DECAY CAPABILITIES OF BASIDIOMYCETES COLONIZING AIR-SEASONING RED OAK AND BLACKGUM RAILROAD TIES

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ABSTRACT

Fungi cultured from air-seasoning blackgum and red oak timbers were assessed for their ability to cause wood decay using two hardwoods and one softwood species in an AWPA E10 soil block test. Weight losses were greatest for bigleaf maple and tended to be much lower on southern pine. Almost a quarter of the 35 taxa tested caused less than 5 % weight loss, suggesting they posed a relatively low decay risk, even under ideal laboratory conditions; despite all fungi tested having the ability to depolymerize wood. Three of the four fungi causing the largest weight losses were brown-rot fungi, although brown-rot fungi represented an only small proportion of the total isolates from the original hardwood timbers. These results illustrate the wide array of decay capabilities of fungi colonizing air-seasoning red oak and blackgum timbers, and the potential of many isolates to negatively affect wood properties through biodeterioration.

Keywords: Blackgum, brown-rot, fungal decay resistance, red oak, soil block test, white rot.

INTRODUCTION

Large timbers, such as railroad ties, are commonly air-seasoned to remove moisture prior to preservative treatment. Air-seasoning is simple and economical, but it also potentially exposes wet, untreated timber to fungal attack. Some of the first studies of decay during air-seasoning were performed on railroad ties, using fungal fruiting bodies as decay indicators (Humphrey and Richards 1939). These studies led to the development of guidelines for proper air-seasoning, including keeping timbers out of direct soil contact, creating adequate air-flow, and removing woody debris around the site, and minimizing the potential for standing water (Humphrey and Richards 1939, AWPA 2017a, AREMA 2019). The goal is to minimize fungal attack during air-seasoning and ensure the timbers are subjected to some form of sterilization (usually as heating over a minimum temperature such as 67 ºC for 75 minutes) during preservative treatment.

While fungal colonization is inevitable for untreated timbers exposed to the elements, surprisingly little research exists on the ability of these latent or colonizing fungi to cause decay during this period (Sexton *et al.* 1992, Chee *et al.* 1998). Most soil block tests have focused on wood durability and protective treatments

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(AWPA 2017b). Other isolate studies have tracked fungi past the point of advanced decay and considered decay as a community function (Butcher 1968, Rayner and Boddy 1988), or isolated only the most identifiable fungi in relation to decay (Eslyn and Lombard 1983, Zabel *et al.* 1980, Zabel *et al.* 1985).

Developing a better understanding of the decay capacities of individual fungi colonizing timber during air-seasoning might lead to the development of practices to limit the growth of certain organisms, or at least help managers better understand the risks associated with the process. The goal of this study was to evaluate the decay capabilities of fungi isolated from air-seasoning ties of two timber species commonly used by North American railroads, blackgum and red oak.

MATERIALS AND METHODS

Blackgum (*Nyssa sylvatica*) and red oak (*Quercus* section *Lobatae*) ties (88 of each species measuring 175 mm by 225 mm by 2,55 m long) were air-seasoned for 6 or 11 months respectively, at a tie yard in Guthrie, Kentucky. Colonization by basidiomycetes, the most common and impactful wood decay fungi, was assessed at the start of seasoning for each tie species to identify latent fungi and those that may have colonized the wood during harvest and transport. Similar assessments were made after 3 and 6 months for blackgum or 6 and 11 months for red oak to identify temporal community shifts and fungi that may have colonized during the air-seasoning process itself. Three increment cores were removed from each tie at each time-point and were cultured on benomyl (50 ppm) amended 1 % malt extract/1,5 % agar as previously described (Rogers 2019). Benomyl retards the growth of ascomycetes, increasing the likelihood of basidiomycete isolation (Carey and Hull 1989). The isolations focused on basidiomycetes because these fungi tend to predominate in this non-soil contact environment, and most are more aggressive wood decayers (Zabel and Morrell 2020). Furthermore, ascomycete molds such as Penicillium or Aspergillus tend to predominate in isolation studies unless steps are taken to minimize their growth. The isolates were identified by isolation and amplification of DNA as previously described (Cappellazzi *et al.* 2018). Cultures of the test fungi were maintained on 1,5 % malt extract agar until needed.

