

DOUBLE-STAINING TO IMPROVE VISUALISATION OF WOOD DECAY HYPHAE IN WOOD SECTIONS*

by

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Summary

Combining fluorescent-labeled wheat germ agglutinin (a chitin-specific lectin) with conventional histological stains offers a simple, efficient method for studying fungal hyphae in deteriorating wood. Cell walls stain dark red with safranin O, providing excellent contrast for the green-fluorescing hyphae. Staining sections with brilliant vital red markedly enhances the visibility of fungal bore holes.

Key words: Decay, hyphae, fluorescence microscopy.

Introduction

Detecting incipient colonisation by decay fungi in wood before substantial weight loss occurs has always been a challenge (Wilcox, 1978; Krahmer et al., 1982; Gibson et al., 1985). We have evaluated the use of fluorescent-labeled lectins with specificities for certain wood and fungal cell-wall fractions to improve hyphal visualisation (Morrell et al., 1985). Lectins are plant-derived chemicals that react with specific carbohydrates or carbohydrate-containing compounds. Fluorescent-labeled lectins permit detailed examination of the point of localisation with a fluorescence microscope. The most promising lectin, wheat germ agglutinin (WGA), is highly specific for residues of N-acetylglucosamine, the principal component of chitin. Because the cell walls of most higher fungi contain chitin, WGA appears to be a highly specific probe for visualising hyphae in wood.

While fluorescent-labeled WGA dramatically improved our ability to visualise decay hyphae in wood at low levels of weight loss, normal wood autofluorescence sometimes interfered with observation of the hyphae. In this study, we evaluated the ability of several common laboratory stains to decrease autofluorescence and improve contrast between hyphae and wood.

Materials and Methods

Blocks from southern yellow pine (*Pinus* spp.) and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] were decayed to selected weight losses by a variety of fungi, and sections 20 μ m thick were cut with a sliding microtome. Sections first were stained with a standard histological stain (Johansen, 1940), rinsed thoroughly in distilled water, and blotted dry on glass slides. The WGA, coupled to the fluorochrome fluorescein isothiocyanate (FITC; Vector Laboratories, Burlingame, CA) and diluted 1:500 in phosphate-buffered saline (PBS) to form a stock solution, was dropped on the sections. After incubating in darkness for about 5 minutes, the sections were rinsed thoroughly with PBS and distilled water and then mounted in water on glass slides. Cover glasses were placed over the stained sections and the sections were observed immediately through a Leitz fluorescence microscope. Incident illumination was provided by a xenon light source through a Ploemopak Module H-2. Photomicrographs were taken using Ektachrome 200 colour film.

Results and Discussion

Unstained sections reacted with FITC-labeled WGA provided good contrast between hyphae and autofluorescing wood, although hyphae in double-stained sections were a brighter green (Table 1). Wood stained with safranin O retained a reddish colour under the filter specific for the green-fluorescing FITC-WGA, making the hyphae more visible. Other stains gave the wood a yellow colour. A comparison of hyphae stained with picro-aniline blue alone and with FITC-labelled WGA alone indicated that the latter reaction improved visualisation of fungal hyphae with little interference from normal wood structure (Figs. 1-3). Hyphae are usually

