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ORIGINAL ARTICLE

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# Gaseous treatment with allyl isothiocyanate to control established microbial infestation on wood

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**Abstract** Applicability of gaseous treatment with allyl isothiocyanate (AIT) was evaluated for controlling the established microbial infestation of wood in the laboratory. Small sapwood specimens from five wood species measuring 20mm (T) × 20mm (R) × 10mm (L) were first preincubated on 2% malt-agar medium with placing them on a fully grown monoculture of seven test fungi. After 7 weeks of preincubation they were transferred to fresh medium and exposed to AIT fumigant at concentrations of 2360 and 23600ppm AIT in the air of a petri dish to determine threshold values of exposure periods for each test fungus. The 2360ppm concentration was not effective for *Penicillium funiculosum*, *Gliocladium virens*, or *Rhizopus stolonifer* for any of the wood species–exposure period combinations. Those test fungi could grow even at 23600ppm after 48h exposure when *Cryptomeria japonica* and *Fagus crenata* were used as wood substrate. Growth of other test fungi was inhibited at 2360ppm with a few exceptions in the case of *Aspergillus niger*. The required periods of exposure to suppress microbial regrowth were different with wood species–test fungi combinations. As two wood-decaying basidiomycetes, *Trametes versicolor* and *Fomitopsis palustris*, were easily controlled at 2360ppm after 24h exposure regardless of wood species, AIT treatment proved applicable to the control of internal decay of utility poles and other relevant products in service.

**Key words** Gaseous treatment · Allyl isothiocyanate · Wood-decaying fungus · Mold · Sapstaining fungus

## Introduction

Allyl isothiocyanate (AIT) previously proved to be effective in controlling microbial growth on wood substrate when applied with a preventive gaseous treatment.[1] Further work, however, was needed to demonstrate applicability of AIT to eradication of microorganisms colonizing wood and inhibition of further microbial infestation in utility poles and posts.

Gaseous treatment has been commercially carried out to inhibit internal decay of utility poles in service, and a few fumigants were tested for their potential in practical use.[2] A few fumigants such as methylisothiocyanate, trichloronitromethane, and sodium *N*-methyldithiocarbamate have already been evaluated for their applicability as a remedial treatment for controlling internal decay of poles.[3–5] The effect of these fumigants was mainly tested on Douglas fir and southern yellow pine in the United States,[6–9] and only a few papers were concerned with other wood species.[10–13] Nineteen decay fungi were isolated from preservative-treated Douglas fir transmission poles installed in the northeastern United States. Of those, *Poria carbonica* Overh. and *Poria placenta* (Fr.) Cke. were most frequently isolated,[14] and they were often used in the subsequent tests.

Present investigations were designed to determine the comparative tolerance of selected wood-inhabiting microorganisms to AIT fumigant using small wood blocks under laboratory conditions. Threshold exposure periods at desired concentrations of AIT were also determined with the test organisms.

## Materials and methods

### Wood specimens

Sapwood specimens were prepared from five wood species in dimensions of 20mm (T) × 20mm (R) × 10mm (L). Wood species tested were *Fagus crenata* Blume, *Cryptomeria japonica* D. Don, *Pinus densiflora* Sieb. et Zucc., *Pseudotsuga menziesii* (Mirb.) Franco, *and Picea jezoensis* Carr. *var. hodoensis* Rehd.

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### Test fungi

Seven test fungi were selected from those commonly used in Japanese standardized tests: two decaying fungi [*Trametes versicolor* (L.: Fr.) Pilat (FFPRI 1030) and *Fomitopsis palustris* (Berk. et Curt.) Gilbn. & Ryn. (FFPRI 0507)]; one sapstaining fungus [*Aureobasidium pullulans* (de Bary) Arnaud (IFO 6353)]; and four molds [*Aspergillus niger* van Tieghem (IFO 6341), *Penicillium funiculosum* Thom (IFO 6345), *Gliocladium virens* Miller, Giddens & Foster (IFO 6355), and *Rhizopus stolonifer* (Ehrenberg: Fries) Vuillemin SN 32 (IFO 31005)].

### Preincubation

A monoculture of the test fungus was inoculated on 2% malt agar medium in a petri dish (8.9 cm diameter and 2.0 cm height); it was incubated at 26° ± 2°C and over 85% relative humidity (RH) for 1–2 weeks until a mycelial mat thoroughly covered the medium. Five specimens of each wood species were then placed on the mycelial mat in a petri dish, and the assembled units were incubated for 7 weeks so fungal growth was well developed over the specimens.