The decay capability of each isolate was evaluated using procedures described in American Wood Protection Association Standard E10 (AWPA 2017b). Briefly, 19 mm sapwood cubes of southern pine (*Pinus* sp.), red oak (*Quercus* section *Lobatae*), and bigleaf maple (*Acer macrophyllum* Pursh.) were cut from clear, defect-free lumber. Decay capability was evaluated on both hardwoods and softwoods because white-rot fungi have a preference for hardwoods, while brown-rot fungi tend to be more aggressive on softwoods (Schmidt 2006, Goodell *et al.* 2008). All three wood species would be classified as decay susceptible (Scheffer and Morrell 1998). The blocks were oven dried at 100 °C, weighed, briefly vacuum soaked in water (30 % to 50 % moisture content), and finally sterilized by autoclaving at 121 °C for 15 minutes.

Decay chambers consisted of 454 ml glass French squares that were half-filled with a moist garden loam meeting the water-holding requirements of the standard. A wood feeder strip (28 mm x 34 mm x 3 mm thick) of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) or alder (*Alnus rubra* Bong.) was placed on the top of the soil, with hardwood and softwood strips assigned to receive hardwood or softwood test blocks, respectively.

Bottles were sterilized for 75 minutes at 121 °C. After cooling, the bottles were inoculated with two 5 mm diameter agar plugs cut from the actively growing edge of a culture of the selected test fungus. A representative pure culture isolate of each genus or species was selected at the finest taxonomic level available. Two distinct forms of *Punctularia strigosozonata* with and without conidia that may also have had different degradation capabilities were evaluated. The chambers were incubated at 26 °C for 3 weeks to allow the test fungus to cover the feeder strip.

In total, 35 cultures representing 26 genera were selected for decay tests (Table 1). Only three of the fungi evaluated are known to cause brown-rot decay. This reflects the tendency for white-rots to selectively colonize hardwood timber above ground and the preponderance of heart-rot fungi that cause white-rot in hardwoods (Hepting 1971, Zabel and Morrell 2020). In addition to fungi isolated from ties, *Trametes versicolor* (L:Fr.) Pilát (Isolate MAD 697), *Rhodonia placenta* (Fr.) Niemelä, (Isolate MAD 698), and *Gloeophyllum trabeum* (Pers.) Murrill (Isolate MAD 617) were included as reference species since their decay capabilities have been well characterized (AWPA 2017b, De Groot 1998). Decay chambers with no fungi were also included as a

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control. Each isolate was evaluated on 8 blocks per wood species (936 blocks in total).

Once the test fungus had covered the feeder strip, two sterile test blocks of a given wood species were placed, cross section down on the feeder following autoclave sterilization described above. The chamber caps were loosely screwed on (to allow for some air-exchange, but minimize contamination) and incubated for 16 weeks at 26 °C. At the end of the incubation period, the blocks were removed, scraped clean of any adhering mycelium and oven-dried at 100 °C before being weighed. Differences between initial and final weight were used to calculate weight loss (%), which was used to compare of the capability for each fungal isolate to decay each respective wood species.

There is no specific weight loss that indicates when wood has been damaged by a fungus, but previous studies suggest that weight losses as low as 5 % can be associated with significant losses in other physical properties such as bending strength (Wilcox 1978). Weight losses below 3 % are usually attributed to handling and possible leaching of wood extractives into the soil. The AWPA Standard states that weight losses for decay tests with any of the three reference decay fungi must exceed 40 % for the test to be considered valid (AWPA 2017b); however, it is important to recognize that the test fungi used in the E10 soil block test were selected for their ability to aggressively attack susceptible sapwood species under the test conditions. These conditions may not be suitable for decay by all fungi, including our isolates. For this reason, the fungal isolates here were classified by their ability to cause weight losses of ≤5 %, 6 % to ≤11 %, 11 % to ≤20 %, 21 % to ≤30 %, and >30 %. Weight losses were categorized by timber species as well as a combined average for all three wood species tested. These ranges correspond to Wilcox's summation that decay below 5 % mass loss can be difficult to detect (but with large losses of strength), mass loss of up to 10 % may be considered the "early stage" of decay, and mass loss above this level shows advanced decay (1978). Other studies suggest that wood strength decreases in a gradient correlating to decay from 0 % to 30 % mass loss with complete loss of physical properties above 30 % mass loss (Winandy *et al.* 2001).

