

# A NOTE ON THE SENSITIVITY OF A CLOSED-TUBE BIOASSAY TO VOLATILE METHYLISOTHIOCYANATE RESIDUES IN FUMIGANT-TREATED WOOD<sup>1</sup>

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## ABSTRACT

A closed-tube bioassay with *Poria placenta* was used to measure residual fungistatic vapors in wood fumigated with methylisothiocyanate (MIT). This bioassay showed an inverse linear relationship between fungal growth and the amount of MIT in the wood samples. For fumigated increment-core sections of unseasoned Douglas-fir heartwood (4.8 mm in diameter by 25 mm long), the sensitivity of the bioassay ranged from 2 to 10 µg MIT per section. Closed-tube-bioassay response was also determined for small slivers of seasoned ponderosa pine and Douglas-fir, and was influenced by the size of the samples.

*Keywords:* Closed-tube bioassay, methylisothiocyanate, fumigant, Douglas-fir, ponderosa pine, residues, sensitivity, *Poria placenta*.

## INTRODUCTION

Volatile fungicides, or fumigants, can effectively control internal decay in utility poles, marine piles, and other large wood structural members (Graham 1973; Helsing et al. 1984; Morrell et al. 1984; Morrell et al. 1986). In the United States, use of fumigants to prevent wood decay is widely accepted (Goodell and Graham 1983). When fumigants are used to treat wood, they not only initially reduce populations of decay fungi, but they also provide residual protection, with fungistatic fumigant vapors remaining in the treated wood for years (Helsing et al. 1984). These residual vapors have been determined by extraction and gas chromatography (GC) (Morrell and Scheffer 1985; Zahora and Corden 1985b), as well as by a closed-tube bioassay (CTB) developed by Scheffer and Graham (1975).

In the CTB, a sample of fumigated wood is sealed in a test tube with a culture of a test fungus. Inhibition of fungal growth (compared with growth in controls using untreated wood) indicates the relative amount of vapors remaining in the fumigated sample. The CTB has certain advantages over gas chromatography for field use in determining when fumigant retreatment is necessary. The CTB is simpler to use, requiring only basic equipment and training in sterile culture work, and it can measure the combined fungitoxic effect of the fumigant and heartwood extractives in the wood samples. Previous use revealed that the CTB can detect residual fumigant vapors in wood years after treatment (Thies and Nelson 1982; Helsing et al. 1984), but quantitative information on its sensitivity is lacking.

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This note describes experiments to determine the sensitivity of the CTB to concentrations of methylisothiocyanate (MIT) in fumigated wood. MIT is a major volatile fungitoxicant present in Vorlex<sup>®</sup> (MIT and chlorinated C<sub>3</sub> hydrocarbons) and is produced from Vapam<sup>®</sup> (sodium N-methyl dithiocarbamate), two of the three fumigants registered by the U.S. Environmental Protection Agency for use in wood. MIT can also be encapsulated to reduce handling dangers, and has great potential as an effective wood fumigant (Zahora and Corden 1985a).

#### MATERIALS AND METHODS

To conduct the closed-tube bioassay, we used 9 × 100-mm borosilicate test tubes with Teflon<sup>®</sup>-lined screw caps. At the bottom of each test tube, 2 ml of 2.5% malt extract (1% agar) was slanted to form an agar surface 5 to 6 cm long. The leading edge of the agar in each test tube was then inoculated with a plug (2 mm in diameter) from an actively growing culture of *Poria placenta* (Fr.) Cke (Madison, FP-94267A). The tubes were incubated for 2 to 3 days (20–23 C), yielding fungal growth 1 to 4 mm from the inoculation plugs. After marking the leading margin of fungal growth, we sealed a wood sample to be tested inside the neck of each test tube. One set of controls contained no wood; another contained nonfumigated wood. All test tubes were stored at a steep inverted angle to prevent direct wood/agar contact and to allow fungitoxic vapors to diffuse upward. After 8 to 12 days, fungal growth in each test tube containing fumigated wood was measured and compared with that in the control tubes.

Three experiments were conducted to determine the relationship between the amount of MIT remaining in fumigated wood and growth suppression of *P. placenta* in the closed-tube bioassay. In our initial experiment, we used small blocks 0.5 cm (with grain) × 1.0 × 1.0 cm cut from seasoned Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) heartwood (10 to 20 growth rings/cm) and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) sapwood (5 to 11 growth rings/cm). The blocks were initially fumigated for more than 7 months in an MIT-saturated atmosphere (agricultural-grade MIT, NOR-AM Chemical Co., Wilmington, Delaware). These blocks were then aerated in a fume hood to reduce the MIT concentrations. Groups of blocks were periodically sampled for MIT content and CTB growth response. Each block was split in half radially. One half was then split again into a series of small slivers (9 to 76 mg) for use in the CTB. The other half was extracted and analyzed for MIT content through GC procedures.

