Efficacy of methyl bromide used at reduced rates to control larvae and adults of goldenhaired bark beetle, *Hylurgus ligniperda*

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Abstract Pine (*Pinus radiata* D. Don) logs exported from New Zealand must undergo fumigation to ensure they are free of phytosanitary insects to meet the requirements of importing countries. The goldenhaired bark beetle, *Hylurgus ligniperda* (F.), is one of the major hitch-hiker species that can infest New Zealand pine logs. Adults and larvae can be present in the bark and cambium layer of freshly cut logs. To examine ways of reducing rates of methyl bromide fumigation, diet containing both life stages (extracted from pine logs) was exposed to 0, 49 or 73 g/m³ methyl bromide for 16 h in 28-litre fumigation chambers at 5, 10, 15 or 20°C. Both the 49 g/m³ and 73 g/m³ methyl bromide exposures resulted in 100% mortality at all temperatures. Average mortality among controls was: larvae 7%, adults 23%. The results indicate that the present methyl bromide fumigation rates used for pine logs exported from New Zealand could be reduced.

Keywords methyl bromide, fumigation, pine logs, *Pinus radiata* D. Don, quarantine treatments.

INTRODUCTION

The New Zealand Government (as Party to the Montreal Protocol) and the public have become increasingly concerned about the use of methyl bromide to fumigate export pine (*Pinus radiata* D. Don) logs. Methyl bromide use in New Zealand increased from 270 tonnes in 2009 to 406 tonnes in 2010 (New Zealand Ministry for the Environment 2011). The increase in methyl bromide use is due solely to an increase in log trade to countries that require fumigation (United Nations Environment Programme 2010). In response to safety concerns, controls on the use of methyl bromide have become more stringent

regarding buffer zones around fumigation locations (New Zealand Environmental Protection Authority 2011). Additionally, total recapture or destruction of methyl bromide after fumigation will be required by 2020 to ensure no fumigant escapes into the atmosphere. Other methods to reduce the amount of methyl bromide used to fumigate logs are being studied, including research to accurately define the optimum amount of methyl bromide required to control pests on export logs.

Insects of major quarantine importance include the goldenhaired bark beetle, *Hylurgus ligniperda*

(F.) (Coleoptera: Scolytidae); the black pine bark beetle, *Hylastes ater* (Paykull) (Coleoptera: Scolytidae); and the burnt pine longhorn beetle, *Arhopalus ferus* (Mulsant) (Coleoptera: Cerambycidae).

Hylurgus ligniperda is native to central Europe, Asia and Mediterranean regions but it has established in numerous countries including Japan, Sri Lanka, South Africa, Brazil, Chile, North America, Australia and New Zealand (Haack 2001). First recorded in New Zealand in 1974, *H. ligniperda* adults are 7–8 mm in length, 2 mm in width and black in colour (Bain 1977), and today generally comprise over 90% of all bark beetle populations in New Zealand pine forests (Bain 1977; Reay & Walsh 2001; Brockerhoff et al. 2006).

The predominant host in New Zealand for *H. ligniperda* is *Pinus* species (Lee et al. 2007). Adults breed in freshly dead host material including cut stumps, logs and debris from trees after harvest. *Hylurgus ligniperda* is bivoltine with two generations per year that correspond to two peaks of adult flight activity – one during spring and the other during autumn. The immature stages are easily transported on unprocessed logs or lumber, packing materials, pallets and dunnage. One of the current treatments used to provide quarantine security against these pests is fumigation with methyl bromide.

Comparison of the methyl bromide fumigation schedules for pine logs from New Zealand shows that the dose rates required by three Asian trading partners differ significantly (Table 1). Two different temperature regimes are specified

according to the ambient air temperature at the time of fumigation. Temperatures below 15°C require higher dose rates to have the same efficacy as treatments above 15°C within the specified 16 or 24 h fumigation times.

