# Evaluation of ethanedinitrile (EDN) as a methyl bromide alternative for eradication of European House Borer (EHB)

Emery, R.N.#[1], Ren, Y.L.*[1,2,3], Newman, J.[2,3], Thalavaisundaram, S.[4]
[1]Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia
[2]School of Veterinary and life Sciences, Murdoch University, WA, 6150, Australia
[3]Cooperative Research Centre for National Plant Biosecurity, GPO Box 5012, Bruce, ACT 2617, Australia
[4]BOC limited, 10 Julius Avenue, North Ryde NSW 2113, Australia

*Corresponding author, Email: y.ren@murdoch.edu.au
#Presenting author, Email: rob.emery@agric.wa.gov.au

DOI: 10.14455/DOA.res.2014.150

## Abstract

European House Borer (EHB) *Hylotrupes bajulus* Linnaeus, is considered a serious invasive threat because it is a destructive pest of seasoned coniferous timber including pine, fir and spruce. If allowed to become established it can cause major structural damage to buildings. The pest is able to live in a variety of climatic conditions however it prefers temperate habitats. Methyl bromide is widely used for quarantine treatment but it is being phased out due to its listing as an ozone depleting substance. Furthermore, complete control of EHB with methyl bromide in timber at low temperatures and high moisture content is difficult. Currently, there are no known chemical or biological controls for EHB larvae in timber and wood packaging and the obvious way to reduce the quarantine threat posed by EHB is to develop reliable and effective means to disinfest wooden materials at the source. There is an urgent requirement for an alternative effective fumigant for the control of EHB larva in timber and wood. Ethanedinitrile (EDN) is a new fumigant (patented under the chemical name "cyanogen") and it is known to be highly toxic to insect pests of timber and is fast acting. It is believed to have particular potential as a quarantine treatment for timber. EHB larvae of differing lengths were found to have varying tolerance to both EDN and methyl bromide with the larger EHB found to be more tolerant. In all experimental fumigations with larvae of a range of lengths and at varying exposure times (6 and 24 hours), EDN showed more toxicity than methyl bromide. EDN achieved 100% mortality at 40 mg/L, 25°C and 70% r.h. for 24 hours of exposure with timber blocks artificially infested with EHB. However, under the same experimental conditions, methyl bromide at 48 mg/L only achieved a mortality range of 97.3-100%.

Keywords: ethanedinitrile, methyl bromide alternative, European house borer, timber fumigation

## 1. Introduction

European House Borer (EHB) *Hylotrupes bajulus* Linnaeus (Fig. 1) is considered a serious invasive threat because it is a destructive pest of seasoned coniferous timber including pine, fir and spruce. If allowed to become established it can cause major structural damage to buildings. EHB is able to live in a wide variety of climatic conditions however it prefers temperate habitats. The damage is done by EHB larvae that hatch from the eggs and it can live in the larval state for two to twelve years until it matures and emerges from the timber as an adult beetle to begin the cycle again. The timber can be repeatedly infested until no sound wood remains and structural collapse may occur. Wood infested by EHB larvae is hard to identify and is often only detected after the mature beetle has emerged from the timber to take flight. EHB generally infests roof timbers but is also known to infest architraves, door frames and timber articles such as pine furniture, packaging and pallets.

EHB is native to Europe, where it has been the subject of research and control for more than 100 years. In Germany in the 1930's it was regarded as the most serious pest of softwood timbers with surveys of houses showing average infestation rates of 46%, and up to 80% of buildings infested in some areas (M Grimm, pers comm). Since then, the use of treated timbers and changes in construction methods has minimised the damage caused by EHB in Germany. In South Africa it was first found in the 1879, in pine packing imported from Italy. In the first 30 years after introduction it caused little concern, but by the 1940's EHB showed itself to be a destructive pest that could only be controlled by use of treated pine. In the United States, EHB has been active since its introduction from Europe more than 100 years ago, and is now regarded as a significant pest in states where it is established.

