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Toxicity of Ethanedinitrile to *Anoplophora glabripennis* (Coleoptera: Cerambycidae) Larvae

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ABSTRACT The mortality of naked *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) larvae exposed to ethanedinitrile (cyanogen; C₂N₂) varied with temperature, time of exposure, and dose of ethanedinitrile. The concentration × time (Ct) product of ethanedinitrile over a range of temperatures (4.4, 10.1, 15.6, and 20.1°C) decreased with increasing temperature, for both 3- and 6-h exposures. The Ct products varied with time of exposure at different temperatures. The variations in mortality at different temperatures are described with a slope ratio ($Y = [\text{slope}]_{6h} / [\text{slope}]_{3h}$). At different temperatures, the concentration of ethanedinitrile and the duration of exposure play different roles in killing *A. glabripennis* larvae. These results suggest the control of *A. glabripennis* larvae within wood is achievable.

KEY WORDS *Anoplophora glabripennis*, fumigant, ethanedinitrile, cyanogen, toxicity

Anoplophora glabripennis Motschulsky (Coleoptera: Cerambycidae) is native to parts of Asia, and it is considered a serious invasive threat because it attacks and kills many varieties of hardwood trees, such as maple, elm, horse chestnut, ash, birch, poplar, and willow (Barak et al. 2002). *A. glabripennis* could significantly disrupt forest ecosystems if it became established outside its native range. The larval stage of the *A. glabripennis* is the most destructive stage for timber and timber products, such as wood packaging. The potential introduction of *A. glabripennis* is serious threat worldwide, because it can be found in the wood packaging of imported goods from China.

Methyl bromide is widely used for quarantine treatment. However, methyl bromide was phased out by 2005 because of its listing as an ozone-depleting substance under the Montreal Protocol (UNEP 1996). In addition, it is hard to achieve complete control of *A. glabripennis* larva with methyl bromide at low temperatures and in timber with high moisture content. Currently, there is no known chemical or biological control for *A. glabripennis* larvae in timber and wood packaging. In all infestations, trees are being removed, chipped, and burned. The obvious way to reduce the quarantine threat by *A. glabripennis* is to develop reliable and effective means to disinfect wooden packing materials at the source. There is, therefore, an urgent requirement for the development of an alternative

effective fumigant for the control of *A. glabripennis* larvae in timber and wood packaging.

Ethanedinitrile (C₂N₂) is a patented new fumigant (patented under the chemical name cyanogen) and is thought to have particular potential as a quarantine treatment for timber (O'Brien et al. 1995, Viljoen and Ren 2001, Wright et al. 2002). The physical and chemical behavior of ethanedinitrile is well documented (Brotherton and Lynn 1959). It is a colorless gas with an almond-like odor. Hooper et al. (2003) reported that the toxicity of ethanedinitrile to stored-product insects increases with both relative humidity and concentration of CO₂. Ethanedinitrile is highly toxic to stored-product insects and is fast acting (Hooper et al. 2003). Ethanedinitrile has a threshold limit value of 10 ppm, which compares favorably with other fumigants: 5 ppm for methyl bromide and 0.3 ppm for phosphine. It is not listed as a gas contributing to global warming or ozone depletion.

The Commonwealth Scientific and Industrial Research Organization Entomology, the Institute of Animal and Plant Quarantine of China, and USDA–Animal and Plant Health Inspection Service (APHIS)–Plant Protection and Quarantine (PPQ) have been assessing the efficacy of ethanedinitrile. This article reports on the initial toxicity studies undertaken in Lanzhou, People's Republic of China, where we were able to obtain the *A. glabripennis* larvae from infested logs.