RESULTS AND DISCUSSION

Mass losses ranged from slight weight gains to 76,3 % for bigleaf maple exposed to *T. versicolor* (Table 1). Weight gains in soil block tests are common and may reflect some migration of salts and other materials from the soil into the blocks (AWPA 2017b). Mass losses for the three reference fungi were dependent on the particular wood species. Exposure of *G. trabeum* or *R. placenta* to southern pine resulted in weight losses of 32,9 % and 35,2 %, respectively, which was slightly below the required 40% target level in the AWPA E10 standard. Mass losses for pine blocks exposed to *T. versicolor* only experienced 18,1 % weight loss; but white-rot fungi tend to perform poorly on pine sapwood or softwoods in the soil block test (AWPA 2017b). We also did not allow the test to proceed for the full 24 weeks recommended in the AWPA E10 Standard for white rot fungi because we wanted all fungi to be exposed to the material for the same length of time. Therefore, weight losses may be under representative of a full test.

Bigleaf maple blocks exposed to the three reference fungi all experienced average weight losses well above the 40 % minimum, while weight losses for red oak blocks exposed to *G. trabeum* and *R. placenta* exceeded the 40 % minimum, but those exposed to *T. versicolor* did not. The limited weight losses found with the latter fungus were interesting as an isolate of this same species from the seasoning ties caused just over 40 % weight loss in red oak. The results illustrate the inherent variability associated with decay tests, but they also indicate that conditions were generally suitable for aggressive fungal attack of the wood species.

Weight losses were highest on bigleaf maple blocks, reflecting the high decay susceptibility of this wood (Scheffer and Morrell 1998, FPL 2021). Almost a third of the isolates exposed to bigleaf maple caused weight losses over 30 % (Table 2). The percentages of isolates causing more than 30 % weight loss on red oak or southern pine were much lower (11,4 % and 8,6 %, respectively). The lower levels of weight loss with pine are consistent with the fact that most of the isolates tested were white-rot fungi and these species tend to perform poorly on softwoods (Goodell *et al.* 2008).

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Table 1: Fungi evaluated for their ability to cause weight loss of blocks of three timber species in an AWPA soil block test.

