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# *Lentinus Edodes* Colonization of Oak is Enhanced by Log Treatment With Methyl Bromide

Elmer L. Schmidt,\*

**ABSTRACT** — Two or more years of outdoor incubation of inoculated oak logs are required before shiitake (*Lentinus edodes* [Berk.] Sing.) mushrooms appear; methods to accelerate the rate of oak sapwood colonization should reduce this incubation period. Observed increased mold growth of oak sapwood fumigated with methyl bromide (MB) prompted an experiment to determine whether such treatment would enhance oak colonization by *L. edodes*. Red and bur oak bolts (1 m long) were inoculated three weeks after felling. Half of the bolts were fumigated with MB (100 g per m<sup>3</sup> of space beneath a plastic tarp) for three days, then vented for five days prior to inoculation. Bolts were incubated in a partly-shady location and sampled for fungus with an increment hammer one year after inoculation. *L. edodes* was recovered from 40% (31/78) of the plugs plated from untreated red oak, and from 92% (72/78) of those formerly fumigated with MB. Bur oak yielded 67% (24/36) from untreated and 94% (34/36) from fumigated bolts. These data indicate enhanced oak colonization by the shiitake fungus after MB fumigation.

## Introduction

The use of low-grade, small diameter oaks for production of the increasingly popular edible shiitake mushroom (*Lentinus edodes* [Berk.] Sing.) (Figure 1) could provide added value from these under-utilized local species. Commercial production of this fungus has begun in the east and west coastal regions of the United States and in Canada (1,2,3). Logs inoculated with shiitake may require two years of outdoor storage before mushroom production begins (4,5). This log colonization period varies according to climatic conditions, and may be reduced to 6 months by encasing inoculated logs in dry, absorbent cellulosic material and protecting the assembly from external moisture (6). However, such manipulations add to the cost of production as compared to outdoor log storage. A method to shorten the spawn run based on increasing the rate of log colonization while minimizing labor inputs would be desirable.

During recent experiments using methyl bromide (MB) to eradicate the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt., from infected bur (*Quercus macrocarpa* Michx.) and red (*Q. rubra* L.) oaks (7,8,9), heavy mold growth on gas-treated oak sapwood was noted. Whether such increased fungus growth was due to the elimination of competitive organisms (10,11) or a substrate modification (e.g. cellulose methylation) remains unanswered, but these observations prompted an experiment to determine whether such treatment would enhance oak sapwood colonization by *L. edodes*.

## Materials and Methods

Sixteen red oak and 10 bur oak bolts (1 m long, 10-20 cm dia.) were cut from a total of five individual trees of each species in late September 1982. After two weeks of storage outdoors, half of the bolts were encased in a six mil polyethylene tarp and fumigated with 100 g of pure MB per m<sup>3</sup> of space under the tarp. The tarp was removed and bolts were aired for



Figure 1. Shiitake mushrooms fruiting from *Quercus macrocarpa* oak log sections (forced-fruited in greenhouse, January, 1984).

five days. Treated and untreated bolts were then inoculated by packing *L. edodes* spawn (pure culture grown on rye) into 18 holes (2 cm dia. x 4 cm deep) per bolt. Holes were covered with duct tape to retard the drying of spawn. Inoculated bolts were placed horizontally on 10 cm high supports in a grassy, partly-shaded area for incubation. Bolts were rotated 180° about the longitudinal axis every six to eight weeks during the summer.

One year after inoculation, selected bolts (five treated and five untreated for each species) were sampled for *L. edodes* using an increment hammer. Cores (2 cm long, 0.5 cm dia.) were taken in a line on either side of fungus colonized inoculum holes at approximately 3, 6, and 9 cm from the hole

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edge. Wood on either side of 13 holes was sampled from the red oak bolts and about six holes from the bur oaks for both treated and untreated material. Plugs were surface disinfected by flaming and placed on malt extract agar plates containing 20 ppm benomyl to selectively favor culture of wood-rotting basidiomycete fungi. Cultures of the strain of *L. edodes* used in inoculation were also established on this medium to provide reference colonies for comparing morphology of those growing from plugs. All cultures differing in gross morphology from reference plates were checked microscopically (x400) for hyphal characteristics. Ten percent of those cultures visually matching *L. edodes* colony types were also confirmed on the basis of microscopic hyphal characters.

