Relative methyl bromide tolerances of *Arhopalus fesus* (Mulsant), *Hylurgus ligniperda* (F.) and *Hylastes ater* (Paykull) adults

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Abstract The adult stages of three key New Zealand forest pests, burnt pine longhorn beetle (*Arhopalus fesus*), golden-haired bark beetle (*Hylurgus ligniperda*) and black pine bark beetle (*Hylastes ater*), were fumigated at 10 or 20°C for 4 h to determine their mortalities at increasing dosages of methyl bromide as a first step towards developing a new fumigation schedule for New Zealand export logs. *Hylastes* adults were the most methyl bromide tolerant of the three species ($LD_{99}=41.6 \text{ g/m}^3$) when fumigated at 10°C ($P<0.01$). When fumigated at 20°C, *Hylastes* were not significantly more tolerant than *Hylurgus* adults. The highest methyl bromide concentration required to control *Hylastes* adults is much lower than the phytosanitary requirement for export logs to India and China, which requires 120 g/m³ for 16 h at $\leq 15^\circ\text{C}$. This study suggests that a reduction in methyl bromide usage may be possible for logs exported from New Zealand, but further work on other life stages is required.

Keywords methyl bromide, fumigation, quarantine treatments, pine logs.

INTRODUCTION

The export of radiata pine (*Pinus radiata* D. Don) logs is a major and growing sector of New Zealand's forestry industry (Mead 2013). This industry is worth an estimated \$1.65 billion in the year ending 30 June 2013 (Ministry for Primary Industries 2013a). A significant proportion of logs exported to India and China are fumigated with methyl bromide on the wharf prior to shipment in order to meet the requirements of importing countries (Self & Turner 2009; Somerfield et al. 2013).

Burnt pine longhorn beetle (BPL), *Arhopalus fesus* (Mulsant) (Coleoptera: Cerambycidae);

golden-haired bark beetle (GHBB), *Hylurgus ligniperda* (F.) (Coleoptera: Curculionidae); and the closely related black pine bark beetle (BPBB), *Hylastes ater* (Paykull) (Coleoptera: Curculionidae), are forest insect pests that may be found on pine logs exported from New Zealand (Pawson et al. 2009). BPL adults show a particular preference for recently burned trees, but they also attack logs, stumps and standing, dead or dying pine trees (Hosking 1978). GHBB are primarily found under the bark of recently-felled trees, especially in those that have direct contact with the ground, and the roots and bases

of dead trees (Bain 1977; Hosking & Bain 1977; Hoebeke 2001). BPBB feed from the inner bark of recently felled trees, especially when these are in contact with the ground, and also from the bark around the root collar of seedlings (Milligan 1978).

The methyl bromide fumigation schedule for export logs varies depending on the importing country's phytosanitary requirements (Ministry for Primary Industries 2013b). In the case of China, the primary market for New Zealand logs, methyl bromide fumigations of 120 g/m³ for 16 h at 5 to 15°C or 80 g/m³ for 16 h at >15°C are required (Ministry for Primary Industries 2012).

There is little information on the tolerance of New Zealand forest pests to methyl bromide that is relevant to phytosanitary treatments for export logs. Cross (1992) suggested adults and larvae of BPL and BPBB had a concentration × time (CT) of <100 g h/m³. No other details, such as the temperature at which the fumigations were done, were provided. Somerfield et al. (2013) obtained 100% mortality of GHBB adults and larvae at a CT of 1168 g h/m³ at 5°C and a CT of 784 g h/m³ at 10 to 20°C.

The present study builds on the work of Somerfield et al. (2013) by determining possible ranges of reduced rates of methyl bromide to control bark beetles. The aim was to evaluate methyl bromide efficacy against BPL, GHBB and BPBB adults over a 4 h fumigation period at 10 or 20°C.

MATERIALS AND METHODS

Preparation of insects

BPL adults were hand-collected during February and March 2014 at night near flood lights at timber processing mills near Nelson (Eves Valley mill, Brightwater) and Napier (Pan Pac mill, Whirinaki), New Zealand. The BPL adults were placed in 1 litre plastic containers with lightly moistened paper towelling to provide humidity and to prevent damage to the insects during shipping. The plastic containers were placed in polystyrene boxes and air-freighted from the collection sites to the laboratory.

BPL adults were prepared for fumigation tests by placing 25 active adults into a 1 litre vented plastic container with moist paper towelling to prevent desiccation. Each test replicate for a set of fumigation parameters (i.e. concentration, temperature) consisted of two plastic containers (50 adults).