Table 1 shows methyl bromide application rates that vary from 72.5 g/m³ (Japan) to 120 g/m³ (China) for temperatures ≤15°C and from 33 g/m³ to 80 g/m³ for temperatures above 15°C with the highest rates required for logs exported to China.

Based on historical data and statistical analyses of the available methyl bromide efficacy information for New Zealand log pests, Armstrong et al. (2011) recommended that the current methyl bromide rates could be reduced to 49 g/m³ or 73 g/m³ for fumigations at 15°C or 15°C, respectively, for 16 h fumigations. However, the rate reductions recommended by Armstrong et al. (2011) must be tested under controlled conditions to demonstrate that the lowered rates will provide quarantine security against the target pests.

This paper presents the results of assays to establish the efficacy of methyl bromide fumigation, delivered at the two concentrations recommended by Armstrong et al. (2011), against *H. ligniperda* adults and larvae at temperatures ranging from 5°C to 20°C.

MATERIALS AND METHODS

Preparation of insects

Pine logs (50–60 cm long and 12–30 cm diameter) were infested with *H. ligniperda* several months before tests were initiated to provide larvae and

Table 1 Methyl bromide log fumigation schedules specified by China (Ministry for Primary Industries 2012) at export, and by Japan (Plant Protection Station, MAFF Japan 2011) and Korea (Yu et al. 1984) at import at two different fumigation temperatures above or below 15°C.

Country	Temperature (°C)	MeBr rate (g/m ³)	Time (h)
China	≥ 15	80.0	16
Japan	≥ 15	48.5	16
	≥ 15	33.0	24
Korea	≥ 15	49.5	16
China	≤ 15	120	16
Japan	≤ 15	72.5	16

adults from the same cohort for each test. The infested logs were stored in a humidity- and temperature-controlled room at 11°C until 8 weeks before testing, when the temperature was increased to 14°C to facilitate the development of larvae through to adults. Relative humidity was held at 70% ±5% during storage to prevent the insects from desiccating.

Hylurgus ligniperda larvae and adults were obtained by removing the logs from storage and prising the bark off each log using screwdrivers. The frass and debris from the inside of the bark and from the surface of the log were swept into a tray using a hearth brush. The insects were hand-sorted from the frass and debris, and temporarily held in plastic tubs lined with a layer of moistened paper towel to prevent desiccation.

An artificial bark diet for larvae and adults was made from pieces (2-3 cm diameter) of pine bark. The bark was pulverised in a blender at low speed and the ground bark was sieved through 2.36 mm and 1.18 mm Endecott sieves. Bark particles (1.18 to 2.36 mm) were mixed (2:1 vol/vol) with fine bark particles (≤1 mm). The bark particles were sprayed with distilled water and mixed until uniformly moist.

Clear plastic screw-top containers (200 ml volume) were used to hold the larvae or adults during fumigation tests. Each container had a 30 mm diameter hole cut into the lid and the base. The holes were secured with a circular piece of stainless steel mesh (30 mesh/inch) that was heat-sealed in place to allow gas exchange but prevent the insects from escaping. Separate containers, laid on their side, were used for larvae or adults. Five grams of the moist bark diet was placed in a layer inside each container. Larvae or adults (40-50 healthy individuals of mixed age) were placed on top of the diet. A piece of moistened paper towel was placed on top of each insect/diet mix to maintain humidity. Lids were then screwed on the containers. The containers were kept on their side during fumigation to facilitate gas circulation, and remained on their side until post-fumigation mortality assessment was completed.

Treatment procedure

Fumigations were done in 28-litre chambers consisting of modified vacuum desiccators (Model 55300-00, Labconco, Kansas City, Missouri, USA) fitted with circulation fans to mix the atmosphere within the chamber and customised door clamps to eliminate leakage. In addition to the insects, a data logger (Temprecord Scientific Recorder ILTE-953101C, Temprecord International Limited, Auckland, NZ) was placed in each chamber to monitor temperature. A vacuum (approximately -30 kPa) was drawn from each chamber. The vacuum facilitated the introduction of calculated amounts (up to 500 ml) of pure methyl bromide gas into each chamber using a 1-litre syringe (Model S1000, Hamilton Company, Reno, Nevada, USA). After the methyl bromide was introduced, each chamber was returned to normal atmospheric pressure. The atmosphere within the chambers was mixed for 5 min. They were then placed in a temperature-controlled room at 5, 10, 15 or 20°C (Table 2).

