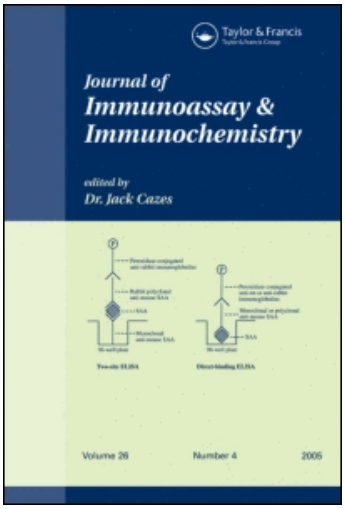

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Dyed Particle Capture Immunoassay for Detection of Incipient Brown-Rot Decay

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DYED PARTICLE CAPTURE IMMUNOASSAY FOR DETECTION OF INCIPIENT BROWN-ROT DECAY

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ABSTRACT

An immunological method for detecting incipient fungal decay in wood is described. Monoclonal antibody to extracellular β-1,4-xylanase of the brown-rot fungus *Postia placenta* was immobilized in a defined capture zone to a strip of hydrophobic polyester cloth. Polyclonal-labeled latex particles were applied near one end of the polyester assay substrate. The resulting test strip was dipped into extracts from wood blocks inoculated with various wood decay fungi. Extracts flowed laterally through the antibody-labeled latex particles and the capture zone. When antigen was present in the extract, the antigen was complexed by the monoclonal and labeled-polyclonal antibodies to form an observable particle complex in the zone of capture. Wood samples were tested at various stages of decay and correlated with wood weight loss. Incipient brown-rot decay was detected with the particle capture immunoassay at less than 2 percent wood weight loss.

KEY WORDS: Dyed particle capture immunoassay, *Postia placenta*, incipient decay, brown-rot fungi

INTRODUCTION

Many types of organisms deteriorate wood, but the most prevalent economic loss is caused by fungi. Brown-rot, the most destructive type of decay, can rapidly cause structural failure. As little as a 1% weight loss in wood can result in a 50% loss in strength as measured by toughness (1).

Detecting incipient stages of decay in wood structures has been such a problem that research efforts in several disciplines have been directed towards the development of a successful field test. Visual and microscopic inspection of borings from wood, sounding of wood, radiography, sonics, and electronic resistance (2,3) have been used for detecting advanced decay, but initial stages of decay are difficult to detect with these methods. Results of direct staining of core samples with chemical indicators are subject to interpretation (4). The Shigometer,[1] an electronic-type detector, has been used for detection of early internal decay in trees and utility poles. This instrument gives unreliable results when used on wood products with a moisture content between 38% and 45% (5). Visual inspection and culturing are still traditionally used to evaluate wood samples for signs of fungal decay, but the shortcomings of these methods can lead to misdiagnosis. Misdiagnosis can lead to unnecessary replacement of uninfected wood or inappropriate remedial treatments in an effort to halt the spread of decay.

[1] The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Recently, immunological methods have been so successful in detecting early fungal infection in agricultural crops, that commercialized field test kits are on the market for several economically important plant pathogens. Various types of immunoassays, such as enzyme-linked immunosorbent assay (ELISA) (6,7,8,9) and dot blot (10), have been successful in detecting brown-rot decay *in vitro*. However, a diagnostic field test method is needed by timber inspectors who lack scientific expertise and laboratory facilities. Our laboratory has studied several systems including ELISA, dot-blot, and agglutination assays (6). We found that agglutination with latex beads was adequately sensitive for a presumptive test, although ELISA was necessary to verify and quantify decay. Neither of these methods was easily transferrable to a field test kit, and both lacked the sensitivity for incipient decay that could be incorporated in a more recent technology known as particle capture immunoassay (PCI). The PCI is a significant advance in technology for all fields of diagnostics. This method incorporates the increased sensitivity and specificity of a double antibody sandwich ELISA (11,12) with the simplicity of a test strip that can be easily transported to the field for use by the layperson. Further simplicity was added by utilizing dyed latex particles and eliminating colored enzyme/substrate reagents. This is the first report of a dyed PCI utilizing antibodies to fungal components for the diagnosis of incipient brown-rot decay.