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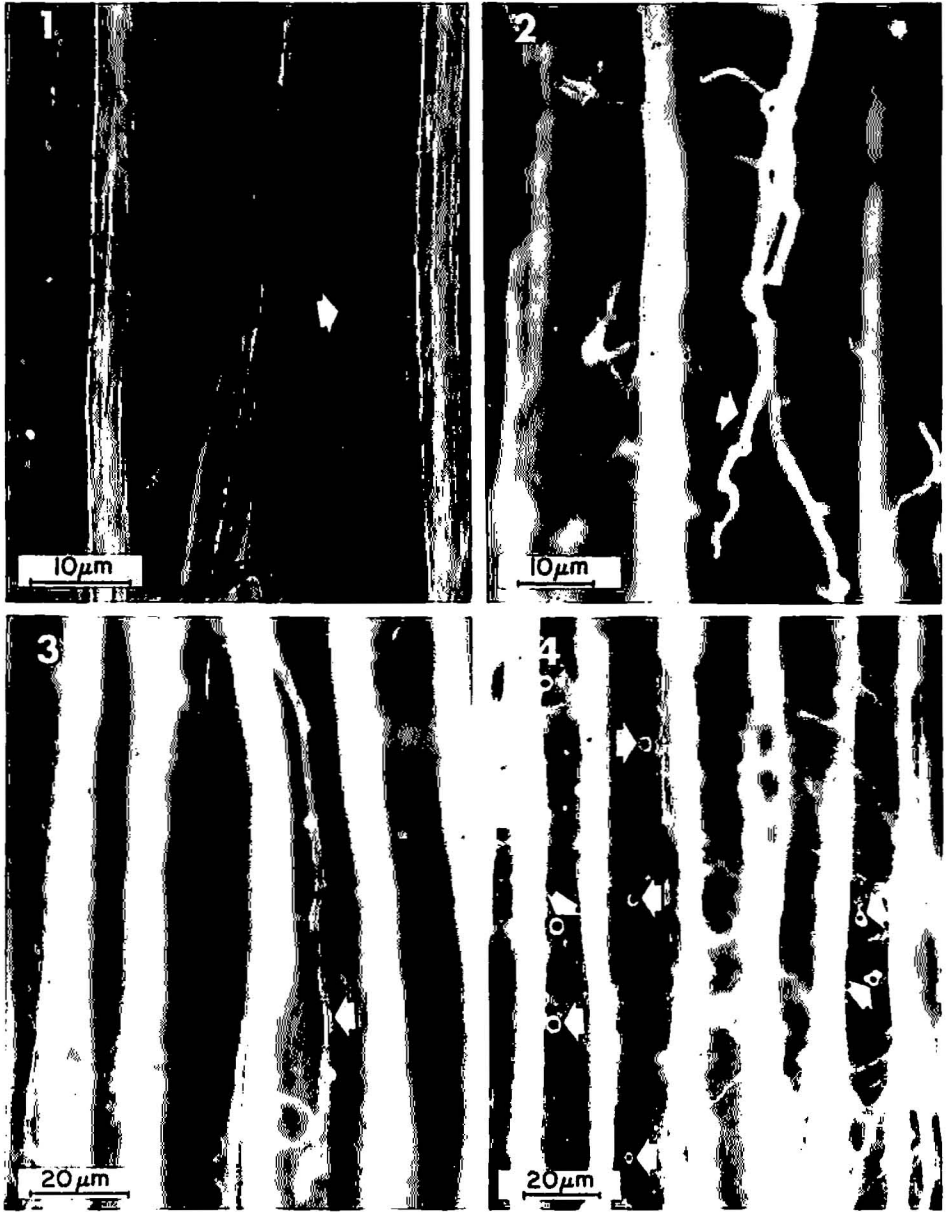


Fig. 1–3. Longitudinal sections of southern yellow pine exposed to *Porta placenta* (Fr.) Cke, illustrating hyphae (arrows) stained with picro-aniline blue (Fig. 1) and with fluorescein isothiocyanate-labeled wheat germ agglutinin (Figs. 2 & 3). The wood in Fig. 2 was not stained; the wood in Fig. 3 was stained with acid fuchsin. — Fig. 4. Longitudinal section of decayed Douglas-fir wood stained with brilliant vital red, followed by FITC-labeled WGA. Note the fluorescence around the borders of fungal bore holes (arrows) in the tracheid walls.

Table 1. Appearance of wood and hyphae double-stained with histological stains and FITC-labeled WGA.

Histological stain	Colour under fluorescent light		
	Wood	Hyphae	Other features
(none)	Greenish yellow	Green	
Safranin O	Brownish red	Bright green	
Acid fuchsin	Yellowish	Bright green	
Alizarin red	Yellowish	Bright green	
Brilliant vital red	Yellowish	Bright green	Bore holes yellow
Phloxine	Yellowish	Bright green	

difficult to detect at early stages of decay, especially in wood with spiral thickenings on the tracheid walls (e.g. Douglas-fir), but the brightly fluorescing green hyphae resulting from treatment with FITC-labeled WGA were observed even at low magnifications.

Double-staining with brilliant vital red and FITC-labeled WGA produced a yellow fluorescent rim around fungal bore holes through the wood (Fig. 4). Many more bore holes were apparent with this stain than with the other stains tested.

Hyphae stained with FITC-labeled WGA lost their fluorescence with time, especially when exposed to fluorescent light. Therefore, most observations were made within 30 minutes after staining. However, the bright green fluorescence on the hyphae could be recovered, even after drying during storage, by restaining with the lectin. This second reaction was more permanent than the initial reaction.

Fluorescent-labeled lectins provide a valuable tool for studying fungal decay of wood at the initial stages of deterioration, when conventional stains are more variable. Combining these compounds with histological stains improves the lectin contrast and increases the visibility of scattered hyphae.

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