Materials and Methods

Fumigation Chamber. Bioassay fumigations were conducted in stainless steel fumigation chambers (45 by 45 by 50 cm) held within a temperature-regulated

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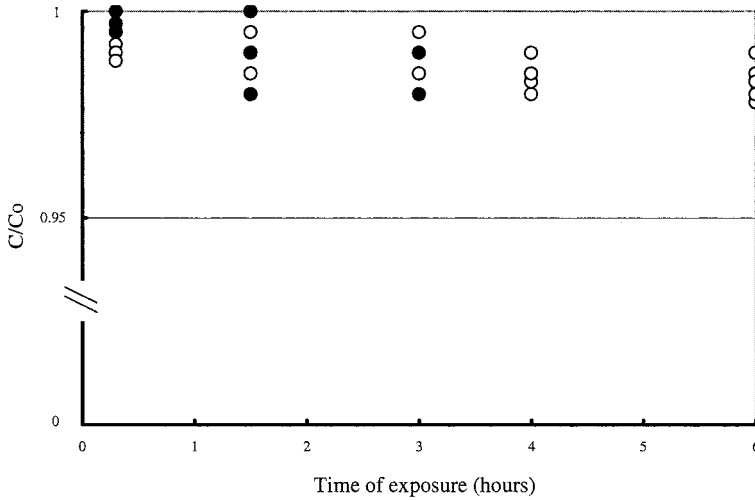


Fig. 1. The ratio (C/C_0) of measured ethanedinitrile concentration (C) in the fumigation chamber to applied fumigant concentration (C_0), at temperature range of 4.4–21.1°C and dose range of 1–80 mg/liter for 3-h (●) and 6-h (○) exposure.

shipping container (THERMO KING, Ingersoll-Rand Asia Pacific, Seoul, Korea) with a capacity of 28.2 m³. The container temperature could be adjusted to between 0 and 50°C, and temperature variation in the set point was 5–18°C.

Measurement of Ethanedinitrile Purity and Concentration. Ethanedinitrile was supplied by the United Fluoride China, People's Republic of China. The purity of the gas was measured on a model 40-001, GOW-MAC gas density balance (GOW-MAC Instrument Co., Bethlehem, PA), after separation on a 1 m by 5 mm i.d. Porapak Q 100/120 mesh (catalog no. 2702, Alltech Associates, Deerfield, IL) at 105°C and carrier (N₂) flow of 150 ml/min. The reference gas was tetrafluoroethane (>99.9% pure). The purity of ethanedinitrile was 85% with CO₂ and air being the main impurities.

During the fumigation, the concentrations of ethanedinitrile and CO₂ in the fumigation chamber were measured with a XK-3 Fumigant monitor (Beijing CIPQ Instrument Co., Beijing, China) and a CO₂ detector (Shanghai, China) respectively. The XK-3 Fumigant monitor was fitted with a thermal conductivity detector. There was a CO₂ interference requiring the use of a CO₂ detector to calculate calibration factors of CO₂.

***A. glabripennis* Larvae.** Mixed aged *A. glabripennis* larvae were collected by splitting naturally infested fresh poplar (*Populus* spp.) logs obtained near city of Lanzhou (35° 34' N, 103° 18' E), Gansu Province, People's Republic of China. The moisture content of the logs was typically 41–54% wet basis, and the core temperature of logs was 6–10°C. Five larvae were placed in paper cups (200 ml) filled to 30% capacity with fresh poplar sawdust. The cups were then transferred into the fumigation chambers and held within the refrigerated container for 24 h at 4.4, 10.0, 15.6, or 21.1°C.

Calculation of Fumigant Dosage. To obtain a fumigant concentration in the chamber required the calculation of the dose or volume of ethanedinitrile at the experimental ambient temperature and pressure. The following equation (Atkins and Paula 2002) was used for the calculation of the dose of fumigant:

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

where V is volume of fumigation chamber (liters), P is atmospheric pressure (mmHg), T is temperature (°C), C is intended concentration (mg/liter), V_f is dose volume of fumigant (ml), M is molecular weight of fumigant, and N is purity of fumigant (%) or 85%.

Preparation of Fumigant. Ethanedinitrile was transferred from a gas cylinder into a Tedlar gas bag (20 liters) and left for 1-h equilibration to ambient temperature and pressure. The calculated volume of gas was drawn with a 2-liter Jumbo syringe (P/N 009920, A12Z9648, SGE International Pty Ltd., Ringwood, Victoria, Australia), and stored in Tedlar gas sampling bags (1–5 liter) until application.

