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Short communication

Choice tests and neighbor effects during fungal brown rot of copper- and non-treated wood

Feng Jin Liew, Jonathan S. Schilling*

University of Minnesota, Bioproducts & Biosystems Engineering, 2004 Folwell Avenue, Saint Paul, MN, USA

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ABSTRACT

In previous work, a 'choice' test was used to test wood durability in mixtures of wood types. Results demonstrated that a filamentous fungus (*Gloeophyllum trabeum*) made a 'choice,' degrading white pine significantly less when less-durable woods were readily available. For this experiment, we substituted white pine with copper-treated pine and used three fungi (*G. trabeum*, *Serpula himantioides*, and *Wolfiporia cocos*) representing a range of copper tolerances. Southern pine blocks treated commercially with micronized copper azole or treated in-house using copper ethanolamine were used as treated wood substrates. Trials were soil-block tests, using two-block combinations to include non-treated, non-durable pine or as single-block treatments. After 12 weeks of decay, copper tolerance by *S. himantioides* and *W. cocos* was evident while *G. trabeum* was not copper tolerant. Availability of non-durable pine blocks, however, had no effect on decay in copper-treated wood. This contrasts with our past results using white pine. The reason for the discrepancy may relate to fungal mechanisms of copper tolerance (e.g., constitutive oxalic acid secretion) which may differ from mechanisms to overcome extractives in white pine. More fungal research using choice trials to assess these 'neighbor effects' is logical, given increased use of mixed, composite, and hybrid materials.

1. Introduction

'Choice' tests are often used to test substrate preferences among organisms that actively disperse and that select substrates. As opposed to 'no-choice' tests (one substrate type), choice tests allow the colonizing organism to select from multiple substrate options. This offers insight into host specificity versus the host range of an organism, although it is best complemented by other measures of specificity (Van Driesche and Murray, 2004). Among wood-degrading organisms, choice testing is typically limited to termite trials (e.g., Hapukotuwa and Grace, 2011) such as the E1 protocol (AWPA, 2009). In the case of wood-degrading fungi, no-choice tests such as the D 1413 protocol (ASTM, 2007) are normally used due, in part, to ease of interpretation and to tradition when testing spore-forming fungal pests. The miniaturized EN113 agar-block test (Bravery, 1978) has been extensively used and may include a non-treated control block with two other treated blocks within the same microcosm; however, the motivation for using the test is generally to generate treatment efficacy results quickly, not to test for substrate preference. Choice testing to gauge preference using multiple wood substrates is not common, excepting our own work testing preference among multiple available wood substrates (Schilling and Norcutt, 2010) and work such as Yoshimura and Takahashi (2000) using a range of wood preservative levels to determine a toxicity threshold.

Choice tests for wood-degrading fungi, however, could be useful in predicting durability of wood or its fibers as a component in mixed, composite, or hybrid materials. This is due to the following: 1) wood-degrading fungi typically have a wide host range in culture but narrower host associations in nature (Gilbertson, 1981; Valášková and Baldrian, 2006), 2) colonization of wood by decay fungi is often via hyphal growth and not spores (Boddy et al., 2009), 3) wood-degrading fungi can actively reallocate and shift hyphal growth in response to substrate availability (Tlalka et al., 2008), and 4) filamentous fungi can translocate elements between substrates. In short, filamentous fungi can 'choose' to colonize a preferred substrate, avoiding or selectively mining elements from other substrates.

Given this potential, the presence of one material type might affect the durability of another piece of wood, both in agar-block efficacy trials and in buildings where environment may limit fungal colonizers and their diversity. We term this the 'neighbor effect.' Previously, in a choice decay trial, we found neighbor effects

* Corresponding author. Tel.: +1 612 624 1761; fax: +1 612 625 6286.
E-mail addresses: liewx010@umn.edu (F.J. Liew), schillin@umn.edu (J.S. Schilling).

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in wood mixtures when the durability of Eastern white pine (*Pinus strobus*) in the presence of aspen and spruce blocks was 2-fold higher than in no-choice microcosms containing only pine (Schilling and Norcutt, 2010). This was complemented by an increase in aspen and spruce decay, relative to the no-choice microcosms containing only aspen or spruce. White pine alone had limited durability, but would rank on the upper end of 'moderately durable' when aspen or spruce was nearby, at the expense of any durability in those wood types. Given the biology of filamentous fungi, this is not surprising. However, it demonstrates that the standard test of natural durability might not reflect its performance in a mixed-material system. This could have consequences, perhaps good or bad, when predicting durability in modern building materials and designs.

The goal of this trial was to employ a choice test for several brown rot wood-degrading fungi, in this case degrading wood impregnated with or without copper formulations. The design is similar to our past study with white pine, but uses one wood substrate type and three fungal species with a range of copper tolerances in order to focus effects on copper-treated substrates and on neighbor effects.