MATERIALS AND METHODS

Fungal Cultures

Brown-rot fungi: *Postia placenta* (Fr.) M. Lars. & Lomb., MAD-698; *Gloeophyllum trabeum* (Pers.: Fr.) Murr., MAD-617; *Antrodia car-*

bonica Overh. HHB-5104; *Lentinus lepideus* (Fr.), MAD-534; *Serpula incrassata* (Berk. & Curt.) Donk, MAD-563; and *Coniophora puteana* (Schum.: Fr.) Karst., MAD-515; white-rot fungi: *Phanerochaete chrysosporium* Burds. ME-446; *Trametes versicolor* (L.:Fr.) Pil. MAD-697; *Ganoderma applanatum* (Pers.) Pat. 7823-S; sap stain fungus: *Ceratocystis coerulescens* (Munch) Bakshi, C-262; mold fungus: *Trichoderma harzianum* Rifai. ATCC-20476. Cultures were maintained on 2% malt extract agar (MEA) plates at 4°C.

Antigen Extraction

Southern yellow pine wood blocks (7 by 7 by 3 mm) were decayed by the standard ASTM soil block method (13). Groups of three blocks were taken from the soil bottles every 3 days, brushed, air-dried, reconditioned at 27°C, 70% relative humidity (RH), and weighed. Each group was ground to 20 mesh in a Wiley Mill and extracted in 1.5 ml H_2O plus 0.1 % Triton X-100 (Sigma, St. Louis, MO) for 2 h at 25°C.

Antibody Preparation

Polyclonal antibody: *P. placenta*-decayed sweetgum (*Liquidambar styraciflua*) was extracted in 50 mM Tris, 0.85% NaCl, and 0.1 mM EDTA, pH 7.0, and ultracentrifuged at 36,000xg for 2 h. Hemicellulases were fractionated by passing through a 60- by 2-cm Sepharose 6B (Pharmacia, Piscataway, NJ) column equilibrated with 50 mM Tris-HCl buffer and subsequently passed through a 90- by 26-cm Fractogel TSK HW-55 (F) (EM Science, Gibbstown, NJ) column equilibrated with 50 mM Tris-HCl buffer, pH 7.0, 0.5M NaCl (14). Fractions containing hemicellulase activity

as determined by the microadaptation of the Nelson–Somogyi reducing sugar assay (15) were pooled, dialyzed, mixed 1:1 with Freund's Complete Adjuvant (Difco, Detroit, MI), and injected subcutaneously at multiple sites in New Zealand white rabbits. At 14-day post-inoculation, rabbits were boosted intramuscularly with hemicellulase:Freund's Incomplete Adjuvant (Difco) and bled 10 days later (16).

Monoclonal antibody: Murine monoclonal antibodies (mAbs) were produced (17) to the extracellular β-1,4-xylanase fraction of the brown-rot fungus, *Postia placenta*, as previously described (18). Purified IgG at a concentration of 1 mg/mL in phosphate-buffered saline (PBS) was used to coat the capture zone of the test strip.

Antibody-Labeled Polystyrene Beads

Blue polystyrene beads, 1 mL of 0.785 μm diameter, (Seradyn, Inc., Indianapolis, IN), washed in PBS to remove surfactant, were resuspended with sonification and passively adsorbed to 2 mL purified polyclonal IgG (1.4 mg protein) for 24 h at 4°C. Bound beads were centrifuged to remove unbound antibody and blocked with 0.1% bovine serum albumin (BSA) (Sigma, St. Louis, MO) in PBS for 1 h. The blocking solution was removed by centrifugation and pelleted beads were resuspended to 3% solids in PBS with 0.1% Triton X-100 and 0.02% Na azide (Sigma).

Particle Capture Test Strip

Antixylanase monoclonal antibody (Ab_1) was passively bound to a 1- by 6-cm strip of polyester cloth (E.I. DuPont Nemours & Co., Wilmington, DE) 4 cm from one end of the strip and dried at 40°C (Fig.