Bioassay Procedures for *A. glabripennis* Larvae. The fumigation chambers were conditioned at 4.4, 10.0, 15.6, and 21.1°C in a temperature-regulated shipping container overnight. Ten paper cups (each cup containing five larvae without sawdust) were placed in the fumigation chamber. The local ambient air relative humidity was 45–55%. The chambers were sealed before application of fumigant in the container. After removal of the same volume of air as the injected fumigant, the fumigant was sucked into the chamber. The control larvae were maintained in a sealed chamber without fumigant until completion of exposure. At the end of the fumigation period, the treated and control chambers were opened for 1 h of aeration. The larvae were transferred to new cups, 30% full of fresh

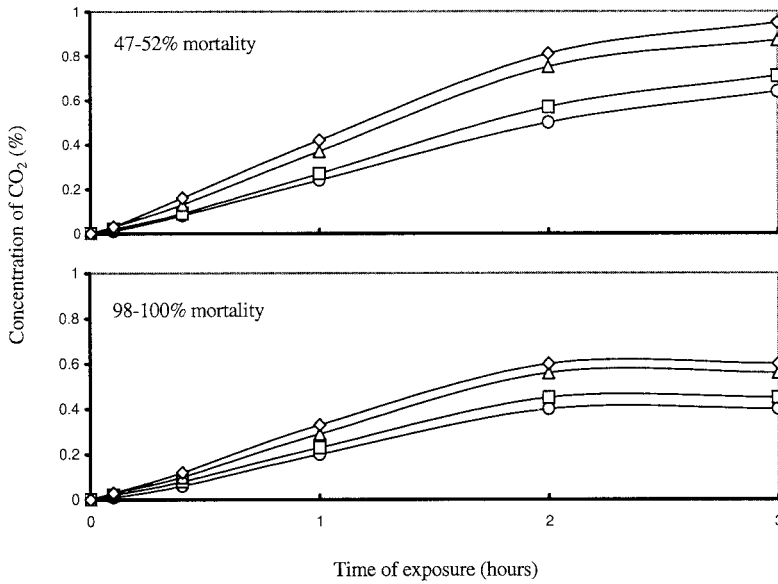


Fig. 2. The levels of CO₂ in the fumigation chambers during exposure of *A. glabripennis* larvae to different applications of ethanedinitrile at different temperatures (○, 4.4°C; □, 10°C; △, 15.6°C; and ◇, 21.1°C) and mortality ranges of 47-52 and 98-100% for 3-h exposure.

sawdust, and then incubated at 25°C and 75-85% RH. Live and dead larvae were counted after 4 d. Larvae showing any movement were considered to be alive. For these assays, the control mortality was always zero.

The concentrations of ethanedinitrile and CO₂ were monitored at timed intervals (from 10 min after application until opening the chambers) over the ex-

posure period (3 and 6 h) and were used to calculate the product Ct = concentration × time.

Calculation of Mortality. The toxicity of ethanedinitrile to *A. glabripennis* larvae was described as the mortality of 50 larvae for at least 12 different Ct products. The L(Ct)₅₀ and L(Ct)_{99.5} values were calculated by probit analysis using a computer program produced by P. C. Annis of the Commonwealth Sci-

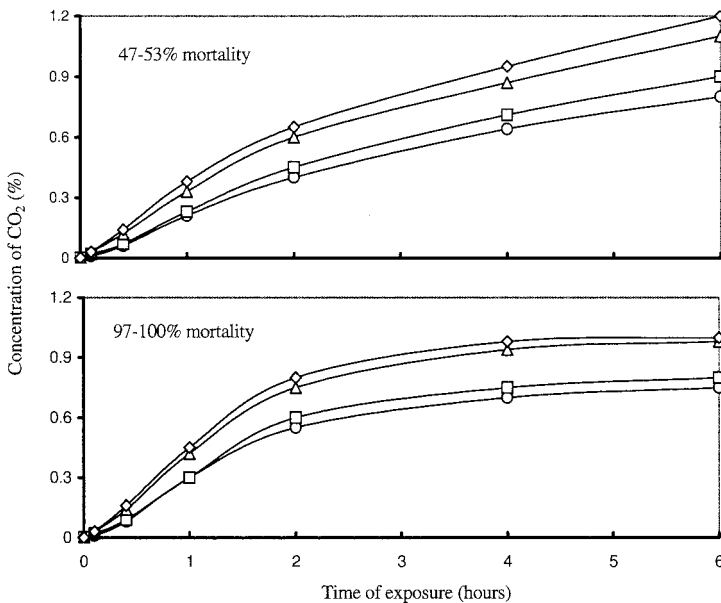


Fig. 3. The levels of CO₂ in the fumigation chambers during exposure of *A. glabripennis* larvae to different applications of ethanedinitrile at different temperatures (○, 4.4°C; □, 10°C; △, 15.6°C; and ◇, 21.1°C) and mortality ranges of 47-52 and 98-100% for 6-h exposure.