### Exposure of fungus-infected wood specimens to gaseous AIT

After preincubation, a set of five specimens infected with a monoculture was transferred onto a fresh medium with a 3 $cm^2$ Toyo filter paper no. 2 containing 100 $\mu$l of AIT solution. The filter paper was taped to the inside of a petri dish lid as a volatile AIT source. The assembled units were sealed with vinyl tape to maintain the AIT concentration unchanged during exposure periods. Treatment stock solutions were prepared from Wasaohro, commercially available synthetic AIT[1] with corn oil to supply the desired fumigant concentrations of 2360 and 23 600 ppm in the air of a petri dish during exposure periods (2–168 h). Exposure periods were selected from 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h based on combinations of fungal species wood species AIT concentrations. The test concentrations were measured as previously described.[1]

### Incubation and inspection

A set of five fungus-infected wood specimens were placed on fresh 2% malt agar medium in a petri dish after exposure to vaporized AIT and incubated at 26° ± 2°C and over 85% RH for 5 weeks. Thus, five replicates were prepared for each wood species fungal species treatment concentration exposure period. Fungal growth around the wood specimens was inspected weekly to determine threshold exposure periods needed for fungal eradication on the basis of fungal regrowth.

Effectiveness of gaseous AIT treatment was expressed as percentage protection calculated from the number of wood specimens that contributed to fungal regrowth around them:

$$\text{Protection (\%)} = [(5 - \text{number of wood specimens with fungal regrowth})/5] \times 100$$

## Results and discussion

### Relative tolerance of test fungi to fumigant treatment with AIT

The first experiments were conducted at 2360 ppm. The regrowth of two decay fungi, *F. palustris* and *T. versicolor*, was readily suppressed after a short exposure time (2–24 h) regardless of wood species. A sapstaining fungus, *A. pullulans*, similarly tended to sustain serious damage after a short fumigant treatment with AIT (2–24 h). These results strongly support the potential of AIT as a fungistatic fumigant agent for remedial treatment of poles and piles, although the residual effect of AIT is unknown. Residual protection, which is dependent on the volatile elements remaining in the treated wood, is advantageous to maintenance of the treated wood poles and piles because it provides a longer retreatment cycle.[6]

Four mold fungi proved to be more tolerant to AIT vapor than others. Table 1 shows the durations needed for fungal regrowth when fungus-infected wood specimens were exposed to AIT vapor for 24 h. This fumigant treatment did not succeed in inhibiting growth of the molds (*P. funiculosum*, *G. virens*, *R. stolonifer*), and the molds restarted growing on the 2% malt agar around wood specimens within a week. A higher concentration of 23 600 ppm was therefore applied to the three molds in the subsequent tests. *A. niger* was more tolerant than wood-decaying basidiomycetes and started growing during the second week of incubation.

### Effect of exposure periods and wood species on fungal regrowth

Percentage protection was calculated from the data on how many wood specimens of five replicates contributed to fun-

**Table 1.** Time (wk) required for fungal regrowth after 24 h exposure to 2360 ppm AIT

| Wood species | Test fungi[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *A.n* | *P.f* | *G.v* | *R.s* | *A.p* | *T.v* | *F.p* |
| *Fagus crenata* | 2 | 1 | 1 | 1 | >5 | >5 | >5 |
| *Cryptomeria japonica* | 2 | 1 | 1 | 1 | >5 | >5 | >5 |
| *Pseudotsuga menziesii* | 2 | 1 | 1 | 1 | >5 | >5 | >5 |
| *Pinus densiflora* | 2 | 1 | 1 | 1 | >5 | >5 | >5 |
| *Picea jezoensis* | 2 | 1 | 1 | 1 | >5 | >5 | >5 |

[a] *A.n*, *Aspergillus niger*; *P.f*, *Penicillium funiculosum*; *G.v*, *Gliocladium virens*; *R.s*, *Rhizopus stolonifer*; *A.p*, *Aureobasidium pullulans*; *T.v*, *Trametes versieolor*; *F.p*, *Fomitopsis palustris*