EHB was first detected in Australia in 1953 in prefabricated pine houses imported from Austria and other parts of Europe (Duffy, 1957). These infested houses were found in Queensland, New South Wales, Victoria and South Australia, but not Western Australia. In fact, similar houses imported into Western Australia in the late 1940's and 1950's were all fumigated or heat treated during importation, and no signs of EHB were ever found. The detections in the eastern states of Australia were made within a few years of importation, while the pest was still in the imported timber, and EHB was never found breeding and surviving in the Australian environment outside of the imported houses. This incursion was subjected to an eradication program and, by 1970 it was considered to have been eradicated from Australia (Eldridge and Simpson, 1987).

In January 2004 an inquiry by a member of the public to the Department of Agriculture and Food Western Australia (DAFWA), Pest and Disease Information Service, resulted in the recovery of an adult female EHB from a decorative pine beam within the house. Subsequently, a final instar larva was recovered from a second pine beam, having been located by the audible scraping made as the larva fed. Compared to the incursion in the Eastern States, the detection of EHB in Perth was much more serious and potentially difficult to deal with because the pest was found to have established in the Western Australian environment and was no longer confined to an identified imported item.

In Western Australia kiln dried plantation gown *Pinus radiata* and *Pinus pinaster* timbers largely replaced Australian native hardwoods in building construction since about 2001. More than 80% of this structural pine is not treated with preservatives, and is at risk from attack by EHB. The replacement cost of untreated pine in house construction in Western Australia is estimated to be about $3 billion (Grimm, 2005).

EHB was formally listed as a quarantine pest and its spread is controlled by the Western Australian Government through the Agriculture and Related Resources Protection (European House Borer) Regulations 2006, which came into force on 7th February 2006 (ARRPA, 2006). Under the regulations, the movement of untreated pine is not permitted unless it has been stored or treated in accordance with the regulations and with approval of the Department of Agriculture and Food. The International Standards for Packaging Materials (ISPM 15) recognises the risk of forestry and timber pests and diseases being spread in stored product timber packing and dunnage in international trade. Furthermore, inter-state import regulations also require treatment of EHB host material, including packaging, originating from the Restricted Movement Zones in Western Australia.

Therefore, in accordance with the ARRPA Regulations, Western Australian pinewood, packaging and dunnage is required to be:

- Treated with a preservative in accordance with the Australian Standard AS/NZS 1604; or
- Heated to a core temperature of more than 60°C for not less than 30 minutes; or

- Fumigated with methyl bromide; or
- Fumigated with another fumigant chemical in a manner approved by an authorised person.

Methyl bromide is widely used for quarantine treatment however it has been listed as an ozone depleting substance under the Montreal Protocol and phased out in all EU countries and some other developed countries. In addition, it is hard to achieve complete control of EHB using methyl bromide in timber at low temperatures and high moisture content. Currently, there are no known chemical or biological controls for control of the EHB larvae in timber and wood packaging. The obvious way to mitigate the international and national quarantine threat EHB poses is to develop reliable and effective means to disinfest wooden materials at the source and there is an urgent need for the development of an alternative effective fumigant for the control of the EHB larva in timber and wood.

Ethanedinitrile (EDN) is a new fumigant (patented under the chemical name cyanogen). It is highly toxic to timber insects and is fast-acting. Accordingly we believe it has particular potential as a quarantine treatment for timber. There is currently no available scientific research to support the established Australian Quarantine Inspection Service (AQIS) schedule for EHB fumigation with methyl bromide. The aims of our research was to conduct bioassays to verify the quarantine schedule for treatment of EHB with methyl bromide, and to conduct bioassays to determine the toxicity of EDN for EHB larvae in timber and provide and alternative method for eradication.

Previous research by DAFWA had established a colony of EHB at the South Perth laboratories and, as the program was drawing to a close, the colony was scheduled for destruction. This colony was securely moved to Murdoch University to conduct bioassays to determine toxicity of EDN for EHB larvae in timber.

