EFFECTS OF WOOD MIXTURES ON DETERIORATION BY A FILAMENTOUS BROWN-ROT FUNGUS

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Abstract. Wood-degrading fungi import elements to meet physiological demands in wood, but little is known about interactions with different wood types. This is despite increased use of wood composites, in which durability can be tested but not well predicted. Blocks of nondurable aspen and spruce and moderately durable eastern white pine were degraded using the brown-rot fungus *Gloeophyllum trabeum* in soil- and agar-block microcosms for 16 wk. Block configurations were either a single species (monosubstrate) or mixed (polysubstrate). At 8 and 16 wk, total wood weight losses were the same in monosubstrate and polysubstrate microcosms; however, white pine degradation was consistently less in polysubstrates than in monosubstrates with decay in aspen and spruce compensating to achieve equal overall weight loss. Nondegraded pine had higher extractives and lower nitrogen levels as compared with the other woods. Carbon fractions and cation contents in degraded pine were typical of brown rot, suggesting the fungus reallocated resources to less durable aspen and spruce when given the option. Data demonstrate that wood durability can be influenced significantly by other wood types. Although this could influence the spatial pattern of decay in mixed materials, overall durability in small-particle size wood composites may also be predictable based on single-species performance.

Keywords: Wood-plastic composites, polyculture, natural durability, co-metabolism, translocation.

INTRODUCTION

Plant lignocellulose polymers (lignin, cellulose, and hemicelluloses) are intimately linked on a molecular level and resist biological degradation compared with polymers like starch. Molecular-level barriers are complemented by anatomic-level barriers such as density of vascular bundles and presence of epicuticular waxes (Himmel et al 2007). This "recalcitrance" resists biodegradation in nature, and wood is a particularly resistant lignocellulosic material.

Wood biodegradation is also limited by high carbon/nutrient ratios and the presence of biocidal extractives. Nitrogen (N) levels are low in wood with carbon/nitrogen (C/N) ratios normally an order of magnitude higher than in nonwoody plant tissues (Cowling and Merrill 1966). Increasing C/N or lignin/N often correlates well with decreasing wood biodegradation rates (Merrill and Cowling 1965; Melillo et al 1983), although wood extractives are a critical rate-determinant often overlooked outside of the field of wood protection (Harmon et al 1986; Downs et al 1996). Wood extractives such as terpenoids and tropolones are complex and likely synergistic in their modes of action (Schultz and Nicholas 2000). Although wood-degrading microbes can detoxify extractives (Burnes et al 2000; Dorado et al 2000, 2001), extractive contents within a wood species still broadly correlate well with durability in field exposure (Windeisen et al 2002).

The availability of exogenous substrates should also be considered when filamentous fungi degrade wood. These include nearby nonwoody

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materials and soil, but also could include other wood types in a mixture or in a structure. Fungal hyphae connect different substrates, and translocation of elements between substrates is well documented (Boddy 1999; Lindahl and Olsson 2004; Watkinson et al 2006). The actual role of elements imported from exogenous sources is not always straightforward. Iron, for example, is a likely requirement for brown-rot fungi, but Schilling and Jellison (2006) reported that substantial amounts of iron were imported by brown-rot fungi without observing any increase in wood decay rate compared with iron-free treatments. In the case of N, low-level N additions can stimulate wood degradation (Allison et al 2009), and translocation of N from soil into wood chip mulch is well known (Frey et al 2000; Homyak et al 2008); however, different forms of N give different results (Reid 1983), and excessive N can suppress fungal degradative mechanisms (Tien and Kirk 1984).

In this study, we tested whether degradation of a wood species by a filamentous fungus can be significantly affected by other wood types present, like in composites and mixed materials. Our working hypothesis was that adding low-durability woods such as aspen, a component in many composites, to a mixture with more durable woods would increase the amount of decay. We based our hypothesis on the composting practice of "biostimulation," adding easily degradable substrates to enhance degradation of recalcitrant materials. We kept our treatment structure simple and used increased replication to account for added statistical error in a mixture, and we used blocks rather than powder to characterize individual weight loss and chemistry changes.