GHBB and BPBB adults were removed from infested logs by prying the bark away with a screwdriver. Preparation of GHBB was previously explained by Somerfield et al. (2013) and preparation of BPBB was done in similar way. Briefly, 12 h before treatment 25 beetles were transferred from the logs to 6.4 cm diameter stainless steel screened tea ball infusers each containing a 15 mm × 15 mm × 15 mm cube of artificial bark diet (Rogers et al. 2002) with pulverised pine bark substituted for pine wood. Each test replicate for a set of fumigation parameters (i.e. concentration, temperature) consisted of two tea ball infusers (50 adults).

Treatment procedure

Fumigations were done in 28-litre chambers (Labconco® desiccators, Kansas City, Missouri, USA) fitted with an internal fan to circulate the atmosphere in the chamber for the duration of the fumigation. The fumigation chambers were placed in a temperature-controlled room at either 10 ± 2°C or 20 ± 2°C.

Preliminary 4-h fumigations using 0, 2, 4, 8, 16, 32, 64 and 128 g/m³ methyl bromide were carried out at both 10 and 20°C to identify efficacious dose ranges for further testing that would provide data points between 50% and 100% mortality to enable curve fitting for each species. Using a dose range identified from the preliminary fumigation tests, a series of dose-mortality fumigations were carried out to determine the LD₉₉ for each of the three species at 10 and 20°C. Each set of fumigation parameters (methyl bromide concentration and temperature) was replicated three times and each replicate consisted of fumigated (treatment) and unfumigated (control) insects.

Table 1 gives the methyl bromide concentration (g/m³) and temperature combinations that were

Table 1 Methyl bromide fumigation parameters used to determine the LD₉₉ for the adult life stage of *Arhopalus ferus* (BPL), *Hylurgus ligniperda* (GHBB) and *Hylastes ater* (BPBB) at 10 or 20°C for 4 h.

Species	Replicate ¹	Methyl bromide at 10°C (g/m ³) ²	Methyl bromide at 20°C (g/m ³) ²
BPL	1	0–24	0–16
	2	0–16	0–16
	3	0–16	0–16
GHBB	1	0–64	0–40
	2	0–48	0–32
	3	0–40	0–36
BPBB	1	0–88	0–64
	2	0–88	0–64
	3	0–72	0–64

¹There were eight treatments per replicate giving a total of 7200 insects. After the first and second replicates the range of concentrations tested were sometimes modified to obtain data points between 50% and 100% mortality to enable curve fitting for each species to calculate the LD₉₉ values.

²The concentrations chosen were at regular intervals (e.g. 2, 4 or 8 g/m³) over the range given.

tested. If the methyl bromide concentrations were found to produce greater mortality (too many data points near or at 100% mortality) than was desired, the concentrations were reduced in later replications to improve the calculations of the LD₉₉.

Individual replicates were carried out on separate days. After the completion of each fumigation, chambers were aerated for 5–10 min using a vacuum pump connected to a fumigant scrubbing system (Nordiko Quarantine Systems Pty Ltd, Sydney). After the 5–10 min aeration, the containers of insects were removed from the chambers and further aerated under a fume hood for 18 h to ensure that no methyl bromide remained.

Delivery and monitoring of methyl bromide

Delivery of methyl bromide across the range of concentrations tested (Table 1) was achieved by

injecting a calculated amount of pure (100%) methyl bromide into a sealed 28 litre chamber using a plastic gas syringe. The calculated amounts of methyl bromide that were injected into the chambers were between 14 ml (~2 g/m³) and 908 ml (~128 g/m³). Before injecting the methyl bromide into each chamber, an identical amount of air was removed so that the amount of methyl bromide injected would equal the partial vacuum created and return the chamber to ambient pressure.

Concentrations of methyl bromide in the chambers were monitored by collecting headspace samples at 5, 120 and 225 min after the methyl bromide was injected into each chamber. Methyl bromide 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 temperature of the oven and detector were maintained at 120°C and 300°C, respectively. The flow rates of carrier gases (helium and air; BOC Gases, Auckland NZ Ltd) were 100 and 400 ml/min, respectively. Samples were withdrawn from the chamber using a gas-tight syringe. A 3 ml sample was injected into a sample loop and a 1 ml sub-sample was analysed. The concentration of methyl bromide was measured on a part per million (ppm) basis, which was determined by a five-point calibration curve using dilutions of pure methyl bromide with air. Concentrations in ppm were converted to g/m³ (1 g/m³ is approximately 253 ppm at 20°C) to relate the data to a dose and for reporting.

Mortality assessment

After fumigation, treated insects were held at 20 ± 5°C for 18 h to allow any live insects to recover from the effects of treatment before assessing mortality. BPL adults were assessed as either dead or alive. Beetles that were incapable of walking or remaining in an upright position but showed some leg movement were kept for an additional 48 h for observation and final determination of survival.