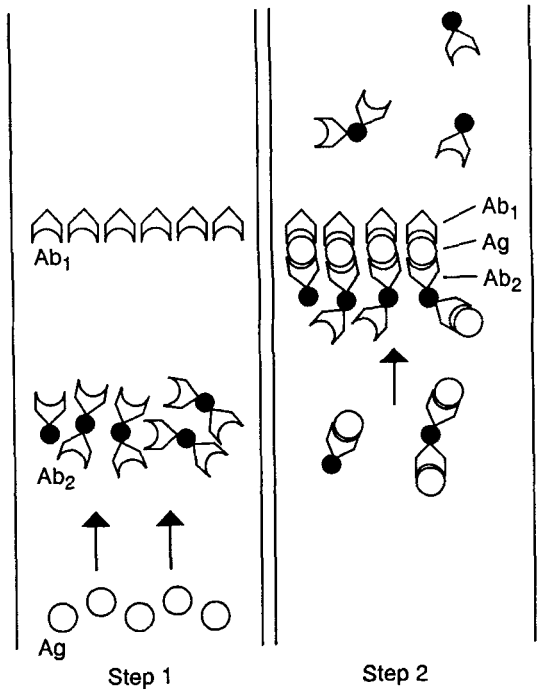

Figure 1—Particle capture immunoassay (PCI): Step 1, test strip is dipped into fungal extract, and extract (antigen) is wicked upward; step 2, fungal antigens captured by Ab_2-labeled beads are sanwiched by a second immobilized antibody (Ab_1) to form colored line for positive result.

1). The entire cloth strip was saturated with 10% sucrose and 0.2% BSA in water for 1 h at 25°C, dried at 40°C, recoated with the sucrose/BSA mixture, then completely dried at 40°C. Polyclonal antibody-labeled colored polystyrene beads (Ab_2) were applied to the coated cloth strip in a line 1 cm from the bottom of the test strip.

RESULTS

The dyed particle capture test strip is shown schematically in Figure 1. In step 1, the prepared test strip is dipped into a fungal extract

that is wicked upward through the cloth. Step 2 shows that antigen from the fungal extract, which is specific for Ab_2, is captured by the labeled particles and further carried to the immobilized Ab_1. In positive tests, the antigen was sandwiched between the polyclonal antibody-labeled beads and the monoclonal antibody, and an easily visible colored line resulted 4 cm from the bottom of the test strip in the reaction zone. In a negative test, the polyclonal antibody-labeled beads passed through the zone of capture resulting in no colored line.

The PCI was tested for sensitivity and specificity to six brown-rot fungi, three white-rot fungi, a sapstain fungus, and a mold fungus. The six brown-rot fungi were detected with the PCI test at less than 2% wood weight loss (Table 1); *S. incrassata*, *L. lepideus*, *P. placenta*, and *A. carbonica* were detected at less than 1% wood weight loss. Weight losses shown in Table 1 were obtained after 3 days of exposure to the test fungi. Samples were collected twice weekly for 4 weeks, and results were the same for the remaining test dates. The white-rot, sapstain, and mold fungi were PCI negative during the 4-week test period. An antigen control (undecayed wood extract) and an antibody control (normal mouse serum) exhibited negative reactions in the PCI test.

In the initial test, there were slight cross-reactions with two of the white-rot fungi, but no cross-reaction with the third white-rotter or the other nondecay fungi tested. Cross-reactions were eliminated by adsorbing cross-reacting antigen out of the antibody preparation prior to preparing the test strips (Fig. 2).

Douglas-fir power poles, which had been remedially treated with boron to arrest fungal decay, were sampled 18 months post-treatment. Core borings from 11 poles were cultured for viable fungi and tested in the PCI.

TABLE 1

Ability of PCI to Detect Fungal Antigen From 11 Fungal Extracts at Low Wood Weight Losses

Fungus	Weight loss (%)	Particle capture immunoassay[a]
S. incrassata	0.8	+++
L. lepideus	0.0	+
P. placenta	0.4	+
A. carbonica	0.4	++
C. puteana	1.1	+
G. trabeum	1.9	++
G. applanatum	0.0	–
T. versicolor	2.2	–
P. chrysosporium	3.2	–
T. harzianum	0.0	–
C. coerulescens	0.0	–
Antigen control[b]		–
Antibody control[c]		–

[a]Reactions were rated visually:
(–) no reaction to (+++) strongest reaction.
[b]Undecayed wood extract.
[c]Normal mouse serum.