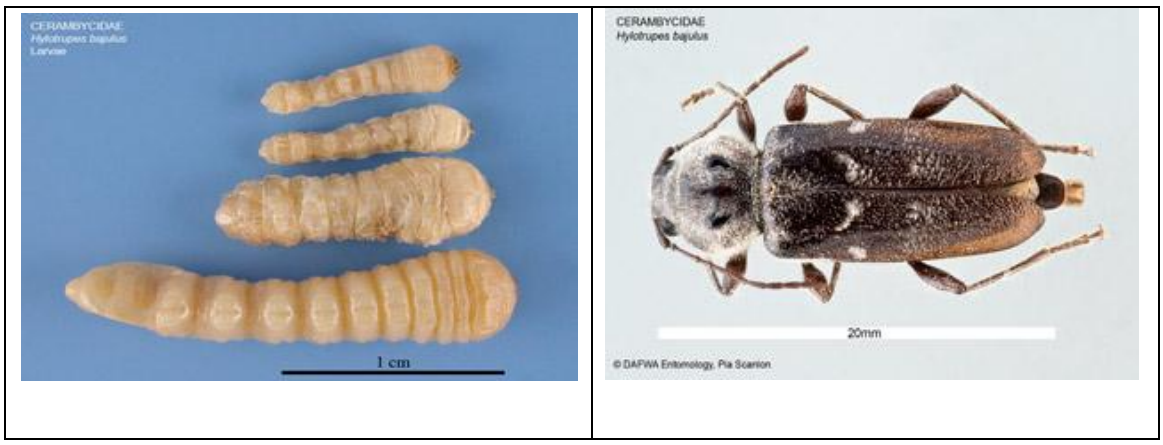


**Figure 1** European House Borer, *Hylotrupes bajulus*, larvae and adult.

## 2. Materials and Methods

### *2.1. Phase 1: Fumigate naked EHB larvae*

#### *2.1.1. Collection of European house borer larvae and adults*

Mixed aged EHB larvae and adults were collected by splitting naturally infested logs obtained from the DAFWA South Perth quarantine laboratory. The moisture content of the logs was typically 10-11% wet basis and the core temperature was 24-26°C. Larvae were held in paper cups (200 ml) filled to 30% capacity with fresh sawdust and five larvae per cup. Adults were held in mesh cages (35 mm x 35 mm) each containing 5 insects.

*2.1.2. Fumigation of "naked" (i.e. without timber) European house borer larvae and adults*

The fumigation chambers were 2.4 litre desiccators equipped with a ground glass stopper fitted with a septum (Alltech Associates Australia, Cat. No. 15419). The volume of each desiccator was estimated by the weight of water it held at 25°C. Ten glass jars (50 mm x 70 mm i.d.) each containing 10 EHB larvae were placed in the unsealed desiccators and left to acclimate overnight at 25°C and 70% r.h. prior to the fumigation treatment.

The dosages and exposure periods for larvae experiments are listed below:

| EDN mg/L | | MB mg/L | |
|---|---|---|---|
| 6 hr | 24 hr | 6 hr | 24 hr |
| 5 | 3 | 5 | 3 |
| 10 | 6 | 15 | 6 |
| 20 | 9 | 25 | 9 |
| 30 | 12 | 35 | 12 |
| 40 | 15 | 45 | 15 |

For EHB adults, 20 metal mesh cages (35 mm x 35 mm i.d.) each containing 5 insects were fumigated in the desiccators at 25°C and 70% r.h. Each treatment was replicated three times.

The dosages and exposure periods for adult experiments are listed below:

| EDN mg/L | | MB mg/L | |
|---|---|---|---|
| 6 hr | 24 hr | 6 hr | 24 hr |
| 1 | 0.4 | 5 | 1.0 |
| 1.5 | 0.5 | 6 | 1.7 |
| 2.2 | 0.6 | 7 | 2.0 |

At the completion of the 6 and 24 hour fumigations, the desiccators were opened and aired for 1 h in a fume cupboard. After aeration the insects (treated and untreated) were placed in recovery glass jars (50 mm x 70 mm i.d.) containing 10 g of fresh sawdust, and then incubated at 25°C and 70-75% r.h. Live and dead larvae and adults were counted after 4 days. Larvae showing any movement were considered to be alive. For these assays, the control mortality was always zero.

