Techniques

# Effect of Fluorescent-Labeled Lectins on Visualization of Decay Fungi in Wood Sections

J. J. Morrell, D. G. Gibson, and R. L. Krahmer

Assistant professor, graduate research assistant, and professor, respectively, Department of Forest Products, Oregon State University, Corvallis 97330. Present address of second author: Reichhold Chemicals, Inc., P.O. Box 1482, Tacoma, WA 98401.
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## ABSTRACT

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Fluorescent-labeled lectins were used to stain white, brown, and soft rot fungi in microtomed sections of wood from several coniferous species; the sections were then observed with incident fluorescence microscopy. Fluorescein-labeled wheat germ agglutinin greatly enhanced hyphal visibility of all fungi over that obtained with the conventional safranin-O/picro-aniline blue method. Thus, fluorescent-labeled lectins provide an improved tool for observing fungi in wood sections at stages when detection with conventional light microscopy varies, and they could prove useful for following the course of decay from spore germination to complete wood breakdown.

*Additional key words*: Douglas-fir (*Pseudotsuga menziesii*), *Poria placenta*, southern pine (*Pinus* spp.), western red cedar (*Thuja plicata*)

The ability to detect fungi in wood before substantial damage occurs is an important part of any decay prevention program. However, by the time decay hyphae are readily visible, substantial wood degradation may have already occurred (10). This dilemma has spurred interest in indicators that detect the chemical changes associated with wood decay as well as in stains that enhance the appearance of fungi in wood (3,6). These techniques have proven useful, but wood and fungal variability have limited their usefulness.

Ideally, indicators should detect components universally present in fungal-colonized wood but absent from noncolonized wood. Indicator specificity must be such that the indicator does not react with any compounds normally present in wood. One component of wood colonized by fungi is chitin, a long chain, linear polymer of repeating *N*-acetylglucosamine units present in the cell walls of most fungi (1). Indicators that react with chitin could detect fungal presence in samples before the culturing process, thereby screening cores that contain fungi and reducing the amount of culturing required.

Towards this goal, we have evaluated the reactivity of plant-derived lectins as potential fungal indicators. Lectins are proteins that selectively bind with various carbohydrates and glycoproteins (7); they have been used to determine the identity of specific compounds present within various cell types (5,7,8). Although each lectin is highly specific, reacting with only one or two carbohydrates, the wide variety of lectins available permits their usage in a variety of biological systems (5,7,8). Location of the compound for which a lectin is specific can be readily visualized by coupling nonreactive portions of the lectin with fluorochromes (tetramethylrhodamine isothiocyanate [RITC] or fluorescein isothiocyanate [FITC]). These compounds fluoresce when excited with ultraviolet (UV) light and, with appropriate filters, can be located by viewing through a microscope.

In our procedures, several lectins were selected to react with the *N*-acetylglucosamine present in chitin or some portion of the wood hemicellulose for the purpose of identifying a rapid method for

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detecting the presence of fungi in wood while improving the ability to visualize fungal invasion.

## MATERIALS AND METHODS

Reactivity and specificity of a number of fluorescent-coupled lectins (Vector Laboratories, Inc., Burlingame, CA) (Table 1) were initially tested in hyphal fragments of the decay fungi *Poria placenta* (Fr.) Cke. [Madison L80355P] and *Sistotrema brinkmanii* (Bres.) Erikss. [OSU 3UD9 Scappose] and the nondecay fungus *Hyalodendron lignola* Diddens [OSU-9BGI]. These fungi were grown in 1.25% malt extract broth for 7 days and filtered; the resulting mycelial mass was rinsed several times with phosphate-buffered saline (PBS), pH 7, before storage in PBS at 0 C. A thawed hyphal sample of each fungal species was placed on a slide to dry, and a dilute solution (1:1,000 in PBS) containing the specific fluorescent-coupled lectin was added to the dry mycelium. After 15–20 minutes, the lectin solution was rinsed from the mycelium three times with PBS and the mycelium was mounted in glycerin on a glass slide. Slides were examined for the presence of fluorescent-labeled lectin on the hyphae by viewing them through a Leitz fluorescence microscope with incident illumination from a xenon light source through either a Ploemopak Module M-2 for RITC or a Module H-2 for FITC.