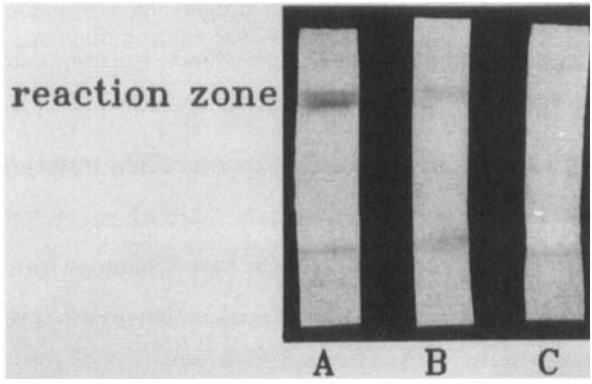

Figure 2—Effectiveness of cross-adsorption in eliminating interfering reactions. A, reaction zone shows a positive reaction from a brown-rot fungus; B, slight reaction from interfering antigens before cross-adsorption of antibody; C, elimination of interfering reaction by cross-adsorption of antibody.

Cultures on Taylor's medium were negative for viable wood decay fungi. Four samples were positive for the PCI test; two strong positives and two slight positive results (data not shown).

DISCUSSION

An immunodiagnostic field test for the detection of incipient brown-rot decay is described. The PCI combines the sensitivity of a double antibody sandwich ELISA with the simplicity of a dipstick test. In previous studies, ELISA was the most sensitive immunoassay for detection of brown-rot fungi (6,7,8,9). The multiple steps and reagents required to perform ELISA and the neccessity for specialized equipment to obtain a quantitative result rendered it undesirable for a field test despite its sensitivity. The PCI test strip is a two-step test that requires no special equipment, reagents, or expertise to read the results.

Blais *et al.* (19,20) introduced hydrophobic polyester cloth as an immunoassay substrate in 1989 in the field of veternary science. This substrate proved to have the ideal wettability, mobility, and passive binding qualities that were desired for a field immunoassay. Nitrocellulose, which is most commonly used as an immunoassay substrate, lacked the strength and wettability of polyester cloth, and possessed the ability to irreversibly bind the antibody-labeled beads regardless of the methods used to block unlabeled sites.

Initially, there were slight cross-reactions with nonbrown-rot organisms. Palfreyman *et al.* (10) showed that undesirable cross-reactions with interfering antigens can be effectively cross-adsorbed out by treating the reacting antibody with the antigen. Elimination of one or more interfering reactions can successfully increase the specificity of the antibody. Cross re-

actions from *G. applanatum* and *P. chrysosporium* were subsequently eliminated by cross-adsorption. As other organisms are screened in the PCI assay, cross-adsorption may be further utilized to enhance the assay's specificity.

It was hoped that the development of an immunodiagnostic field test for brown-rot would serve the dual purpose of evaluating the efficacy of remedial treatments. Core borings from 11 power poles gave initial field test data for the efficacy of boron to arrest existing decay. Remedial treatment for fungal decay occurred 18 months prior to sampling. Culturing samples from 11 poles resulted in no viable fungi, although the PCI gave two strong positive and two weak positive results. Direct culturing was not totally effective in determining if viable fungi are present. Addition of antibiotics to Taylor's medium did not prevent the growth of bacterial contaminants on four plates, and two samples were overgrown with mold. Still, the negative culture results illustrated one shortcoming of the PCI test for use in determining efficacy of remedial treatments; that is, fungal antigens detected by PCI need not come from viable fungi. These poles were previously infected with wood decay fungi, and the boron indeed appeared to have effectively arrested decay. Antigens from nonviable fungi were equally sensitive to the PCI test, so the PCI assay can not be recommended for evaluating efficacy of remedial treatments to "in-service" wood products known to be previously infected with brown-rot fungi. However, it can be used for diagnosing the initial infection.

This is the first report of a double antibody sandwich PCI for the diagnosis of brown-rot decay fungi. It is the first immunological field test for early detection of brown-rot in wood and requires no special equipment or scientific expertise. Accurate diagnosis of incipient brown-rot decay

prevents excess replacement of wood and excess remedial treatment. The PCI assay will provide a means of accurately diagnosing incipient decay which is essential for increasing the service life of "in-service" wood products.

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