MATERIALS AND METHODS

Microcosms

Soil-block microcosms 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. In the soil-block setup, wood blocks were placed and degraded on top of birch feeder strips. In agar-block microcosms, thick plastic mesh was used instead of birch and was placed on 20-mL Type A minimal nutrient agar (2% w/v) in 150 × 25-mm petri dishes. Agar was supplemented with basal salts to 0.1 the normal NH_4NO_3 addition (Highley 1973).

Inoculation and Incubation

The wood-degrading fungus used in both of these trials was *Gloeophyllum trabeum P. Karst.* (ATCC isolate 11539). The fungus was maintained on 20 mL of 2% (w/v) malt extract solidified with 2% Bacto agar (Difco). After 2-wk growth, 100-mm^2 plugs were removed and added to microcosms. Two plugs were used to inoculate agar-block microcosms; four were used in soil-block microcosms (Fig 1).

Aspen (*Populus* sp.), spruce (*Picea* sp.), and eastern white pine (*Pinus strobus*) blocks for each trial were each cut from single or adjacent rips of a single board. Blocks were cut and split once longitudinally to create blocks with a 25-mm length, 20-mm width, and approximate 12.5-mm height, leaving a nonplaned surface face down in the microcosms. Blocks were oven-dried (103°C, 48 h), weighed, autoclaved (1 h), and added to microcosms coincident with inoculum additions in agar-block microcosms or after 2-wk growth in soil-block microcosms. Three blocks were added per agar-block microcosm and six per soil-block microcosms (Fig 1). In both microcosm types, "monosubstrate" microcosms contained only one wood type, whereas "polysubstrate" microcosms contained the three different wood types. In soil-block polysubstrates, there were 12 total replicates (n = 12) with 4 replicates with each wood species in the middle position between blocks of the other wood types, whereas there were 12 replicates for monosubstrates (n = 12). In the agar-block polysubstrates, to test the effect of position on degradation rates, there were 6 replicates in each configuration (n = 6 × 3 wood types) and 6 monosubstrate replicates (n = 6).

Monosubstrate and polysubstrate controls without fungal inoculum were included to monitor

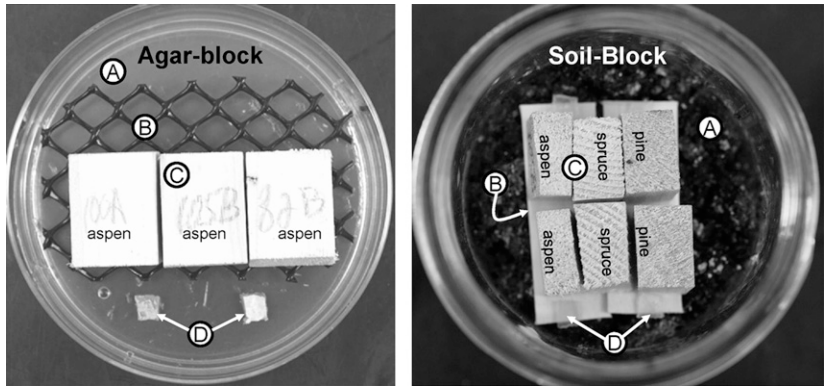

Figure 1. Agar- and soil-block microcosm designs used. Growth media (a) were low-nitrogen minimal nutrient agar or a standard soil mix. Block supports (b) were thick plastic mesh on agar or birch feeder strips on soil. Three blocks (c) were added to agar-block microsms and six added to soil-block microcosms with two or four inoculum plugs (d) added. A "monosubstrate" configuration is shown on agar and a "polysubstrate" is shown on soil.

contamination (none was detected) and to serve as baseline nondegraded material. Replication for weight-loss data from noninoculated agar-block control microcosms was lower than in treatments because some were sacrificed for baseline characterization data. All microcosms were incubated in the dark at room temperature, and Week 8 harvests from soil-block microcosms were made aseptically, including the removal of control blocks.