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], southern pine (*Pinus* spp.), and western red cedar (*Thuja plicata* Donn ex D. Don) wood blocks were exposed to a variety of hymenomycetous decay fungi and soft rot fungi for periods ranging from 5 days to 2 mo (Table 2). Specifically, Douglas-fir samples were decayed with brown or white rot fungi (agar-block test [4,6]) and soft rot fungi (vermiculite burial test [9]), southern pine samples with brown rot fungi (agar-block test [4,6]), and western red cedar samples with brown rot fungi (modified soil-block test) (Table 2). All blocks were dried for storage after fungal exposure. Later, the dried samples were soaked in water before sectioning with a sliding microtome. Sections 20 $\mu$m thick were cut from the radial and tangential block faces. Unstained sections from each wood species were mounted on glass slides in glycerin and examined to assess the degree of autofluorescence produced with the selected fluorescence filter systems. Autofluorescence was determined to be at a low enough level that the fluorescing compounds being tested could easily be discerned. A few sections were treated with hot water extracts from Douglas-fir bark or sodium borohydrate to quench wood autofluorescence, but these techniques proved unsatisfactory.

Other microtomed sections were reacted with the selected lectins as follows: the section was dried on a glass slide, reacted with a dilute lectin solution (1:500 in PBS) for 15–20 min, and rinsed three times with an excess of PBS to remove unreacted lectin. The sections were blotted dry and mounted in glycerin on glass slides for examination.

The effectiveness of lectins for detecting decay fungi was compared with that of a safranin-O/picro-aniline blue staining technique (11). Sections were first reacted and examined for fungi with fluorescent-labeled lectins. These sections were then removed from the slides, rinsed in distilled water, stained with 1% safranin-O for 2 min, rinsed, and placed in steaming picro-aniline blue for 1