Species	Isolate# or Accession number	Decay Type/Isolated from.	Mass Loss (%)				Ecological note
			Maple	Red oak	Pine	Average	
Acanthophysium bisporus Boidin & Lanq.	MN430921	WR/Oak	-0,22 (0,21)	1,84 (0,16)	0,53 (0,10)	1,08	
Antrodia minuta Spirin	MN430922	BR/Oak	49,13 (9,14)	36,49 (15,54)	35,29 (6,21)	40,3	
Antrodia oleracea (Davidson & Lombard) Ryv.	MN430923		50,23 (4,47)	40,25 (4,71)	35,97 (6,11)	42,15	
Bjerkandra adusta (Willd.) P. Karst.	MN430924	WR/Gum	8,83 (9,34)	8,19 (3,63)	6,02 (3,08)	7,67	
Chondrostereum purpureum (Pers.) Pouzar	MN430925	WR/Gum	1,75 (0,16)	2,70 (0,17)	2,47 (0,21)	2,32	Widespread disease of many commercially important trees (Hepting 1971). Also marketed as a mycoherbicide against trees.
Coprinellus radians (Desm.) Vilgalys *et al.*	MN430926	WR/Gum	23,94 (5,06)	16,95 (9,19)	3,14 (0,43)	14,68	Degrades already decayed hardwoods, but not sound wood (Oliver *et al.* 2010)
Cylindrobasidium sp.	MN430927	WR/Gum & Oak	2,02 (0,90)	2,89 (0,64)	2,57 (0,16)	2,49	
Dichostereum granulosum (Fr.) Boidin & Lang.	MN430928	WR/Oak	14,73 (5,46)	2,91 (1,81)	12,80 (2,74)	10,14	
Ganoderma sessile Murr.	MN430930	WR/Gum & Oak	19,74 (5,31)	22,69 (9,25)	11,87 (8,38)	18,1	
Hydnopolyporus fimbriatus (Fr.) D.A. Reid	MN430932	WR/Oak	42,33 (4,84)	10,19 (4,40)	18,29 (1,90)	23,6	Possible pathogen of softwood seedlings, not often found on live wood (Hepting 1971).
Hypochincium sp.	MN430933	WR/Gum	9,91 (6,73)	1,77 (0,28)	-1,55 (0,32)	3,38	
Laetiporus cincinnatus (Morgan) Burdsall *et al.*	MN430934	BR/Oak	50,52 (18,80)	64,79 (1,69)	59,65 (4,03)	58,32	Common brown-rot on softwoods and a butt-rot of hardwoods (Hepting 1971)
Lenzites betulinus (L) Fr.	MN430935	WR/Gum	7,24 (3,62)	2,03 (0,46)	4,45 (0,84)	4,57	Causes heart-rot of living *Nyssa* trees (Hepting 1971)
Peniophora pini (Brot.) Bondartsev & Singer	MN430936	WR/Oak	51,17 (30,44)	26,05 (17,16)	29,50 (29,64)	35,57	More commonly found on softwoods (Hepting 1971)
Phanerochaete sp.	MN430938	WR/Gum & Oak	45,15 (12,93)	23,52 (8,97)	10,23 (3,94)	26,3	Includes model white-rot species *P. chrysosporium* (Kersten and Cullen 2007). *Phanerochaete* cause decay in many hardwoods (Hepting 1971)
Phlebia fuscoatra Fr.	MN430940	WR/Gum & Oak	10,43 (6,96)	4,25 (0,46)	5,92 (4,67)	6,87	
Phlebia subserialis (Bourdot & Galzin) Donk	MN430941		1,17 (1,27)	1,25 (0,13)	0,96 (0,22)	1,13	
Phlebiopsis flavidoalba Cooke	K14	WR/Gum	16,01 (3,84)	10,76 (2,87)	12,33 (2,01)	13,03	
Pholiota (adiposa) Batsch	K16	WR/Gum	8,79 (3,41)	2,07 (0,52)	6,49 (1,40)	5,78	Causes butt-rot of hardwoods and softwoods (Hepting 1971)
Pholiota (limonella/adiposa)	K17		0,45 (0,31)	2,36 (0,17)	1,36 (0,13)	1,39	
Pholiota squarrosa Oeder	K15 - MN430942		8,16 (1,76)	2,87 (0,22)	8,16 (1,66)	6,4	