Each methyl bromide dose rate and treatment temperature combination was replicated three times (three separate containers). All fumigations were 16 h in duration. Treatments at each temperature were carried out on separate days.

The data loggers showed that temperatures in the chambers at 15 and 20°C were accurate to within 1°C throughout the trial. For 5 and 10°C treatments temperatures were initially up to 6°C higher than required while chambers cooled down during the first 3 h.

Table 2 Temperatures and methyl bromide dose rates used in fumigations of *H. ligniperda* larvae and adults. The ‘✓’ shows treatments included in this study.

Temperature (°C)	Methyl bromide dose (g/m ³)		
	0	49	73
5	✓		✓
10	✓	✓	✓
15	✓	✓	✓
20	✓	✓	

At completion of fumigation the chambers were aerated for 15 min using a vacuum pump, doors were opened and the containers of insects and the data loggers were removed.

Fumigant monitoring

Methyl bromide concentrations in the chambers were monitored by collecting duplicate 3 ml samples with a gas-tight syringe. The samples were analysed with a SRI 8610C gas chromatograph equipped with a flame ionisation detector (FID) and a J&W GS-Q 30 m × 0.53 mm column. Chromatography conditions were: oven temperature isothermal at 100°C, carrier gas helium at 45 PSI, FID at 300°C and injector temperature at 250°C.

In initial tests, methyl bromide concentration measurements were completed shortly after the chamber was returned to normal atmospheric pressure, and again at 2, 4, 8 and 16 h during and at the end of each fumigation. In later tests, methyl bromide concentrations were measured shortly after atmospheric equilibration and at 2, 4 and 16 h because GC analysis showed the methyl bromide concentrations did not change significantly during fumigation.

Mortality assessment

After removal from the fumigation chambers, the plastic containers were aerated for 24 h under a fume hood (air flow 0.1–0.15 m/s measured with Airflow TA 3000, Airflow Developments Ltd., High Wycombe, Buckinghamshire HP12 3QP, England.). Larvae and adults were then removed from each container and counted. Insects showing little or no movement (either moribund or only immobile) were placed in a Pyrex® Petri dish (with lid fitted) and the Petri dish was held for up to 1 min on a temperature-controlled hot plate at 42.5°C. Insects previously showing no activity began moving quickly while dead insects did not respond to the heat stimulus.

Statistical analysis

Mortality percentages were compared using binomial generalized linear models, with allowance for overdispersion where necessary. Analysis was done in Genstat (Version 15, 2012, VSNi Ltd, UK)

RESULTS

Methyl bromide concentrations

Gas concentrations in the chambers were within 2.6 g/m³ of the target concentration with two exceptions: the methyl bromide concentration in one 10°C fumigation was consistently 7.1 g/m³ above the 73 g/m³ target concentration, and in another 10°C fumigation the methyl bromide concentration was consistently 6.2 g/m³ below the 49 g/m³ target concentration. Methyl bromide loss in all chambers during the 16 h fumigation was no greater than 8.6% of their initial dose.

Insect mortality

Methyl bromide doses of 49 or 73 g/m³ both killed 100% of *H. ligniperda* larvae and adults at all temperatures (Table 3). Mortality in the control chambers varied between 3–16% for larvae and 4–34% for adults (Table 3). Mortality from methyl bromide was significantly higher ($P < 0.001$ for adults and larvae at all temperatures except for adults fumigated at 15°C where $P = 0.002$). When comparing mortality according to temperature, it was not possible to separate out methyl bromide dose from temperature due to the complete mortality. In the untreated controls, adults had significantly higher mortality at 5 and 10°C compared to larvae ($P = 0.031$ and 0.019, respectively).