Harvests

At 8 and 16 wk, degraded and nondegraded control blocks were cleaned of surface hyphae, oven-dried, and weighed as before. Weight loss was determined on an oven-dry weight basis. Three replicate control samples of each wood type were milled to 40 mesh in a Wiley mill, homogenized, and analyzed directly for C/N ratio as described subsequently. Because a statistically significant effect of wood combination was observed on *P. strobus* blocks in soil-block microcosms, all polysubstrate white pine blocks (n = 12), one randomly selected monosubstrate pine block from each of the 12 microcosms, and 16 control pine blocks (3 wk) in soil microcosms were milled through 40 mesh and characterized using wet chemistry and inductively coupled plasma optical emission spectroscopy (ICP-OES).

Tissue Characterization

For each wood species, the C/N ratio was determined in nondegraded material using wood milled to 40 mesh and homogenized from three block samples. Total carbon was determined using an empty tube combustion method at 1050°C on a Skalar Primacs analyzer. Total N was determined using a Leco FP-528 Determinator with burn phase at 850°C and using thermal conductivity detection. Results are expressed as an oven-dry weight percentage.

In the *P. strobus* control and degraded wood powder, lignin, cellulose, and hemicelluloses fractions were determined along with acetone-soluble extractives, both expressed as an oven-dry weight percentage. TAPPI standard 222 om-06 (Technical Association of the Pulp and Paper Industry [TAPPI] 2006]) was followed to determine acid-insoluble lignin. Using acid-extracted filtrate, filtered to 0.2 μm, monomeric carbohydrates were determined using high-performance liquid chromatography using a cellobiose internal standard. Glucose levels were measured as glucan content (cellulose + monomeric glucose). Xylose, galactose, arabinose, and mannose levels were measured as hemicellulose fractions. Separations were on a 300- × 7.8-mm Metacarb 87P column (Varian) at 80°C using water as eluent at a flow rate of 0.3 mL min^{-1}. A de-ashing guard column

(Bio-Rad) was used, and detection of the filtrate sugars was via refractive index with peak area quantitation. Standard conversion factors (eg cellulose-to-glucose) were used for yield calculations. Extractives in homogenized samples of the controls, monosubstrate, and polysubstrate samples were analyzed by weight following TAPPI standard 204 cm-07 (TAPPI 2007) using acetone as a Soxhlet extraction solvent.

Cation Analysis

Powder samples from the same *P. strobus* blocks used for wet chemistry characterization were ashed at 485°C and dissolved in 10% HCl for analysis of the cations, aluminum, boron, calcium, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc. The ICP-OES instrument used was an ARL 3560 (Thermo Scientific).

To standardize cation data as well as wet chemistry characterization data, the data (reported as dry weight percentage or as mmol/kg) were adjusted to account for mass loss incurred from fungal degradation and metabolism of lignocellulose components by multiplying data by the fraction of mass (final mass/initial mass) remaining after degradation.

Statistics

To compare monosubstrates vs polysubstrates, t-tests at $\alpha = 0.05$ were used within each wood type per harvest of agar- and soil-block trials. Percentage data were log transformed to normalize before analysis. To test the effect of block position on degradation to determine if means could be pooled, analysis of variance was used with the intention that if protected at $\alpha = 0.05$, means comparisons would be performed; however, none were protected and means were pooled.