Samples were extracted in ethyl acetate for 7 to 10 days. These extracts were analyzed on a Varian 3700 Gas Chromatograph equipped with a flame-photometric detector. A glass column (3-m × 4-mm inner diameter) packed with 10% Carbowax<sup>®</sup> 20M on 80/100 Supelcoport<sup>®</sup> solid support was operated at the following conditions: column temperature, 170 C; injection and detector temperatures, 220 C; and nitrogen-flow rate, 75 cc/min.

To reduce the aeration time required for lowering MIT concentrations to the range of CTB sensitivity, we repeated this experiment with a new set of wood blocks that had been exposed initially to milder fumigant treatments. These blocks were exposed to a saturated atmosphere of MIT (purified) for 15 minutes, and then stored in glass jars sealed with Teflon<sup>®</sup>-lined lids. After a 24-day equilibration period, the blocks were removed from the jars, aerated for 3 days, and re-equil-

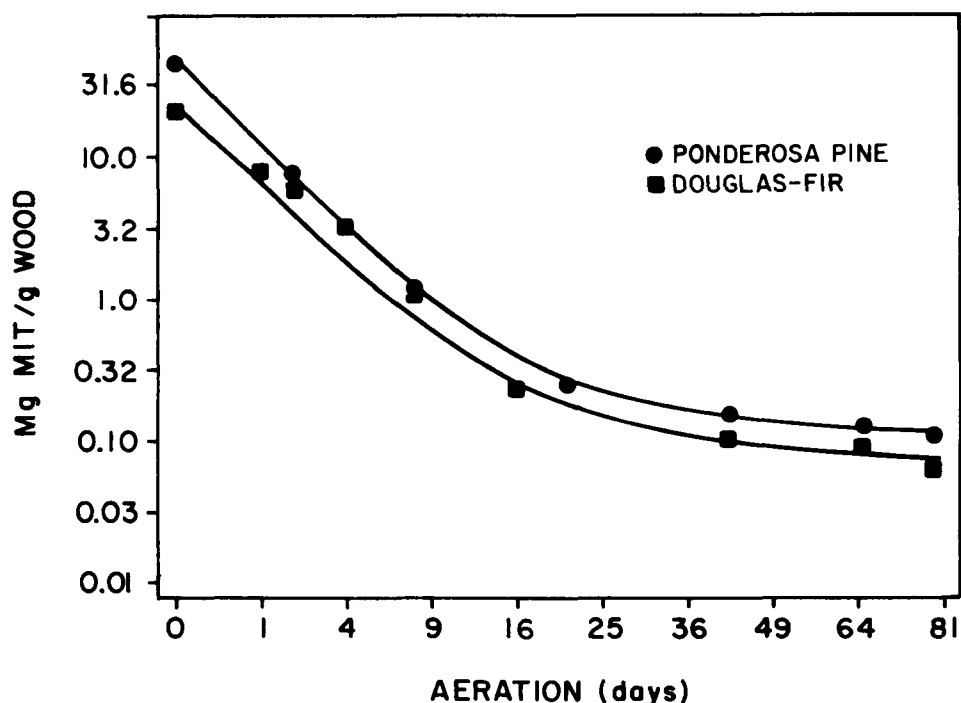


FIG. 1. Rate of methylisothiocyanate (MIT) loss from seasoned blocks of Douglas-fir heartwood and ponderosa pine sapwood (0.5 cm grain direction  $\times$  1.0 cm  $\times$  1.0 cm) during aeration in a fume hood. Blocks were initially exposed to saturated MIT vapors for over 7 months.

ibrated for 5 to 10 days. The blocks were then split in half, and analyses were conducted for MIT content and CTB growth response as described above.

In these experiments we assumed a uniform distribution of MIT within each block for regression analyses. Because volume and surface area of test samples will influence MIT release from wood, the MIT content tested in these initial CTB tests was varied by using different sizes of wood slivers.

To minimize the influence of sample size on the CTB results, a final experiment was conducted in which the size of the wood sample was held constant and only the MIT content varied. This experiment used increment-core sections that were 4.8 mm in diameter by 25 mm long, weighed about 0.2 g, and averaged two growth rings/cm. They had been cut from increment cores taken from an unseasoned Douglas-fir log (46 cm in diameter). The sections were from heartwood 9 cm from the cambium layer. (Sapwood depth was 6 to 7.5 cm). Groups of 16 sections were fumigated for 10 to 60 minutes by a continuous-flow apparatus with a 0.42-liter fumigation chamber (Zahora and Corden 1985b). The MIT vapor concentration was  $0.67 \mu\text{g/ml}$  air with a flow rate of 15 ml/min. A small magnetic fan circulated the air-fumigant mixture in the chamber.