**Table 2.** Percentage protection after desired exposure periods to 2360 ppm AIT vapor (*F. crenata*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *A. niger* | | | | | |
| 12 | 0 | 0 | 0 | 0 | 0 |
| 24 | 100 | 0 | 0 | 0 | 0 |
| 48,72 | 100 | 0 | 0 | 0 | 0 |
| 96 | 100 | 20 | 0 | 0 | 0 |
| 120,144,168 | 100 | 100 | 100 | 100 | 100 |
| *A. pullulans* | | | | | |
| 2 | 10 | 100 | 40 | 0 | 0 |
| 4 | 100 | 100 | 20 | 0 | 0 |
| 6 | 100 | 100 | 100 | 100 | 100 |
| *T. versicolor* | | | | | |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 4,6,8,12,24 | 100 | 100 | 100 | 100 | 100 |
| *F. palustris* | | | | | |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 4 | 40 | 0 | 0 | 0 | 0 |
| 6 | 20 | 0 | 0 | 0 | 0 |
| 8 | 100 | 80 | 0 | 0 | 0 |
| 12 | 20 | 0 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |

**Table 3.** Percentage protection after desired exposure periods to 2360 ppm AIT vapor (*C. japonica*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *A. niger* | | | | | |
| 2,4,6,8,12 | 0 | 0 | 0 | 0 | 0 |
| 24,48,72 | 100 | 0 | 0 | 0 | 0 |
| 96,120,144,168 | 100 | 100 | 100 | 100 | 100 |
| *A. pullulans* | | | | | |
| 2 | 100 | 40 | 0 | 0 | 0 |
| 4 | 100 | 60 | 0 | 0 | 0 |
| 6,8 | 100 | 40 | 0 | 0 | 0 |
| 12 | 100 | 20 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |
| *T. versicolor* | | | | | |
| 2 | 100 | 0 | 0 | 0 | 0 |
| 4 | 100 | 80 | 20 | 0 | 0 |
| 6,8 | 100 | 100 | 100 | 100 | 0 |
| 12,48 | 100 | 100 | 100 | 100 | 100 |
| *F. palustris* | | | | | |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 4,6,8,12 | 100 | 0 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |

**Table 4.** Percentage protection after desired exposure periods to 2360 ppm AIT vapor (*P. menziesii*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *A. niger* | | | | | |
| 8,12,24,48,96,120 | 100 | 0 | 0 | 0 | 0 |
| *A. pullulans* | | | | | |
| 2 | 100 | 80 | 80 | 40 | 40 |
| 4,6,8,12,24,48 | 100 | 100 | 100 | 100 | 100 |
| *T. versicolor* | | | | | |
| 8 | 40 | 0 | 0 | 0 | 0 |
| 12 | 80 | 20 | 20 | 20 | 20 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |
| *F. palustris* | | | | | |
| 8 | 100 | 100 | 60 | 0 | 0 |
| 12 | 100 | 60 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |

**Table 5.** Percentage protection after desired exposure periods to 2360 ppm AIT vapor (*P. densiflora*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *A. niger* | | | | | |
| 4,6,8,12,24,48,72,96,120 | 100 | 0 | 0 | 0 | 0 |
| *A. pullulans* | | | | | |
| 4 | 100 | 100 | 40 | 0 | 0 |
| 8 | 100 | 100 | 100 | 40 | 0 |
| 12 | 100 | 100 | 100 | 60 | 20 |
| 24 | 100 | 100 | 100 | 100 | 100 |
| *T. versicolor* | | | | | |
| 4 | 100 | 100 | 0 | 0 | 0 |
| 8,12,24 | 100 | 100 | 100 | 100 | 100 |
| *F. palustris* | | | | | |
| 4 | 100 | 0 | 0 | 0 | 0 |
| 8 | 100 | 0 | 0 | 0 | 0 |
| 12 | 100 | 20 | 0 | 0 | 0 |
| 24 | 100 | 100 | 100 | 100 | 100 |

gal regrowth after exposure to 2360 ppm AIT vapor. Results for each wood species and four test fungi (*A. niger*, *A. pullulans*, *T. versicolor*, *F. palustris*) are shown in Tables 2–6. A longer exposure resulted in better protection in most cases as expected, whereas a shorter exposure could not inhibit fungal regrowth after prolonged incubation periods.