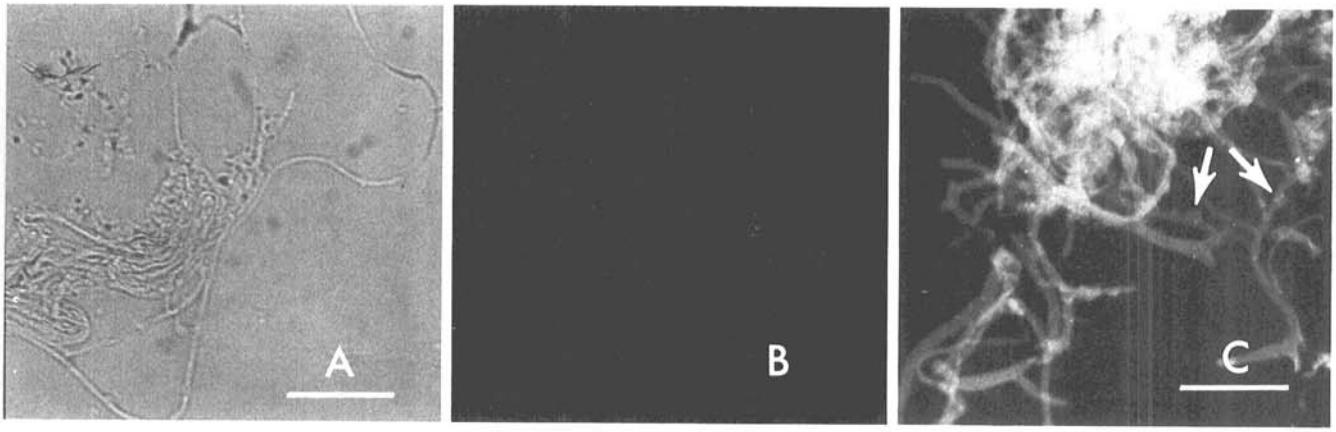


**Fig. 1.** Micrographs of hyphae of *Poria placenta* (×392) showing their appearance: **A,** under normal bright-field illumination; **B,** under incident illumination from a xenon light source, observed with a Leitz fluorescent microscope equipped with Ploemopak Module H-2 for fluorescein; **C,** under the same illumination as B following reaction with fluorescein-coupled wheat germ agglutinin. Note the clamp connections (arrows) and dramatically improved visibility of all hyphae. Bar represents 50 $\mu$m, and the scale is the same for **A–C.**

TABLE 1. Specificity of fluorescent-labeled lectins tested on culture-grown hyphal fragments and on wood sections exposed to decay fungi

| Lectin | Specificity | Lectin reactivity[a] | | | |
|---|---|---|---|---|---|
| | | Hyphae[b] | | Wood section[c] | |
| | | ND | D | Hyphae | Wood cell wall |
| Concanavalin A | α-D-mannosyl, α-D-glucosyl | ++ | + | ++ | +++ |
| Soybean agglutinin | *N*-acetylgalactosaminyl | 0 | 0 | 0 | 0 |
| Wheat germ agglutinin | $(\beta$-*N*-acetylglucosaminyl$)_n$ sialic acid | +++ | +++ | +++ | 0 |
| *Dolichos biflorus* agglutinin | *N*-acetylgalactosaminyl-Blood group A | 0 | 0 | 0 | ++ |
| *Ulex europaeus* agglutinin I | L-fucosyl, Blood group O (H) | 0 | 0 | 0 | 0 |
| Peanut agglutinin | β-D-gal(1-3)D-galNac β-D-galactosyl | + | + | 0 | ++ |

[a] Based upon visual assessment where +++ = strongly reactive, ++ = moderately reactive, + = weakly reactive, 0 = nonreactive.
[b] Hyphae were collected from cultures grown in 1.25% malt extract for 7 days, rinsed to remove media, and frozen before use. Dilute lectin solutions (1:1,000 in phosphate-buffered saline) were reacted on slides and examined directly after rinsing. ND = a nonwood decaying fungus, *Hyalodendron griseus*; and D = wood decay fungi, *Sistotrema brinkmanii* and *Poria placenta*.
[c] Wood sections were cut from Douglas-fir and southern pine blocks exposed to *P. placenta* and examined following reaction with dilute lectin solution (1:500 in phosphate-buffered saline).

min. Following rinsing, the sections were remounted in glycerin on glass slides and examined for aniline-blue stained fungal hyphae with a Leitz light microscope.

Photomicrographs were taken on 35-mm color film (Kodachrome 64) and black and white film (Tri-X-400 ASA).

## RESULTS AND DISCUSSION

Of the lectins tested (Table 1), only wheat germ agglutinin (WGA) exhibited any specificity for the hyphal fragments examined. This lectin, which is specific for *N*-acetylglucosamine and sialic acid residues, would be expected to react positively with the *N*-acetylglucosamine-containing chitin. Both RITC- and FITC-labeled WGA reacted strongly with the hyphae, causing them to fluoresce a brilliant red and green, respectively, making them readily visible when compared to controls not treated with lectin (Fig. 1). However, FITC-labeled WGA enhanced hyphal visibility to a greater extent than RITC-labeled WGA and was used for all subsequent tests.

When the lectins were tested on wood sections, FITC-labeled WGA was the only lectin to react positively with hyphae present in the wood cells, although concanavalin A was weakly reactive with both the hyphae and wood cell wall (Table 1). This lectin, which is specific for the hemicellulose components D-mannosyl and D-glycosyl, may prove to be a useful decay indicator. However, the fluorescence observed was not significantly stronger than normal wood autofluorescence.