Table 1. Dose estimates and parameters of regression of probit mortality of log concentration with 6 df for exposure of the naked *A. glabripennis* larvae to ethanedinitrile for 3 and 6 h at different temperatures

Temp (°C [°F])	Time of exposure (h)	L(Ct) ₅₀ (mg h/liter)	L(Ct) _{99.5} (mg h/liter)	Slope ± SE	Heterogeneity (χ ²)
4.4 [40]	3	94.41	282.49	4.96 ± 0.62	5.52
	6	101.39	353.38	4.05 ± 0.69	2.68
10.0 [50]	3	72.61	240.99	5.24 ± 0.80	2.17
	6	74.13	221.82	5.42 ± 0.47	3.26
15.6 [60]	3	48.73	130.50	5.42 ± 0.64	4.97
	6	53.46	126.77	6.87 ± 0.74	3.91
21.1 [70]	3	33.57	95.28	5.69 ± 0.84	3.54
	6	29.51	56.62	9.14 ± 0.56	2.92

entific and Industrial Research Organization Entomology, Australia, based on Finney (1971).

Results and Discussion

Concentrations of Ethaneditrile and CO₂ during Exposure. The decay of ethanedinitrile in the fumigation chamber is shown in Fig. 1, which shows the ratio (C/Co) of measured concentration (C) to applied concentration or initial concentration (Co) against exposure time. The concentrations of ethanedinitrile were nearly constant in the chamber, and the apparent loss of fumigant was <3% in all cases. Therefore, the mean value of concentration can be used to calculate Ct products, and the standard error in fumigation concentration was <2% of the mean value in all cases.

The toxicity of ethanedinitrile can be increased with CO₂ (Hooper et al. 2003). Therefore, the levels of CO₂ produced by *A. glabripennis* larvae were monitored at different temperature and mortality ranges of 47–53 and 97–100% for 3 and 6 h of exposure. During the fumigation, CO₂ was increased by respiration of the larvae, and results are shown in Figs. 2 and 3. For

3 h of exposure, in both 47–52 and 98–100% mortality, CO₂ was <1% (Fig. 2). For 6 h of exposure, in both 47–53 and 97–100% mortality, CO₂ was <1.2% (Fig. 3). In general, CO₂ concentration was increased at high temperature and/or low mortality (Figs. 2 and 3). CO₂ significantly enhanced the toxicity of ethanedinitrile to insects when its concentration was 30%, but 1% has no effect on mortality (Hooper et al. 2003). Experiments on CO₂ enhancement of phosphine toxicity and ethyl formate toxicity to stored-product insects showed that at concentrations of >5% CO₂, there was an enhancement of the fumigant mortality (Ren et al. 1994, Lee 2003). Therefore, 1–1.2% CO₂ (generated by *A. glabripennis* larvae) has no effect on the bioassay results.

Toxicity of Ethaneditrile to *A. glabripennis* Larvae. Mortality of *A. glabripennis* larvae exposed to ethanedinitrile varied with temperature, time of exposure, and dose of ethanedinitrile. The Ct products of ethanedinitrile treatments over a range of temperatures varied with time of exposure (Table 1). In both 3- and 6-h exposures, the toxicity of ethanedinitrile increased with temperature elevation. For both 3- and 6-h exposures, the Ct products of L(Ct)₅₀ values de-