The groups of core sections were aerated for 0 to 120 minutes to obtain a range of final MIT concentrations and then sealed in small glass vials for 9 days to equilibrate. They were then removed from the vials and six were extracted for GC analysis of MIT content; the remaining ten were used to test growth response in the CTB. Because sampling was destructive (individual core sections could be

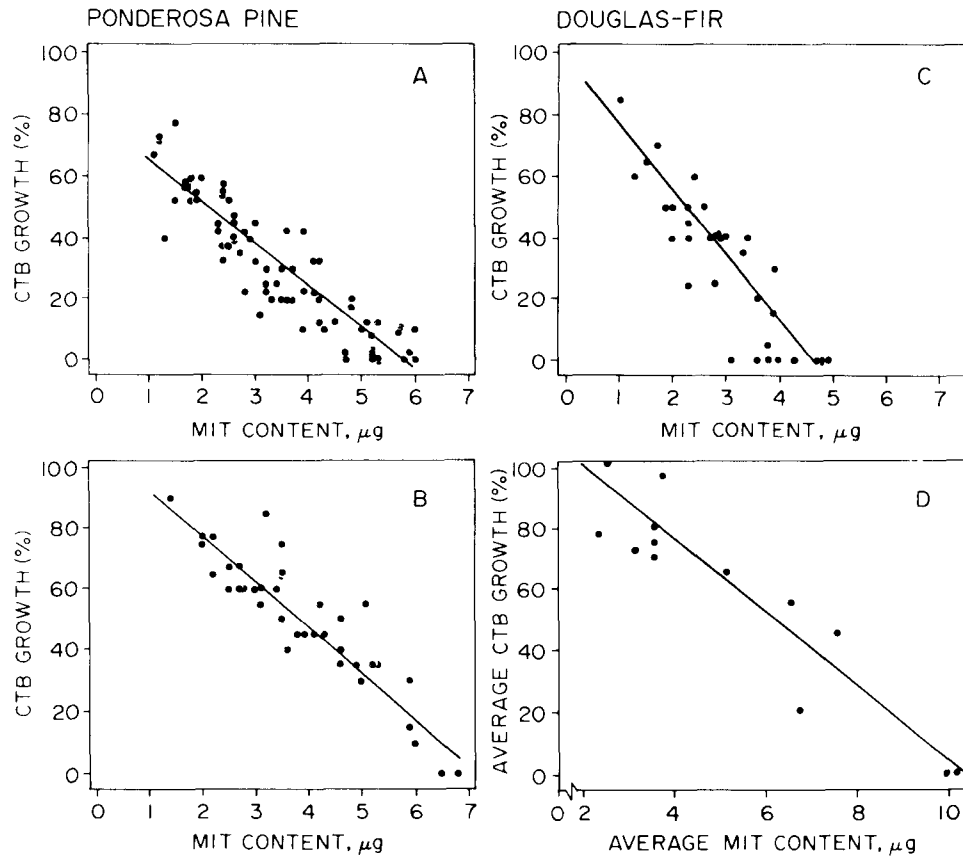


FIG. 2. Influence of methylisothiocyanate (MIT) content in ponderosa pine sapwood and Douglas-fir heartwood on the growth of *Poria placenta* in the closed-tube bioassay. (A) 9 to 50 mg slivers of seasoned ponderosa pine exposed to a saturated MIT atmosphere for 7 months, and aerated for over 65 days. (B) 18 to 76 mg slivers of seasoned ponderosa pine sapwood exposed to a saturated MIT atmosphere for 15 min, and aerated for 3 days. (C) 9 to 43 mg slivers of seasoned Douglas-fir heartwood exposed to a saturated MIT atmosphere for 15 min, and aerated for 3 days. (D) Increment core sections (0.022 g) of unseasoned Douglas-fir heartwood exposed to 0.67  $\mu\text{g}$  MIT/ml air for 10 to 60 minutes, then aerated 0 to 120 minutes. Results are based on the percent of growth compared with that in the control test tubes.

used for either MIT or CTB growth determinations, but not both), MIT content and the CTB growth responses (based on unfumigated wood controls) of each group of sections were averaged for regression analysis.