DISCUSSION

Although there was an initial delay in achieving the target temperatures in the 5°C and 10°C treatments, the target temperature was achieved within 3 h after the treatments were initiated and were accurate thereafter. Delay in achieving the target temperatures was caused by the time required to draw the vacuum on the chambers and add and mix the fumigant at ambient temperature, then cool the fumigation chambers to 5 or 10°C.

Doses of methyl bromide used in the tests were accurate because of the low loading factor (between one and three small containers in a 28-litre chamber). Amounts of material in the chambers were too small for sorption to occur, and the well-sealed chambers prevented any loss of methyl bromide.

The 5 g of bark diet in each container was sufficient to maintain the insects during the 16-h

Table 3 Mean and SE for mortality (%) of *H. ligniperda* larvae and adults fumigated with 0, 49 or 73 g/m³ methyl bromide at 5, 10, 15 or 20°C.

Temperature (°C)	Dose (g/m ³)	Adults			Larvae		
		Mean	SE	n	Mean	SE	n
5	0	25	7	149	3	2	150
5	73	100	0	149	100	0	150
10	0	28	7	120	3	2	150
10	49	100	0	120	100	0	150
10	73	100	0	120	100	0	150
15	0	34	18	152	6	0	149
15	49	100	0	144	100	0	150
15	73	100	0	151	100	0	149
20	0	4	3	150	16	3	120
20	49	100	0	150	100	0	119

trial and facilitated the rapid retrieval of insects after each fumigation. The piece of paper towel remained moist throughout the fumigation and post-treatment mortality assessments. Mortality of adults and larvae in the controls was probably not due to desiccation as humidifiers were provided. Average adult mortality was 22.5% and average larval mortality was 7.1%. Adult *H. ligniperda* had significantly higher mortality than larvae at 5 and 10°C. *Hylurgus ligniperda* larvae may be more cold tolerant, or this difference may reflect the effect of other control conditions on survival of each life stage, such as relative acceptability of diet, average age of each life stage, or other factors.

Fumigation is likely to occur over a range of temperatures in New Zealand, and may be below 10°C in winter. The USDA Treatment Manual (USDA 2012) allows for methyl bromide fumigation over a wide range of temperatures with 4.4°C being the lowest allowed because vaporization becomes increasingly difficult at conditions below 4.4°C. The present results show that methyl bromide is effective at 5°C. Temperatures close to 5°C or less are likely to occur in winter in the South Island. Barak et al. (2011) found that ash (*Fraxinus spp.*) logs fumigated with 112 g/m³ methyl bromide at 4.4°C resulted in complete mortality of fully-developed larval and pre-

pupal emerald ash borer, *Agrilus plannipennis* Fairmaire (Coleoptera: Buprestidae). The effect of methyl bromide may vary with the life stage of the insect species being treated. In the present study complete mortality of larval and adult *H. ligniperda* was achieved. Vincent & Lindgren (1977) fumigated cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), life stages with methyl bromide and found that the 5-day-old cocoons (predominantly pupae and prepupae) were most tolerant to methyl bromide at 4.4–10.0°C but that 1-day-old cocoons containing only larvae were most tolerant at 15.6°C or 21.1°C. Increased exposure times at decreasing temperatures to obtain complete mortality for fruit fly eggs and larvae fumigated with methyl bromide in the range of 15–30°C were reported by Armstrong & Whitehand (2005). Vincent & Lindgren (1975), Bond (1975) and Bond & Buckland (1976) also described the need to increase fumigant times or dose rates to achieve required mortality as temperatures decreased.

Results given here are a first step towards optimising methyl bromide dose rates for control of forest pests on export logs.

CONCLUSIONS

Methyl bromide was effective for controlling *H. ligniperda* adults and larvae directly exposed

to the fumigant, and the efficacy of dose rates lower than those currently being required by some overseas markets was demonstrated.

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