RESULTS AND DISCUSSION

Weight Loss

The total amount of wood weight loss (spruce + pine + aspen) after 8- and 16-wk degradation by *G. trabeum* in individual soil-block microcosms was statistically equal between monosubstrate and polysubstrate treatments; however, the patterns within individual wood types shifted significantly and were robust at both sampling times (Table 1). Eastern white pine blocks in monosubstrate configurations were degraded nearly twice as much after 8- and 16-wk degradation than in polysubstrates (34 vs 15% at 8 wk and 45 vs 26% at 16 wk). Lower pine degradation in polysubstrates was offset by higher

Table 1. Soil-block trial data from aspen, spruce, and Eastern white pine (*Pinus strobus*) degraded by the brown-rot fungus *Gloeophyllum trabeum* in 'monosubstrate' microcosms containing three adjacent wood blocks of the same type or in 'polysubstrate' microcosms containing one of each of the three wood types in each possible configuration.[a]

Wood substrate	Microcosm setup	Replicates (n)	Wk 8 weight loss (%)	Wk 16 weight loss (%)
Aspen	Monosubstrate	12	35.4 (2.5)a	63.9 (1.8)a
	Polysubstrate	12	46.7 (1.8)b	66.5 (1.8)a
	Control	12	0.01 (0.03)	0.89 (0.71)
Spruce	Monosubstrate	12	36.7 (3.6)a	56.4 (4.6)a
	Polysubstrate	12	38.8 (3.6)a	64.8 (0.9)a
	Control	12	0.00 (0.03)	0.04 (0.03)
Eastern white pine	Monosubstrate	12	33.9 (3.0)a	44.7 (3.9)a
	Polysubstrate	12	14.6 (4.0)b	26.1 (4.8)b
	Control	12	0.15 (0.09)	0.56 (0.16)
Combined	Monosubstrate	36	35.3 (1.7)a	55.0 (2.4)a
	Polysubstrate	36	33.4 (3.0)a	52.5 (3.6)a
	Control	36	0.05 (0.05)	0.50 (0.30)

[a] Means (± standard error) followed by the same letter in a column and within a wood species are not significantly different.

degradation in aspen and spruce in those microcosms. Similar to soil-block results, weight loss in agar-block microcosms was higher for eastern white pine in monosubstrates vs polysubstrates (Table 2), although weight loss and statistical power were lower in this design.

These results suggest that this fungus might preferentially initially degrade the least durable components of a composite or of mixed materials but that the effect on total wood mass loss among all the wood present would be minor. This could have valuable implications for materials applications such as designing to direct colonizing fungi away from load-bearing wood components. Likewise, if the overall durability of a composite or mixture could be predicted based on the durability of its individual wood components, regardless of dynamics in individual components, this would be important to understand. Composites are typically formulated and then tested for durability, often with unexpected results; predictive tools would be useful before formulation. It is important to acknowledge, however, that composites contain resins and other components that could alter the decay process and affect this predictive potential. The observations here should not be applied directly, but instead, follow-up research using representative composite materials is in order.

Characterization

Total carbon (dry weight percentage) was 48.9 (±0.5) in aspen, 50.9 (±0.1) pine, and 46.0 (±0.6) spruce. Total nitrogen (dry weight percentage) was 0.213 (±0.004) in aspen, 0.207 (±0.001) pine, and 0.205 (±0.001) spruce. Resulting C/N was 229.8 for aspen, 245.5 for pine, and 229.0 for spruce.

In nondegraded white pine (*P. strobus*), lignin and holocellulose levels (Table 3) were similar to that reported for *P. strobus* as a reference, 27% lignin, 45% cellulose, and 68% total holocellulose (Pettersen 1984). Because *G. tra-*

Table 2. Agar-block trial with aspen, spruce, and Eastern white pine (*Pinus strobus*) degraded by the brown-rot fungus *G. trabeum* in 'monosubstrate' or in 'polysubstrate' microcosms.[a]