## Results and Discussion

*L. edodes* was recovered from 40 percent (31/78) of the increment cores from untreated red oak (Table 1). The remainder (60%) provided cultures of other wood-rotting fungi (*Schizophyllum commune* Fr. and three unidentified basidiomycetes), bacterial contamination, or *Alternaria* sp. In contrast, 92 percent (72/78) of plugs from red oak previously treated with MB were positive for *L. edodes*. Seven of these positive cultures presumed to be *L. edodes* by their similar culture appearance were microscopically checked for the typical clamped hyphae which tended to strand, a characteristic of *L. edodes* (Figure 2).

The improved colonization of bolts on MB treated bur oak was less dramatic than on red oak. Untreated bur oak yielded 67 percent positive cultures (24/36) and treated bolts were 94 percent (34/36) positive for *L. edodes*. Chi-square analysis of counts showed the red oak isolations from treated bolts significantly greater ( $P < .001$ ;  $\chi^2 = 37$ ) than from untreated material. Too few holes were sampled from bur oak to declare the probability of significant difference between the isolation frequencies greater than 50 percent ( $P = 0.5$ ;  $\chi^2 = 4.8$ ).

These data indicate enhanced colonization of oak sapwood by shiitake after MB fumigation. The probability of recovering the fungus was not well-correlated with distance from inoculum hole, perhaps due to organism succession or die-off of *L. edodes* during incubation. These bolts will be monitored for onset of mushroom production to assess whether more

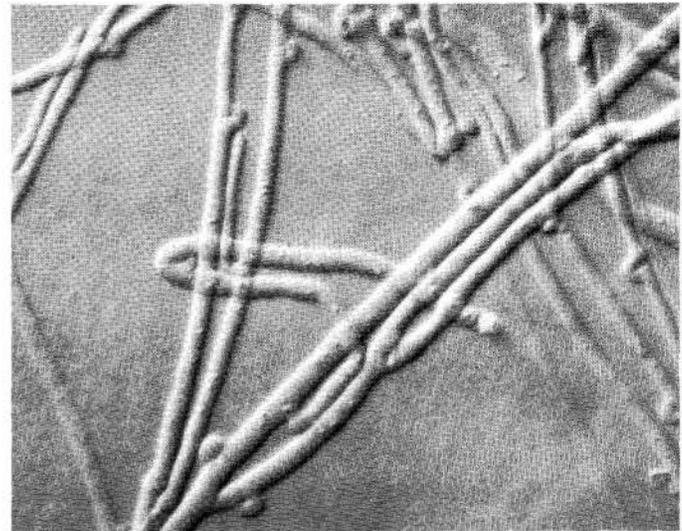


Figure 2. Clamp connections and hyphal alignment of *Lentinus edodes* grown on benomyl-amended malt extract agar (x400-original magnification).

extensive mycelial colonization of MB treated wood shortens required incubation period of inoculated logs, and results in greater mushroom yield over the three to five years of expected production.

## Acknowledgements

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Table 1. Number of samples from which *Lentinus edodes* were recovered from untreated and methyl bromide treated oak after one year of outdoor incubation.

| Hole # | RED OAK   |                 | BUR OAK   |                 |
|--------|---|-----------------|---|-----------------|
|        | Treated   | Untreated       | Treated   | Untreated       |
| 1.     | 5*  | 1               | 6   | 6               |
| 2.     | 5   | 0               | 6   | 6               |
| 3.     | 6   | 1               | 6   | 6               |
| 4.     | 6   | 3               | 6   | 0               |
| 5.     | 6   | 6               | 4   | 3               |
| 6.     | 5   | 1               | 6   | 3               |
| 7.     | 6   | 6               | 34 <sup>c</sup>   | 24 <sup>d</sup> |
| 8.     | 6   | 4               |   |                 |
| 9.     | 3   | 1               |   |                 |
| 10.    | 6   | 0               | * -Number of cores out of 6 yielding <i>L. edodes</i> for isolation about any given inoculation hole. |                 |
| 11.    | 6   | 4               |   |                 |
| 12.    | 6   | 4               |   |                 |
| 13.    | 6   | 5               |   |                 |
|        | 72 <sup>a</sup>                                     | 31 <sup>b</sup> |   |                 |
|        | Chi-square ( $P < .001$ ): $a > b$<br>$\chi^2 = 37$ |                 | $(P = 0.5)$ : $c > d$<br>$\chi^2 = 4.8$   |                 |