cages for approximately 2 h before fumigation. After fumigation, the adults were kept at 20°C in the same controlled temperature room as the eggs and larvae.

Eggs of BPL were obtained from field-collected adults. Groups of adults (n=150-200) were placed in 16-litre plastic containers supplied with sections of wax paper added as a substrate for oviposition. Eggs were collected daily and stored at 11–12°C for up to 10 days. For fumigation, eggs were then transferred individually on to Petri dishes (9 cm diameter × 1.5 cm depth) (n=25 eggs/Petri dish) that contained a layer (2–3 mm depth) of Phytagel™ (Sigma-Aldrich®, Auckland, New Zealand) in the bottom dish. The Phytagel™ provided a moist and hardened substrate for the eggs that prevented desiccation before, during and after fumigation tests. After fumigation, eggs were kept in a controlled temperature room at 20°C and 16:8 h light:dark until hatching (6 to 11 days on average, at least for control eggs).

Larvae of BPL were obtained from a laboratory-reared colony maintained at Plant & Food Research, Mt Albert (Auckland). Larvae were reared individually in test tubes (7.5 cm × 1.2 cm) supplied with a variation of the bark-based artificial diet developed for rearing larvae of the endemic huhu beetle *Prionoplus reticularis* White (Coleoptera: Cerambycidae) (Barrington et al. 2015). First instar larvae were transferred into the tubes and were allowed to feed on the diet for 6 weeks. For fumigation, the 6-week-old larvae were removed from the tubes and then transferred to 24-well tissue culture plates (Interlab, Johnsonville, Wellington) (Figure 2) provided with approximately 1.5 g per cell of the bark-based diet. After fumigation, the larvae were kept at 20°C in the same controlled temperature room as the eggs.

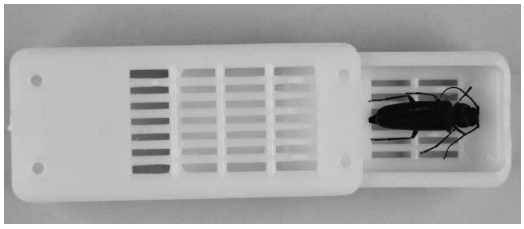

**Figure 1** Queen bee cage used to hold adults of the burnt pine longhorn beetle for fumigation with ethanedinitrile or methyl bromide at 10°C or 20°C for 3 h or 4 h, respectively.

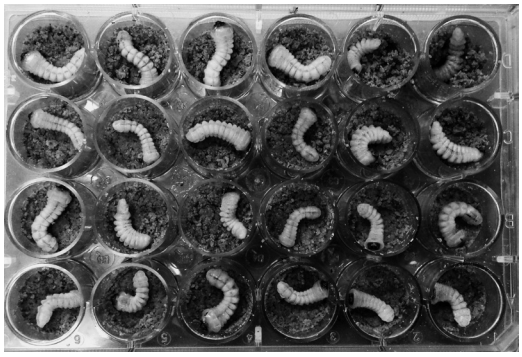

**Figure 2** Twenty-four well tissue culture plates used to hold 6-week-old larvae of the burnt pine longhorn beetle for fumigation with ethanedinitrile or methyl bromide at 10°C or 20°C for 3 h or 4 h, respectively.

**Fumigations with ethanedinitrile and methyl bromide**

Fumigations with either EDN or MB were carried out in 28-litre fibreglass chambers (Labconco® desiccators, Kansas City, Missouri, USA) equipped with internal fans to circulate the air-fumigant mixture. Fumigation chambers were placed inside a temperature-controlled room at either 10°C or 20°C ± 2. A water-saturated sponge (5 cm × 5 cm × 1 cm) was placed in each chamber to prevent desiccation of the insects and the artificial diet (if used). Fumigations with EDN were carried out for 3 h and those with MB for 4 h.

Initial fumigations with both EDN and MB were carried out at both 10°C and 20°C to identify efficacious doses for all BPL life stages to be tested. These results then guided a series of dose-mortality fumigations to allow estimation of the lethal dose required to achieve 99% mortality ($LD_{99}$) of each fumigant for each life stage at respective temperatures. Either eight or nine doses, at regular intervals, were tested across these factors (Table 1). Each combination of fumigant type, life stage and temperature (i.e. replicate) was carried out three times and on different days. Each replicate consisted of a control (no fumigant) and a number treatments consisting of different fumigant doses. For each treatment, 50 individuals were used, so the total number of insects used for each replicate was 450 or 500, when the control and either eight or nine doses were tested. If the doses chosen for a given fumigant or a given temperature yielded too many data points near or at 100% mortality, the doses were reduced in subsequent replicates to provide a more useful range of mortalities for $LD_{99}$ calculations.

After the completion of each replicate, chambers were aerated for 5–10 min, the insects were then removed from the chambers and further aerated under a fumehood for 18 h to ensure that no fumigant remained. Insects were then assessed for mortality as described below.