Wood substrate	Microcosm setup	Block location[b]	Replicates[c] (n)	Wk 16 weight loss (%)
	Monosubstrate	—	6	26.7 (3.3)
	Polysubstrate	ASP	6	28.9 (6.9)
Aspen		APS	6	25.2 (7.5)
		SAP	6	29.0 (3.8)
		Combined	18	26.2 (3.9)
	Control	—	8	−0.1 (0.1)
	Monosubstrate	—	6	20.27 (2.5)
	Polysubstrate	ASP	6	19.6 (4.9)
Spruce		APS	6	18.4 (2.7)
		SAP	6	28.3 (6.4)
		Combined	18	22.1 (2.8)
	Control	—	12	0.0 (0.0)
	Monosubstrate	—	6	20.6 (2.6)
	Polysubstrate	ASP	6	15.7 (6.2)
Eastern white pine		APS	6	12.8 (2.0)
		SAP	6	15.3 (2.0)
		Combined	18	15.0 (2.6)
	Control	—	12	0.0 (0.0)
	Monosubstrate	—	54	22.5 (1.6)
Combined	Polysubstrate	—	54	21.7 (1.8)
	Control	—	32	0.0 (0.0)

[a] In polysubstrates, the effect of block configuration in relation to other wood types is shown for aspen (A), spruce (S), and pine (P).

[b] Block configuration is shown with block location denoted (ASP = aspen on the end with spruce adjacent and pine two blocks distant).

[c] Replicates are whole microcosm jars. Monosubstrate jars contain 3 of the same wood substrate types as within-jar replicates. These within-jar replicates are reflected in the total 54 monosubstrate combined replicates. Means were pooled from block location after no statistical influence of position was found, and after microcosm groupings were not found to be more similar than between microcosms.

Table 3. Characterization and cation contents (mean ± standard error) of Eastern white pine (*P. strobus*) degraded by *Gloeophyllum trabeum* 16 wk in 'monosubstrate' microcosms containing 3 adjacent wood blocks of the same type or in 'polysubstrate' microcosms containing one of each of the three wood types in each possible configuration.

		Control		Monosubstrate		Polysubstrate	
	Tissue constituent	Raw[a]	Adjusted[b]	Raw	Adjusted	Raw	Adjusted
Oven-dry wt. %	Klason lignin	30.9	30.9	45.0	25.0	39.1	28.9
	Glucan	43.7	43.7	32.6	18.1	37.6	27.8
	Arabinan	1.1	1.1	0.7	0.4	0.7	0.5
	Galactan	1.4	1.4	1.0	0.6	1.0	0.8
	Mannan	11.0	11.0	6.1	3.4	7.8	5.8
	Xylan	5.2	5.2	3.9	2.2	3.2	2.4
	Total hemicellulose	18.7	18.7	11.7	6.6	12.7	9.5
	Total holocellulose	62.4	62.4	44.3	24.7	50.3	37.3
	Extractives	4.9	4.9	5.3	2.9	3.5	2.6
mmol/g	Aluminum	0.1 (0.0)	0.1 (0.0)	6.7 (2.3)	3.1 (0.8)	6.2 (0.6)	4.4 (0.4)
	Boron	0.2 (0.0)	0.2 (0.0)	1.1 (0.3)	0.5 (0.1)	1.0 (0.1)	0.7 (0.1)
	Calcium	7.0 (0.4)	7.0 (0.4)	30.5 (5.1)	15.1 (1.8)	17.9 (1.8)	12.8 (1.3)
	Copper	0.0 (0.0)	0.0 (0.0)	0.5 (0.2)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)
	Iron	5.7 (1.0)	5.7 (1.0)	24.2 (6.5)	11.5 (2.3)	17.1 (1.5)	12.3 (1.1)
	Magnesium	4.2 (0.2)	4.2 (0.2)	33.8 (7.1)	16.6 (2.4)	34.2 (2.1)	24.5 (1.4)
	Manganese	0.1 (0.0)	0.1 (0.0)	0.4 (0.1)	0.2 (0.0)	0.5 (0.0)	0.4 (0.0)
	Phosphorus	0.7 (0.2)	0.7 (0.2)	4.3 (1.3)	2.1 (0.6)	4.5 (0.7)	3.0 (0.4)
	Potassium	11.3 (0.5)	11.2 (0.5)	15.3 (2.1)	7.9 (0.8)	16.0 (1.1)	11.4 (0.7)
	Zinc	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.1 (0.0)	0.2 (0.0)	0.1 (0.0)

[a] "Raw" data are expressed as weight percentage of the material oven-dry weight used during wet chemistry characterization or as the concentration of a particular element per gram of material analyzed.