#### RESULTS AND DISCUSSION

Extensive aeration of wood blocks fumigated for 7 months in a saturated MIT atmosphere was required to reduce fumigant concentrations to levels allowing fungal growth in the CTB (Fig. 1). This was unexpected based on the rapid loss of MIT previously observed during aeration of Douglas-fir blocks (at 80% moisture content) after a 24-hour fumigation period (Zahora and Corden 1985b). Apparently, the long fumigation and dry conditions greatly retarded the rate of MIT loss from the blocks. After a 65-day aeration, fungal growth was observed only

in the CTB test tubes containing small slivers (9 to 50 mg) of ponderosa pine (Fig. 2A). CTB growth inhibition was complete above 6  $\mu\text{g}$  MIT, but the lower limit of sensitivity could not be accurately determined because of the high MIT concentrations remaining in the wood. The Douglas-fir slivers as small as 15 mg remained fungitoxic even after 79 days of aeration, and a regression relationship could not be established.

In the ponderosa pine blocks initially exposed to MIT vapors for only 15 min, the total extractable MIT in the slivers showed a strong correlation ( $R^2 = 0.82$ ) with the CTB growth response (Fig. 2B). Although these pine blocks had a much shorter exposure to MIT than in the first experiment, the range of CTB sensitivity was similar, with complete growth inhibition occurring at 7  $\mu\text{g}$  MIT. At similar MIT concentrations, the pine blocks fumigated for 7 months produced greater inhibition of fungal growth than blocks fumigated for 15 minutes. This may be due to impurities in the NOR-AM MIT, or to undetected MIT decomposition products formed during the 7-month exposure. Methyl disulfide (an impurity in the NOR-AM MIT) was found at concentrations equivalent to those for MIT in the aerated blocks that had been fumigated for 7 months. Methyl disulfide alone did not inhibit *P. placenta* growth in the CTB, even at concentrations eight times higher (50  $\mu\text{g}$ /sliver) than those found in the fumigated wood. Nevertheless, a synergistic toxic interaction with MIT cannot be ruled out.

The CTB was sensitive to lower MIT concentrations in slivers of Douglas-fir heartwood (Fig. 2C), with growth completely inhibited at 4  $\mu\text{g}$  MIT per CTB tube. Although this level of MIT correlated well with CTB growth response ( $R^2 = 0.77$ ), other blocks with longer equilibrations showed a higher scatter. The growth response was often more closely related to the size of the wood slivers than to the MIT content of the extracted sample. However, growth was always completely inhibited at concentrations above 4  $\mu\text{g}$  MIT.

MIT concentrations in Douglas-fir heartwood core sections varied among the sections within each treatment group, even after long equilibrations. This variation may be due to an unequal initial adsorption during the short fumigant exposures, coupled with a hysteresis-type effect in which bound MIT equilibrated at different concentrations in dry wood depending on whether wood was adsorbing or desorbing fumigant (Zahora, manuscript in preparation). The CTB growth response was strongly correlated with the extractable MIT content in the core sections ( $R^2 = 0.87$ ); CTB sensitivity ranged from no growth inhibition at 2  $\mu\text{g}$  MIT to complete growth inhibition at 10  $\mu\text{g}$  (Fig. 2D). These core sections required more total MIT to obtain a given growth inhibition than did the smaller wood slivers. These findings support the influence of wood sample size on CTB results.

Growth in the control tubes averaged between 25 and 30 mm. The growth response of *P. placenta* was not influenced by the slivers of seasoned pine sapwood or Douglas-fir heartwood, but growth was inhibited 15% in the CTB tubes containing core sections taken from the unseasoned Douglas-fir log. This may have been the effect of emission of volatile extractives from the unseasoned heartwood.

#### CONCLUSIONS

- 1) The growth of *P. placenta* in the CTB shows a strong inverse linear correlation with the MIT content in fumigated wood. We recommend the use of increment-core sections (4.8 mm in diameter by 25 mm long, containing 0.2 g of wood)

- for use in the CTB, which is sensitive to MIT concentrations ranging from 2  $\mu\text{g}$  (no growth inhibition) to 10  $\mu\text{g}$  (complete growth inhibition) in Douglas-fir.
- 2) The CTB growth response to a given MIT concentration is influenced by the size of the wood sample, with a sensitivity range of 1 to 7  $\mu\text{g}$  MIT for ponderosa pine sapwood slivers and 1 to 4  $\mu\text{g}$  MIT for Douglas-fir slivers when less than 76 mg of wood is used.
  - 3) Volatile extractives from unseasoned Douglas-fir heartwood reduced CTB growth by about 15% from that of controls without wood. No significant effect was observed when seasoned Douglas-fir heartwood or ponderosa pine sapwood was used.
  - 4) The closed-tube bioassay offers a simple, but highly sensitive, method for determining residual fungitoxic vapors in fumigant-treated wood.

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