To achieve the selected doses (in $g/m^3$) of EDN and MB (Table 1), a calculated volume of pure (100%) fumigant was injected into each chamber using a plastic gas syringe (Hamilton, Reno, USA). Concentrations of each fumigant in the chambers were monitored by collecting headspace samples at 5 and 165 min or at 5, 120 and 225 min, for EDN and MB, respectively, after each fumigant was injected into each chamber. Fumigant concentrations were quantified using an Agilent 7890A GC fitted with a flame ionisation detector and a 30 m×0.53 mm capillary column GS-Q (Agilent Technologies Inc., Auckland, New Zealand). The oven and detector temperature were held at 100°C and 300°C, respectively. Samples were withdrawn from the chamber using a gas-tight syringe (Valco Instruments Co., Texas, USA). A 3 ml sample was injected into a sample loop and a 1 ml subsample was analysed. Five-point calibration curves using dilutions of pure fumigant with air were established weekly.

**Table 1** Combinations of temperature, life stage and dose used to determine the $LD_{99}$ for burnt pine longhorn beetle with ethanedinitrile or methyl bromide at 10°C or 20°C for 3 h or 4 h, respectively.

| Fumigant | Temperature | Life stage | Doses tested ($g/m^3$) |
|---|---|---|---|
| Ethanedinitrile | 10°C | Egg | 0, 1, 1.5, 2, 2.5, 3, 3.5, 4 |
| Ethanedinitrile | 10°C | Larval | 0, 2, 4, 6, 8, 10, 12, 14 |
| Ethanedinitrile | 10°C | Adult | 0, 2, 4, 6, 8, 10, 12, 14 |
| Ethanedinitrile | 20°C | Egg | 0, 1, 1.5, 2, 2.5, 3, 3.5, 4 |
| Ethanedinitrile | 20°C | Larval | 0, 2, 4, 6, 8, 10, 12, 14 |
| Ethanedinitrile | 20°C | Adult | 0, 2, 4, 6, 8, 10, 12, 14 |
| Methyl bromide | 10°C | Egg | 0, 4, 6, 8, 10, 12, 14, 18, 22 |
| Methyl bromide | 10°C | Larval | 0, 4, 8, 16, 24, 32, 40, 44, 48 |
| Methyl bromide | 10°C | Adult | 0, 2, 4, 6, 8, 10, 12, 16, 20 |
| Methyl bromide | 20°C | Egg | 0, 2, 4, 6, 8, 10, 12, 14 |
| Methyl bromide | 20°C | Larval | 0, 4, 8, 12, 16, 20, 24, 28 |
| Methyl bromide | 20°C | Adult | 0, 2, 4, 6, 8, 10, 12, 14, 16 |

**Mortality assessments and statistical analysis**

Fumigated BPL adults and larvae were assessed as either dead (no movement) or alive (any type of movement) 24 and 48 h or 24, 48, 72 and 96 h after fumigation, respectively, as per Pranamornkith et al. (2014a,c). Eggs that did not hatch after three consecutive counts over 6 days on average were counted as dead. All mortality data were analysed using the Probit mortality analysis, with a complementary log-log transformation. These allowed calculations of lethal dose ($LD_{99}$) values for all BPL life stages. Since control mortality values were very low, no adjustments for control mortalities were required. Concentration × time (CT) products were calculated for each fumigant/life stage combination tested by multiplying $LD_{99}$ values from Table 2 (in $g/m^3$) by the duration of each fumigation treatment (in h)

## RESULTS AND DISCUSSION

The results confirm that EDN is highly toxic to all BPL life stages tested as naked insects, with toxicity increasing with increasing temperature (Table 2). At both 10°C and 20°C, the eggs were the most susceptible life stage to EDN while there was no significant difference between the larval and the adult life stages (Table 2). Similarly, MB was toxic to all BPL life stages. Larvae were the most tolerant life stage to MB. However, the adults, not the eggs, were shown to be the most

**Table 2** Toxicity ($LD_{99}$; $g/m^3$) of 3-h ethanedinitrile fumigations and 4-h methyl bromide fumigations to burnt pine longhorn beetle eggs, larvae and adults at 10°C or 20°C. $LD_{99}$ and 95% confidence intervals (CI) values based on probit mortality analysis.

| | | Ethanedinitrile | | Methyl bromide | |
|---|---|---|---|---|---|
| Life stage | Temperature | $LD_{99}$ | 95% CI | $LD_{99}$ | 95% CI |
| Egg | 10°C | 1.70 | 1.52-1.97 | 16.04 | 14.83-17.73 |
| Larval | 10°C | 6.72 | 5.93-7.94 | 22.72 | 21.50-24.45 |
| Adult | 10°C | 6.47 | 5.72-7.61 | 9.01 | 8.38-9.89 |
| Egg | 20°C | 0.49 | 0.41-0.62 | 9.12 | 8.40-10.13 |
| Larval | 20°C | 6.50 | 5.67-7.83 | 13.81 | 13.28-14.60 |
| Adult | 20°C | 5.60 | 4.70-7.33 | 7.96 | 7.40-8.74 |

susceptible life stage (Table 2), indicating for the first time a differential response to fumigants across the life stages of the BPL.