[b] "Adjusted" data compensates for the mass of wood consumed by the fungi by multiplying raw data by the weight fraction-of-original remaining after degradation.

beum is a brown-rot fungus that selectively degrades the holocellulose, lignin weight percentage increased over time when not adjusted to compensate for mass loss. The adjusted holocellulose values of 24.7 and 37.3 observed here are typical at 44.7 and 26.1% weight loss, respectively.

Extractives contents in *P. strobus* were nearly 5% in control wood and in the mass-adjusted data had dropped by nearly one-half in individual whole blocks (Table 3). Some mass loss (0.56%) was observed in white pine blocks without the fungus present in control soil-block microcosms, suggesting extractives loss was partly from passive leaching as well as the colonization by the test fungus.

Characterization of degraded white pine did not reveal unusual patterns related to treatments that are not consistent with other brown-rot studies. Carbon fractions were typical for brown rot at each wood weight loss (Curling et al 2002), and mass loss compensation shows that extractives leaching and biotransformation were occurring at similar rates between monosubstrates and polysubstrates.

These data suggest that the test fungus reallocated resources with a preference to colonize and degrade the less resistant aspen and spruce when given the opportunity. Less durable wood did not stimulate decay in more durable wood as hypothesized based on composting principles. Fungal substrate preference may relate both to wood extractives and wood C/N ratio, although C/N differences in this case were minimal. Eastern white pine extractives levels are about 6 wt% using ethanol/benzene extraction (Rowell et al 2005), twice the level found in aspen (*Populus tremuloides*) and three times higher than black or white spruce. In general, isolating effects of extractives from nitrogen availability as well as correlating other durability factors could be useful to weigh the importance of each.

Cation Data

Cation translocation into degrading *P. strobus* blocks was significant in most cases, evidenced by higher cation contents in degraded vs control pine (Table 3). In mass-adjusted data, cation contents were higher for most cations in pine degraded in polysubstrates, whereas weight loss was lower than in monosubstrates. The most notable exception is calcium, which was highest in the more degraded pine in monosubstrates. Either there was an effect of wood combination on cation import or early cation flux was followed by cation export at the later stages of decay.

Cation increases are normal during wood biodegradation (Jellison et al 1997), and although a linear increase in cation concentration as decay progresses might be expected, the gain–loss dynamic observed here (cation flux followed by loss at later decay stages) is consistent with other studies. Ostrofsky et al (1997) reported that wood degraded by *Postia placenta* experienced the highest cation import among the brown-rot fungi tested and that cation contents were higher at moderate than later decay stages with a notable exception in calcium (Ca), as we observed here. Longer-term Ca accumulation in wood has been demonstrated for wood degraded on the forest floor (Smith et al 2007). This gain–loss dynamic for the cations other than Ca may relate to Ca-oxalate crystal formation as a non-exchangeable Ca fraction as opposed to exchangeable, more dynamic pools for other cations such as potassium and magnesium. In any case, we believe these cation trends, similar to carbon fractions, are normal, suggesting the main affect of wood type was to influence colonization preference of the test fungus.

CONCLUSIONS

Gloeophyllum trabeum showed an apparent substrate preference when colonizing mixtures of wood substrates. Preference was for less-durable aspen and spruce when eastern white pine was present, leading to significantly less pine degradation in wood mixtures than when degraded as a pure substrate. This dynamic should be tested with other fungi and certainly in the field where species richness will be high and colonization dynamics may be spatially heterogeneous. These results can direct future research and have useful relevance to selection and predicted durability of composites and mixed wood materials.

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