Fumigant dosage: The dosages or required volumes for the fumigant concentrations were calculated from Eq 1.

$$V_f = \left(1 + \frac{T}{273}\right)\left(\frac{1.7 \times 10^4 \times C \times V}{P \times M \times N}\right) \qquad \text{Eq. 1.}$$

Where: V - volume of fumigation chamber (litre)
P - atmospheric pressure (mmHg)
T - temperature (°C)
C - intended concentration (mg/l)
Vf - dose volume of fumigant (ml)
M - molecular weight of fumigant
N - purity of fumigant (%)

*2.1.3. Determination of methyl bromide and ethanedinitrile*

Methyl bromide and ethanedinitrile was determined on a DPS GC equipped with a flame ionisation detector (FID) after separation on a 30 m x 0.53 mm ID GS-Q column at 100°C with a carrier flow (H2) of 20mL/min.

*2.1.4. Determination of concentration x time products (Cxt) for naked EHB larvae exposure to methyl bromide and ethanedinitrile*

The concentrations of fumigants were monitored at time intervals over the exposure period and were used to calculate the product *Ct* = Concentration x time. The *Ct* products were calculated from Eq. 2, and determined from four replicate measurements.

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

Where: C - fumigant concentration (mg/L)
t - time of exposure (hours)
i - the order of measurement
*Ct* - Concentration x time products (mg h/L)

*2.2. Phase 2: Fumigation of timber infested with EHB larvae*

*2.2.1. Timber artificially infested with EHB larvae (within timber)*

Pine timber blocks were artificially infested with mixed aged EHB larvae by drilling, inserting one larva and plugging with pine dowelling.

*2.2.2. Fumigation chamber and fumigation*

The fumigation chamber (47.8 cm x 40.6 cm internal diameter) was constructed from steel with the net volume of the chamber being 62 litres. The removable lids were fitted with a centrally located septum fitting for introduction of gas during fumigation. The fumigation chamber was fitted with two gas sampling ports which were located on the side of the fumigation chamber near the top and bottom (Fig. 2). Each lid was fitted with gas-tight seals which were pressure-tested before use. The fumigation chambers were well-sealed with a pressure halving time of greater than three minutes.

The infested timber blocks (100 x 100 x 300 mm at about 30% loading ratio) were placed in the chamber and fumigated with methyl bromide at 48 g/m$^3$ and ethanedinitrile at 40 g/m$^3$ for 24 hours at 25°C. Methyl bromide at 48 g/m$^3$ for 24 h is the current AQIS recommended standard quarantine dosage for wood fumigation (AQIS 2002).

The calculation of loading ratio was from as Eq. 3.

$$L = V_t / V_f \qquad \text{Eq. 3.}$$

Where: L - loading ratio (%)
Vt - volume of solid timber (L)
Vf - volume of fumigation chamber (L)

*2.2.3. Determination of mortality*

After fumigation, the top of chamber was opened and vented for 24 hours. The fumigated timber blocks were split and mixed aged EHB larvae were collected and placed in recovery glass jars (50 mm x 70 mm i.d.) containing 10 g of fresh sawdust, and then incubated at 25°C and 70-75% r.h. Live and dead larvae were counted after 4 days and larvae showing any movement were considered to be alive. The experiment was replicated twice (Experiments 1 and 2). For all assays control mortality was zero.

**Figure 2** The fumigation chamber (47.8 cm x 40.6 cm internal diameter) equipped with gas injection and sampling systems.

## 3. Results and Discussion

*3.1. Toxicity of EDN and methyl bromide to "naked" EHB larvae and adults with bioassay*

European house borer larvae of different lengths were found to have varying tolerance to both EDN and methyl bromide with the larger EHB being more tolerant (Table 1). In all experiments with larvae of varying length and different exposure periods (6 and 24 hours), EDN was more toxic than methyl bromide (Table 1).

Experimental treatment of EHB adults shows that EDN was more toxic than methyl bromide with an average Ct ratio of 0.79 (Table 2).