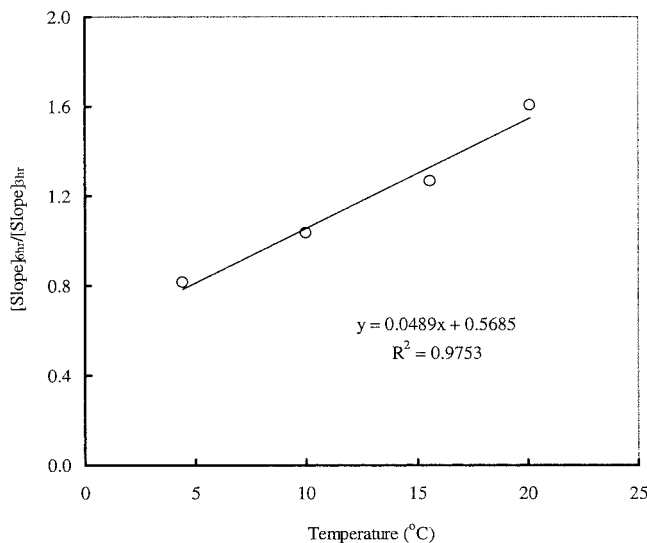


Fig. 4. The ratio ([slope]_{6h}/[slope]_{3h}) of the slope of the probit line for a 6-h exposure ([slope]_{6h}) to slope for a 3-h exposure ([slope]_{3h}), over the temperature ranges of 4.4–21.1°C.

creased three-fold when the temperature increased from 4.4 to 21.1°C (Table 1). The Ct products of $L(Ct)_{99.5}$ values increased 3 and 6 times for 3- and 6-h exposure, respectively. For 3-h exposure, the slope increased with temperature, but the increase in slope was much more pronounced for the 6-h exposure (Table 1). The variation in mortality at different temperatures and times of exposure are described with a slope ratio ($[\text{slope}]_{6h} / [\text{slope}]_{3h}$). This is shown in Fig. 4, which plots the ratio of slope for a 6-h exposure ($[\text{slope}]_{6h}$) to that for a 3-h exposure ($[\text{slope}]_{3h}$) against temperature. This ratio doubled over the studied temperature range. It is described by the following equations:

$$Y = [\text{slope}]_{6h} / [\text{slope}]_{3h} \quad [1]$$

$$Y = 0.5685 + 0.0489T \quad [2]$$

where Y is slope ratio (1 and 2) and T is temperature (°C). Therefore, equation 2 indicates three possible conditions that depend on temperature. For condition 1, when $T = 9^\circ\text{C}$, $Y = 1$, and $[\text{slope}]_{6h} = [\text{slope}]_{3h}$, which indicates changing the exposure time will not change $L(Ct)_{50}$ and $L(Ct)_{99.5}$ of ethanedinitrile to *A. glabripennis* larvae. That is, concentration and time of exposure play an equal role in killing *A. glabripennis* larvae at 9°C. Here, if Ct is given by

$$C^m t^n = K \quad [3]$$

where $m = n = 1$, when $T > 9^\circ\text{C}$, $Y > 1$, and $[\text{slope}]_{6h} > [\text{slope}]_{3h}$ (condition 2), which indicates the toxicity increases with increasing exposure time. That is, time of exposure plays a more important role than that of concentration. Therefore, $m < 1$ and $n > 1$.

For conditions 3, when $T < 9^\circ\text{C}$, $Y < 1$, and $[\text{slope}]_{6h} < [\text{slope}]_{3h}$, which indicates the toxicity increases with increasing concentration of ethanedinitrile. That is, concentration of ethanedinitrile plays a more important role than that time of exposure. Therefore, $m > 1$ and $n < 1$.

These results on the efficacy of ethanedinitrile to naked larvae are very encouraging. The comparable Ct products for methyl bromide and sulfuryl fluoride are higher than for ethanedinitrile (Barak et al. 2002). Ethanedinitrile penetrates through wood or across the grain faster than methyl bromide and carbonyl sulfide (O'Brien et al. 1995, Ren et al. 1997). In addition, ethanedinitrile can dissolve in water, and its toxicity to insects is increased with both relative humidity and CO_2 (Brotherton and Lynn 1959, O'Brien et al. 1995, Viljoen and Ren 2001). Therefore, in comparison with methyl bromide, sulfuryl fluoride, and carbonyl sulfide, ethanedinitrile seems to promise high efficacy of fumigation at high relative humidity and high CO_2 concentrations. It is suggested that quarantine treatment with ethanedinitrile of *A. glabripennis* larvae within wood is possible.

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