The toxicity of MB doses tested here correspond with those previously found for BPL adults and bark beetles, *Hylurgus ligniperda* and *Hylastes ater* adult, treated with similar concentrations (Somerfield et al. 2013; Pranamornkith et al. 2014a). This indicates that a reduction in the MB rates prescribed in the official treatment schedules may be possible for logs exported from New Zealand (Glassey 2013). EDN was significantly more toxic to BPL eggs and larvae compared to MB, with differences in toxicity being as high as 22 times in the case of eggs at 20°C, as shown by the $LD_{99}$ and confidence interval (CI) estimates (Table 2) and the CT products (Table 3). While EDN was also more toxic than MB to BPL adults (Table 3), the differences were not statistically significant.

Concentration × time (CT) products are normally used to compare fumigation efficacy between species and fumigants. Thus, when comparing the CT products for the Asian longhorned beetle larvae, *Anoplophora glabripennis* Motschulsky, another wood-related cerambycid (Ren et al. 2006), with those calculated here for BPL larvae (Table 3), at 10°C and 20°C, the CT products reported by Ren et al. (2006) were found to be almost 10 times higher than those resulting from the present tests. This indicates that there are species-specific differences in EDN toxicity to wood-related cerambycids and confirms that EDN is highly toxic for BPL larvae. They also indicate that low $LD_{99}$ values (Table 2), and therefore low CT products, are achievable. However, higher doses of EDN may be needed if subsequent tests indicate that adjustments must be made to account for fumigant losses caused by sorption, when used to treat wood products (Pranamornkith et al. 2014b; Hall et al. 2015).

Although scientific evidence on the toxicity of EDN to forest insects is scarce, the information that is available consistently shows that EDN is quick-acting and highly toxic to insects and in most cases, more toxic than MB or other fumigants tested in comparison (e.g. phosphine and sulfuryl fluoride), which corresponds with the present findings. Fumigations with naked life stages of the Asian longhorned beetle were carried out using low concentrations for 6 h at 21–25°C (Ren et al. 2005). EDN was very toxic (100% mortality) to all immature and adult stages at a concentration of 11 g/m$^3$. In a follow-up study, the toxicity of EDN to naked larvae of the Asian longhorned beetle was tested at a range of temperatures (4.4, 10.1, 15.6 and 20.0°C) and for 3 or 6 h (Ren et al. 2006). EDN was, once again, shown to be highly toxic to the larvae, even at low temperatures, with mortality varying according to temperature, time of exposure and dose. Toxicity studies of EDN and MB to naked insects of six forest species, including pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera: Curculionidae), fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), Japanese termite, *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae) (Park et al. 2007), *C. brevis*, *Coptotermes cynocephalus* (Light) and *Cryptotermes domesticus* (Havilend) (all Isoptera: Kalotermitidae) (Wright et al. 2002), were also determined. EDN was found to be more toxic than MB to all the species tested.

**Table 3** Concentration × time products (CT; g.h/m$^3$) of ethanedinitrile and methyl bromide to burnt pine longhorn beetle eggs, larvae and adults at 10°C or 20°C. CT data are $LD_{99}$ values from Table 2 (in g/m$^3$) × duration of treatment (in h).

| Life stage | Temperature | Ethanedinitrile | Methyl bromide |
|---|---|---|---|
| Egg | 10°C | 5.1 | 64.2 |
| Larval | 10°C | 22.0 | 90.9 |
| Adult | 10°C | 19.4 | 36.0 |
| Egg | 20°C | 1.5 | 36.4 |
| Larval | 20°C | 19.5 | 55.2 |
| Adult | 20°C | 16.8 | 31.8 |

All together, these previous studies and the present results indicate that EDN is consistently more toxic to forest insects than MB. Thus, control of timber or wood-related insect pests, particularly coleopterans, lepidopterans and isopterans, with EDN seems to be achievable with comparatively low concentrations.

## CONCLUSION

The present findings indicate that EDN is highly toxic to all naked life stages of BPL tested and that it may be an effective alternative pre-shipment treatment to MB for log exports, in accordance with initial findings by Pranamornkith et al. (2014c). Reduced rates of MB also seem promising as indicated by the present results and those by Pranamornkith et al. (2014a) and Somerfield et al. (2013). The next step for gathering a robust efficacy dataset for respective fumigants will be treating logs with the most tolerant life stage of the BPL and investigating factors such as fumigant sorption (Pranamornkith et al. 2014b; Hall et al. 2015) and penetration (M.K.D. Hall, unpublished data). The findings from these studies will be used to develop fumigation schedules for commercial treatment of New Zealand logs with either EDN or reduced rates of MB.

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