Regrowth of *A. pullulans* was readily prohibited for the 5-week incubation period after fumigant treatments of less than 24 h in any wood species. *A. niger* did not have suppressed regrowth even after 120 h exposure to AIT vapor in the presence of *P. menziesii*, *P. densiflora*, and *P. jezoensis*; and no effect of the exposure period was noted in the former two wood species over a range of 4–120 h. For *P. jezoensis* a longer exposure seemed to produce better protection, although the fungus finally started regrowing within 3 weeks of incubation. On the other hand, regrowth of the fungus was well controlled in *F. crenata* and *C. japonica* after 96–120 h and 72–96 h exposure to AIT fumigant, respectively.

Persistence of AIT vapor varied with exposure period for *F. palustris*, and 24 h of exposure could always ensure perfect protection after 5 weeks of incubation in any wood species. The other decay fungus, *T. versicolor*, seemed less tolerant to AIT vapor and could not regrow after 2–24 h of exposure.

**Table 6.** Percentage protection after desired exposure periods to 2360ppm AIT vapor (*P. jezoensis*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *A. niger* | | | | | |
| 8,12,24 | 100 | 0 | 0 | 0 | 0 |
| 48 | 100 | 40 | 0 | 0 | 0 |
| 72 | 100 | 0 | 0 | 0 | 0 |
| 96 | 100 | 0 | 0 | 0 | 0 |
| 120 | 100 | 100 | 40 | 0 | 0 |
| *A. pullulans* | | | | | |
| 2 | 80 | 40 | 0 | 0 | 0 |
| 4 | 100 | 100 | 80 | 80 | 80 |
| 6,8,12,24 | 100 | 100 | 100 | 100 | 100 |
| *T. versicolor* | | | | | |
| 8 | 100 | 60 | 0 | 0 | 0 |
| 12 | 100 | 80 | 60 | 60 | 60 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |
| *F. palustris* | | | | | |
| 8 | 100 | 40 | 0 | 0 | 0 |
| 12 | 100 | 60 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |

**Table 7.** Percentage protection after desired exposure periods to 23600ppm AIT vapor (*F. crenata*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *P. funiculosum* | | | | | |
| 8 | 40 | 0 | 0 | 0 | 0 |
| 12 | 60 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 |
| 48 | 60 | 0 | 0 | 0 | 0 |
| *G. virens* | | | | | |
| 8,12,24,48 | 0 | 0 | 0 | 0 | 0 |
| *R. stolonifer* | | | | | |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 12 | 60 | 0 | 0 | 0 | 0 |
| 24,48 | 0 | 0 | 0 | 0 | 0 |

**Table 8.** Percentage protection after desired exposure periods to 23600ppm AIT vapor (*C. japonica*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *P. funiculosum* | | | | | |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 12 | 100 | 100 | 20 | 0 | 0 |
| 24,48 | 0 | 0 | 0 | 0 | 0 |
| *G. virens* | | | | | |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 12,24 | 100 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 |
| *R. stolonifer* | | | | | |
| 8,12,24,48 | 0 | 0 | 0 | 0 | 0 |

**Table 9.** Percentage protection after desired exposure periods to 23600ppm AIT vapor (*P. menziesii*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *P. funiculosum* | | | | | |
| 8 | 100 | 100 | 40 | 0 | 0 |
| 12 | 100 | 100 | 100 | 80 | 80 |
| 24 | 100 | 100 | 40 | 40 | 40 |
| 48 | 100 | 100 | 100 | 100 | 100 |
| *G. virens* | | | | | |
| 8,12 | 100 | 0 | 0 | 0 | 0 |
| 24,48 | 10 | 100 | 100 | 100 | 100 |
| *R. stolonifer* | | | | | |
| 8 | 100 | 100 | 60 | 0 | 0 |
| 12,48 | 100 | 100 | 100 | 100 | 100 |

**Table 10.** Percentage protection after desired exposure periods to 23600ppm AIT vapor (*P. densiflora*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *P. funiculosum* | | | | | |
| 8 | 100 | 60 | 0 | 0 | 0 |
| 12 | 100 | 100 | 100 | 100 | 100 |
| 24 | 100 | 80 | 40 | 0 | 0 |
| 48 | 100 | 100 | 100 | 100 | 100 |
| *G. virens* | | | | | |
| 8 | 100 | 100 | 100 | 100 | 0 |
| 12 | 100 | 100 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |
| *R. stolonifer* | | | | | |
| 8,12,24,48 | 0 | 0 | 0 | 0 | 0 |