Similarly GHBB and BPBB adults were initially assessed as either dead or alive. Dead GHBB

and BPBB adults were those beetles showing no visible signs of movement. However, in other studies it had been observed that some GHBB and BPBB adults that appeared dead were found to be only feigning death and began moving only when continuously provoked to move. To accurately determine mortality by inducing any live BPBB adults to move and to shorten the time required to ascertain live and dead BPBB, all BPBB classified as dead were further assessed by placing them on a hot plate at $42.5 \pm 0.2^\circ\text{C}$ for 1 min. Any live BPBB placed on the hot plate would start moving within this time. BPL adults were not subjected to further assessment using the hot plate because they do not feign death and quickly respond when touched.

Statistical analysis

Concentration mortality data for each replicate were fitted using the generalised linear model (Dobson 1990) capability of R (R Core Team) with the complementary log-log (clog-log) link (Preisler & Robertson 1989) and the actual methyl bromide concentration as the explanatory variable. Specifically, the assumed form of response was $\log(-\log(1 - p)) = a + bC$, where p = expected mortality, and C = concentration of methyl bromide. The coefficients from the models were used to derive the estimated lethal concentration (LD_{99}), the concentration (g/m^3) to achieve a mortality of $cm + (1 - cm) \times 0.99$, where cm was the control mortality. For each species and

temperature, a geometric mean LD and its associated standard error (SEM) were estimated, from which a 95% confidence interval (CI) was calculated. Non-overlap of the 95% CIs is approximately equal to a test for difference at $P=0.01$.

RESULTS AND DISCUSSION

The preliminary fumigations indicated that methyl bromide concentrations of 24 g/m^3 or 16 g/m^3 at 10°C or 20°C , respectively, were sufficient to cause 100% mortality of BPL adults, and that methyl bromide concentrations of 64 g/m^3 or 40 g/m^3 at 10°C or 20°C , respectively, were sufficient to cause 100% mortality of GHBB adults. However, the preliminary fumigations indicated that higher methyl bromide concentrations of 88 g/m^3 or 64 g/m^3 at 10°C or 20°C , respectively, were required to obtain 100% mortality of BPBB adults.

For each fumigation event the average of three chamber concentrations over time was used for data analysis. Chambers lost approximately $4 \pm 3\%$ of their initial concentrations during the 4 h fumigation period, which is a typical response for this fumigant (Somerfield et al. 2013).

Insect mortality

A summary of the insect mortality of the three species at 10°C and 20°C combined with the mean LD_{99} and 95% confidence interval is presented in Table 2.

Table 2 Calculated LD_{99} (g/m^3) for each replicate, along with the geometric mean and 95% confidence interval, following methyl bromide fumigation of adult life stage of *Arhopalus fesus* (BPL), *Hylurgus ligniperda* (GHBB) and *Hylastes ater* (BPBB) at 10°C or 20°C for 4 h.

Insects	Temp. ($^\circ\text{C}$)	Replicate 1 ¹	Replicate 2	Replicate 3	Mean	95% confidence interval
BPL	10	8.6	12.3	11.8	10.8	8.6, 13.5
	20	7.1	6.3	6.9	6.7	5.4, 8.5
GHBB	10	33.6	25.8	29.2	29.3	25.3, 34.0
	20	19.3	28.5	24.3	23.7	20.5, 27.5
BPBB	10	44.9	36	44.4	41.6	29.5, 58.6
	20	21.4	15.4	18.2	18.2	12.9, 25.6

¹All data analysed using complementary log-log link method. Average mortality for controls from three replicates tested at 10°C and 20°C were: BPL 7%, GHBB 2% and BPBB 0%.

As expected, higher doses are required at a lower treatment temperature (Ren et al. 2006). BPBB adults were the most tolerant to methyl bromide among those tested at 10°C ($P < 0.01$), requiring a dose of 41.6 g/m³ for 4 h to obtain 100% mortality. BPBB were not significantly more tolerant than GHBB adults to methyl bromide at 20°C requiring a dose of 23.7 g/m³ for 4 h. BPL required the lowest methyl bromide dose and was the most sensitive at both treatment temperatures ($P < 0.01$).

Somerfield et al. (2013) reported that 100% mortality of GHBB larvae and adults was obtained when fumigated with methyl bromide rates of more than 49 g/m³ at 10, 15 and 20°C for 16 h. The present results showed that lower methyl bromide concentrations (41.6 g/m³ at 10°C and 23.7 g/m³ at 20°C) and a much shorter exposure time (4 h) were able to obtain 100% mortality for three species including GHBB.

Further work is required on other life stages of these species before a new treatment schedule can be proposed. Factors such as fumigant penetration and sorption by logs must also be taken into account.

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