FITC-labeled WGA permitted more detailed examination of wood-hyphal interactions than was possible with the conventional safranin-O/picroaniline blue stain series used for comparison of

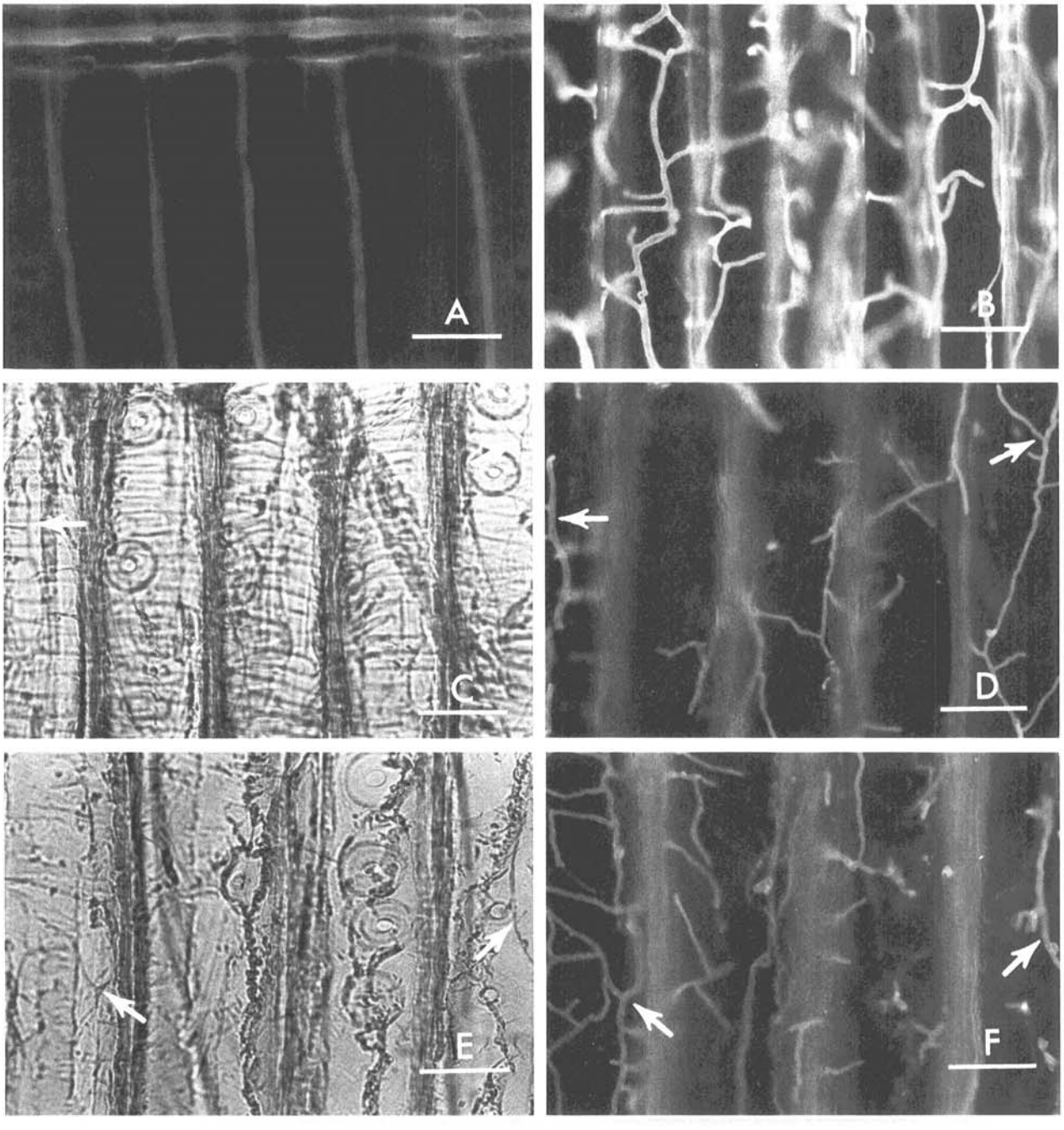


**Fig. 2.** Radial sections from western red cedar (**A,B**), Douglas-fir (**C,D**), and southern pine (**E,F**) stained with a safranin-O/picro-aniline blue series (**C,E**) or fluorescein-coupled wheat germ agglutinin in phosphate buffered saline (**A,B,D,F**) (×392). All sections but **A**, the unexposed control, were exposed to *Poria placenta* for varying time periods. Note the absence of lectin reactivity on the control (A) and the vastly improved visibility of lectin-reacted hyphae, compared with that of the safranin-O/picro-aniline blue series (arrows), on the exposed specimens. Bar represents 50 μm; scale is the same for **A-F**.

TABLE 2. Fungi used to decay wood samples later tested for effects of lectins on visualization of hyphae in wood sections

| Wood species | Decay fungi | Source of culture[a] |
|---|---|---|
| Douglas-fir | Brown rot[b] | |
| | *Poria placenta* (Fr.) Cke. | Madison-698 |
| | *Poria xantha* (Fr.) Cke. | Madison 5096-35 |
| | *Lentinus lepideus* Fr. | Madison-534 |
| | White rot[b] | |
| | *Coriolus versicolor* (L. ex Fr.) Que'l. | Madison-697 |
| | *Irpex lacteus* (Fr.) Fr. | Madison-517 |
| | Soft rot[c] | |
| | *Chaetomium globosum* Kunze ex Fr. | Syracuse |
| | *Phialocephala dimorphospora* Kendrick | Syracuse P-136 |
| Southern pine | Brown rot[b] | |
| | *Poria placenta* (Fr.) Cke. | Madison 698 |
| | *Poria radiculosa* (Pk.) Sacc. | L-11659 sp. |
| | *Lentinus lepideus* Fr. | Madison 534 |
| | *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. | Madison-617 |
| | *Gloeophyllum saepiarium* (Wulf ex Fr.) Karst. | Madison 604 |
| | *Peniophora gigantea* (Fr.) Mass. | FP-105277 sp |
| Western red cedar | Brown rot[d] | |
| | *Poria placenta* (Fr.) Cke. | Madison 698 |