2. Materials and methods

2.1. Wood blocks

Non-treated southern yellow pine (SYP) blocks were cut from a single board into 19 mm cubes. We used approach of Cao and Kamdem (2005) to treat wood with copper ethanolamine (CEA) solution with relatively high (2%) elemental copper concentration (w/w) and with a molar ratio of 4:1 monoethanolamine (J.T. Baker, Phillipsburg, NJ, USA): copper (II) hydroxide (Sigma Aldrich, Saint Louis, MO, USA) (final pH 11.5). Blocks were pressure treated using full cell regime. The copper ethanolamine treated blocks were conditioned at 20 °C and 65% RH to equilibrium moisture content. The retention of CEA in these blocks was 5.6 kg m^{-3}, as determined in 40-mesh powder ashed at 485 °C, dissolved in 10% HCl (v/v), and analyzed via inductively-coupled plasma optical emission spectroscopy (ICP-OES) using an ARL 3560 instrument (Thermo Scientific, Madison, WI, USA). This retention was lower than 8.6 kg m^{-3}, attained for Schilling and Inda (2011), but within the target range (5–10 kg m^{-3}) for this treatment regime.

Micronized copper azole (MCA)-treated SYP was purchased from a retail chain in Minneapolis, MN., USA. The MCA retention (1.6 kg m^{-3}, as determined by ICP-OES) in this material was compliant with the standards of International Code Council Evaluation Services (ICC-ES) for above-ground use, and end-grain blocks (14 mm^3) were from the same stock used in a prior research trial, thus the discrepancy in block volumes between the copper treatments (Schilling and Inda, 2011). This past work showed 4-fold more leachability of CEA than the MCA, which retained 99.1% mass after leaching.

2.2. Fungal isolates

Wood-degrading fungi used in the trial were *Gloeophyllum trabeum* P. Karst. (ATCC isolate 11539), *Serpula himantioides* P. Karst. (ATCC isolate 36335) and *Wolfiporia cocos* (MD isolate 104). The fungi were maintained on 20 mL of 2% (w/v) malt extract (Difco, Franklin Lakes, NJ, USA) solidified with 2% bacteriological grade agar (Difco). After 2 weeks of incubation in the dark with constant temperature at 25 °C, four 1 cm diameter plugs were removed and added on feeder strips in the soil-block jars.

2.3. Mixed substrate 'choice' tests

Soil-block jars were set up following ASTM D 1413-07 (ASTM, 2007) using a wetted 1:1:1 mixture of vermiculite, peat and additive free potting soil as the soil substrate. The mixture in each jar weighed approximately 160 g. In the soil-block set up, wood blocks were placed and degraded on top of two SYP feeder strips (2.5 × 3.4 × 0.3 cm, largest radial face) pre-colonized for 2 weeks by the test fungi.

All blocks were oven-dried (103 °C, 48 h), weighed, autoclaved (1 h), and added to the soil-block jars in single- or mixed-species configurations (Fig. 1). Our earlier mixed-substrate design was used as a template for this set-up (Schilling and Norcutt, 2010). Five replicates were used per block treatment. Decay duration was 12 weeks, targeting moderate decay stages (20–40% wood weight loss) to exacerbate treatment effects on decay rate, not decay extent. Five replicates of controls without fungal inoculum were included in the trial to monitor contamination and to serve as baseline non-degraded material. All soil-block jars were incubated together and in the dark at room temperature. At week 12, blocks from seven treatments and controls were harvested. Hyphae on block surfaces were removed and blocks were oven-dried and weighed to determine weight loss.

A set of treatments was included to test one versus two blocks per jar as a measure of inoculum potential effects, keeping inoculum amount equal. A separate treatment set was also included for CEA-treated blocks and untreated SYP to determine the effect of a gap, using a 5-mm spacing between the blocks as opposed to the other treatments.

2.4. Statistics

All data were tested for normality and transformed, if necessary, prior to analyses. Analysis of variance was used among the treatments to protect post-hoc means comparisons using Tukey's multiple comparison test ($\alpha = 0.05$). These comparisons were among treatments within each fungal isolate and not used to compare among fungal isolates.

3. Results and discussion

After 12 weeks of decay, mean weight loss in SYP blocks in 'no choice' treatments ranged from 22.5% (*G. trabeum*) to 37.5% (*W. cocos*) (Table 1). There was an effect of inoculum potential/wood volume, but only for *G. trabeum*, which had significantly more per-block decay in single-block jars than in dual-block jars (Fig. 2). Because this might be caused by surface area/volume change when blocks are in contact, spaced block treatments were included but

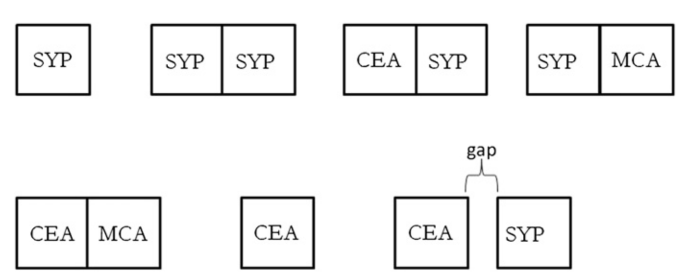

Fig. 1. Experimental set-up. Block treatments were either non-treated southern yellow pine (SYP), copper ethanolamine (CEA) treated as blocks in the laboratory or micronized copper azole (MCA)-treated blocks cut from end-grain of commercially-available lumber. Brown rot fungi were selected to decay these wood block combinations, representing a range from copper intolerant (*G. trabeum*) to copper tolerant (*W. cocos*). The gap between CEA and SYP was 2 cm.