**Table 11.** Percentage protection after desired exposure periods to 23600ppm AIT vapor (*P. jezoensis*)

| Exposure period (h) | Protection (%), by weeks of incubation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *P. funiculosum* | | | | | |
| 8 | 100 | 100 | 40 | 40 | 0 |
| 12,24,48 | 100 | 100 | 100 | 100 | 100 |
| *G. virens* | | | | | |
| 8 | 100 | 0 | 0 | 0 | 0 |
| 12 | 100 | 20 | 0 | 0 | 0 |
| 24 | 100 | 0 | 0 | 0 | 0 |
| 48 | 100 | 100 | 100 | 100 | 100 |
| *R. stolonifer* | | | | | |
| 8,12 | 0 | 0 | 0 | 0 | 0 |
| 24,48 | 100 | 100 | 100 | 100 | 100 |

A 10-fold higher concentration (23600ppm) of AIT fumigant was applied to the remaining three tolerant molds using each wood species for 8–48h of exposure by the same method, because a 2360ppm application failed to control regrowth even after 168h of exposure. The effect of the wood species was prominent for these molds, as shown in Tables 7–11. Regrowth of the molds was not inhibited even

**Table 12.** Threshold values of exposure period[a]

| Wood species | *A. niger* | *A. pullulans* | *P. funiculosum* | *G. virens* | *R. stolonifer* | *T. versicolor* | *F. palustris* |
|---|---|---|---|---|---|---|---|
| *F. crenata* | 96–120 | 4–6 | >48 | >48 | >48 | 2–4 | 12–24 |
| *C. japonica* | 72–96 | 12–24 | >48 | >48 | >48 | 8–12 | 12–24 |
| *P. menziesii* | >120 | 2–4 | 24–48 | 12–24 | 8–12 | 12–24 | 12–24 |
| *P. densiflora* | >120 | 12–24 | 24–48 | 12–24 | >48 | 4–8 | 12–24 |
| *P. jezoensis* | >120 | 4–6 | 8–12 | 24–48 | 12–24 | 12–24 | 12–24 |

Results are given in hours

[a] Applied treatment concentrations of AIT: 2360 ppm for *A. niger*, *A. pullulans*, *T. versicolor*, and *F. palustris*; and 23 600 ppm for *P. funiculosum*, *G. virens*, and *R. stolonifer*

after the longest exposure (48 h) to 23 600 ppm AIT vapor in *F. crenata* and *C. japonica*, whereas such fungal regrowth was completely depressed in *P. menziesii* and *P. jezoensis*. *P. densiflora* indicated an intermediate tendency of these two groups; that is, *R. stolonifer* regrew and two others did not grow after exposure to a high level of AIT fumigant. Threshold values of the exposure period for test fungi are shown in Table 12 when a concentration of 2360 ppm AIT was used for four fungi (*A. niger*, *A. pullulans*, *T. versicolor*, *F. palustris*) and 23 600 ppm for the remaining three fungi (*P. funiculosum*, *G. virens*, *R. stolonifer*).

No correlation was remarkable between fungal regrowth and wood species, although in general fungi belonging to Fungi Imperfecti (*A. niger, P. funiculosum*, *G. virens*) and a zygomycete (*R. stolonifer*) were more tolerant than wood-decaying basidiomycetes. In contrast, one of the Fungi Imperfecti, *A. pullulans*, proved to be less tolerant to AIT fumigant than the other test fungi in the present study. Some factors may be involved in the tolerance of fungi to AIT fumigant treatment: permeability of AIT vapor into wood, structural characteristics of fungal cells, and affinity of AIT to mycelial mat, among others. Nothing, however, is known to account for such discrepancy in tolerance among combinations of wood and fungal species.

## Conclusions

In situ treatment of poles, piles, and large wooden structural members is important to extend their service lives. AIT proved effective in controlling microbial regrowth when the chemical was applied to previously fungus-infected wood specimens as a fumigant for 2–24 h. The results suggested a potential use for AIT in gaseous treatment of wood in service as existing commercial products containing methylisothiocyanate or sodium *N*-methyldithiocarbamate, registered with the US Environmental Protection Agency for application to wood.[8] As the interactions between AIT and wood remain unclear, further investigations should be conducted to understand the mechanism of gaseous treatment of wood and to determine the effectiveness of AIT as a fumigant.

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