[a] Madison, L, and FP all refer to USDA Forest Service, Forest Products Laboratory, Madison, WI; Syracuse refers to SUNY College of Environmental Science and Forestry, Syracuse, NY.
[b] Agar-block test (4,6).
[c] Vermiculite burial test (9).
[d] Modified soil-block test.

the visualization of both brown and white rot fungi in western red cedar, Douglas-fir, and southern pine (Fig. 2). Conversely, the soft rot fungi, which produced both Type I (longitudinal, diamond-shaped cavities) and Type II (cell wall erosion) damage (6), were made more visible only in the eroded areas of the cell wall adjacent to the cell lumen. The cavities produced by these fungi within the $S_2$ (secondary) cell wall layer were characterized by zones of intense fluorescence that obscured most cavity detail. This intense fluorescence may have occurred due to the presence of a slime matrix around the cavity hyphae, which inhibited movement of the nonreacted lectin from the cavity; the presence of a surrounding hyphal matrix has been suggested in a number of cavity formation theories (2).

The enhanced appearance of lectin-reacted hyphae at very early stages of decay could be extremely useful for tracking fungal invasion before wood weight losses become detectable. At this early decay stage, there is little measurable weight loss, although we observed an abundance of hyphae within the wood. Many of these hyphae do not appear to react well with conventional stains and therefore are less easily seen with the light microscope. However, when FITC-labeled WGA (which is specific for the chitin universally present in higher fungi) is used, hyphae can be readily observed and their role in early cell-wall attack more clearly demonstrated. Thus, fluorescent-labeled lectins provide an improved tool for observing and detecting fungi in wood sections at stages when detection with conventional light microscopy is variable (10) and could be a useful technique for following the course of decay from spore germination to complete wood breakdown.

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