**Table 1** Dosage (measured as *Ct* product, mg h/L) estimates of 100% mortality for exposure different length (<2 cm and >2 cm) of EHB larvae to EDN and methyl bromide for the 6 and 24 hour fumigations at 25.0±0.5°C and 70.0±0.8% r.h.

| | **Ct for 6 hr exposure (mg h/L)** | | **Ct for 24 hr exposure (mg h/L)** | |
|---|---|---|---|---|
| Larvae size | <2 cm | >2 cm | <2 cm | >2 cm |
| EDN | 130 | 240 | 150 | 250 |
| MB | 160 | 300 | 180 | 340 |
| EDN:MB | 0.81 | 0.80 | 0.83 | 0.73 |

**Table 2** Dosage (measured as *Ct* product, mg h/L) estimates of 100% mortality for exposure of EHB adults to EDN and methyl bromide for the 6 and 24 hours fumigations at 25.0±0.5°C and 70.0±0.8% r.h.

| | **Ct for 6 hr exposure (mg h/L)** | **Ct for 24 hr exposure (mg h/L)** |
|---|---|---|
| Experiment-1 | | |
| EDN | 9 | 12 |
| MB | 36 | 40 |
| Experiment-2 | | |
| EDN | 13 | 15 |
| MB | 42 | 48 |

*3.2. Toxicity of EDN and methyl bromide to naked EHB larvae in timber blocks*

Fumigation experiments with timber blocks artificially infested with EHB showed that 100% mortality was achieved with EDN fumigation at 40 mg/L (25°C, 70% r.h.) for 24 hours. However, with methyl bromide, mortality ranged between 97.3 and 100 percent at 48 mg/L (25°C, 70% r.h.) for 24 hours. This methyl bromide dosage is the current AQIS recommended quarantine treatment for wood fumigation (AQIS, 2002) and appears to be marginal with respect to achieving eradication of EHB within timber (Table 3).

## 4. Conclusions

European House Borer larvae of differing lengths were found to have varying tolerance to both EDN and methyl bromide with the larger EHB found to be more tolerant. In all experimental fumigations with larvae of a range of lengths and over varying exposure periods (6 and 24 hours), EDN showed more toxicity (about 1.2x) than methyl bromide. In experimental fumigations with adults over varying exposure periods (6 and 24 hours), EDN showed more toxicity (about 3.5x) than methyl bromide. EDN fumigation of timber blocks artificially infested with EHB achieved 100% mortality of larvae at 40 mg/L, 25°C and 70% r.h. for 24 hours of exposure. However, under the same experimental conditions, methyl bromide at 48 mg/L only achieved a mortality range of 97.3-100%.

**Table 3** Mortality of EHB larvae with 24 hours of exposure to EDN (40 mg/L) and methyl bromide (48 mg/L) at 25°C and 70% r.h.

| **Experiment-1** | **Day 1** | | **Day 4** | | |
|---|---|---|---|---|---|
| | **Dead** | **Alive** | **Dead** | **Alive** | **% mortality** |
| EDN | 41 | 0 | 41 | 0 | 100% |
| MB | 29 | 0 | 29 | 0 | 100% |
| Experiment-2 | | | | | |
| EDN | 24 | 0 | 24 | 0 | 100% |
| MB | 36 | 2 | 37 | 1 | 97.3% |

**Acknowledgements**

We wish to thank the Western Australian EHB Response Program and the Forest Products Commission for use of the South Perth quarantine laboratory and for making specimens available for bioassays. We acknowledge BOC Limited for financial support and the Plant Biosecurity Cooperative Research Centre for research collaboration and conference travel support.

**References**

AQIS, 2002. Import case details - public listing, timber packaging and dunnage Treatment T9047. http://apps.daff.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=9006252&intCommodityId=17670 Last accessed: November 2014.

ARRPA, 2006. Agriculture and Related Resources Protection (European House Borer) Regulations. 2006. http://www.ehb.wa.gov.au/html/mangt02_regulations.htm Last accessed: November 2014.

Grimm, M., 2005. Incursion of *Hylotrupes bajulus* Linnaeus (European House Borer) into Western Australia. In: The international research group on wood preservation, 36th Annual Meeting, Bangalore, India.

Howick, C.D., Carr, H., 1971. Status of the European House Borer in Australia and an example of Quarantine action to prevent its establishment. International Pest Control 13, 8-11.

Duffy, E.A.J., 1957. A Monograph of the immature stages of African timber beetles (Cerambycidae). Commonwealth Agricultural Bureaux, London pp 338.