Punctularia strigosozonata (w/o conidia) Schwein.	K18	WR/Gum	21,10 (3,91)	3,11 (0,30)	6,28 (1,47)	10,16	Found mostly in *Populus*. The decay capability of this fungus is variable (Young et al. 2015).
Punctularia strigosozonata (w/ conidia) Schwein.	MN430943	WR/Gum & Oak	44,94 (10,87)	5,65 (1,25)	9,34 (2,46)	19,98	
Radulon americamus Ryvarden	K20	WR/Gum	35,95 (10,26)	25,67 (11,57)	9,64 (4,22)	23,75	Responsible for decay of aspen and poplar in Canadian timber, causing yellow stringy rot in live trees. (Hepting 1971)
Radulon casaerius Morgan	MN430944	WR/Gum	23,19 (5,21)	3,41 (1,17)	13,31 (5,36)	13,3	
Schizophyllum commune Fr.	MN430945	WR/Gum	7,75 (10,60)	4,73 (0,55)	3,02 (0,28)	5,17	Low decay potential but associated with other decay fungi (Humphrey and Richards 1939)
Sistotrema brinkmannii Bres.	MN430946	WR/Gum & Oak	7,24 (12,72)	2,99 (0,44)	1,74 (0,22)	3,99	Low decay potential but associated with other decay fungi (Carey and Hull 1989). Found in treated utility poles (Zabel and Morrell 2020)
Spongipellis delectans Peck	MN430947	WR/Gum	10,04 (3,39)	0,81 (0,24)	4,68 (1,36)	5,18	Major cause of commercial loss of aspen trees (Hepting 1971)
Spongipellis pachyodon Pers.	MN430948		41,50 (5,51)	27,16 (5,51)	21,25 (8,64)	29,97	
Stereum sp.	MN430950		22,25 (5,78)	11,31 (6,78)	7,68 (3,04)	13,75	Heart-rot of living *Nyssa* trees (Hepting 1971) Cultured from mine timbers (Eslyn and Lombard 1983)
Stereum complicatum Fr.	MN430949	WR/Gum	29,92 (3,61)	14,41 (4,19)	5,81 (3,66)	16,71	
*Stereum hirsutum (*Willd.) Pers.	K26		27,38 (5,50)	13,99 (5,42)	14,07 (2,84)	18,48	
*Trametes gibbosa (*Pers.) Fr.	MN430954	WR/Gum	54,16 (9,88)	14,82 (6,28)	21,44 (8,99)	30,14	
Trametes versicolor (L:Fr.) Pilát (from ties)	MN430953	WR/Gum & Oak	76,34 (10,57)	40,65 (24,80)	23,48 (11,11)	46,82	Commonly associated with downed wood (Hepting 1971) and wood in service (Eslyn and Lombard 1983)
Xylobolus frustulatus (Pers.:Fr.) Fckl.	MN430955	WR/Oak	19,20 (6,08)	24,07 (3,96)	3,37 (4,96)	15,55	Rapid decay of oak already colonized by other decay fungi. (Hepting 1971), and from oak mine-timbers (Eslyn and Lombard 1983).
G. trabeum (Pers.:Fr.) Murr.	MAD 617	BR/N/A	51,82 (15,90)	42,73 (16,39)	32,88 (19,77)	52,08	AWPA standard fungi assumed to have high decay potentials or causing damage of commercial significance.
R. placenta (Fr.) Niemelä, K.H. Larss. & Schigel	MAD 698	BR/N/A	50,09 (9,58)	41,58 (4,66)	35,19 (4,48)	38,17	
T. versicolor (L:Fr.) Pilát	MAD 697	WR/N/A	74,38 (11,62)	16,07 (12,5)	18,14 (8,1)	36,2	
No Fungus	-	-	-0,93 (0,40)	1,18 (0,50)	2,30 (0,34)	0,76	

Isolate numbers with MN are accession numbers that correspond to GenBank/DDBJ/ENA submission SUB6273185. Other numbers represent stock cultures of AWPA reference fungi or isolates identified without sequence submissions. Decay types are white rot (WR), and brown rot (BR) and source of blackgum or oak. Mass loss is shown as the mean percent of mass lost with standard deviation in parentheses (n = 8).

Although bigleaf maple is very susceptible to decay, it is interesting to note that over 40 % of the isolates tested did not cause more than 11 % mass loss. The relatively limited decay capabilities of many isolates likely reflect their ecological roles. Many decay fungi found in living trees tend to grow and decay wood slowly and have fairly narrow environmental requirements that may not have been met under the soil block test conditions (Hepting 1971, Scheffer 1986, Schwarze *et al.* 2013). For example, *Chondrostereum purpureum*, *Sistotrema brinkmannii*, *Schizophyllum commune* and *Phlebia subserialis* are common decayers of slash but had only limited decay capabilities in this test (Hepting 1971, Boddy and Rayner 1983, Rayner and Boddy 1988, Sexton *et al.* 1992, Zabel and Morrell 2020). *Punctularia strigosozonata* with arthroconidia caused about twice as much mass loss in all wood types as *P. strigosozonata* without arthroconidia illustrating the variations that can occur in decay capabilities of different morphotypes of the same species.

Table 2: Relative decay capability of 35 fungal isolates as measured by weight loss on different wood species in a soil block test.[a]

Weight Loss Range (%)	Frequency in Category (%)		
	Bigleaf maple	Red oak	Southern pine
≤6	14,7	48,6	8,6
6 to ≤11	26,5	5,7	11,4
11 to ≤21	8,8	17,1	20,0
21 to ≤30	17,6	17,1	20,0
≥30	32,4	11,4	40,0

[a]Categories based upon weight losses listed in Table 1.

Combining weight losses caused by each fungal isolate on all three timber species provides a relative guide to decay capacity (Table 3). Almost a quarter of the taxa tested (23,5 %) caused less than 5 % weight loss and would be considered, at best, minor decayers. It is important to note, however, that these fungi may play other roles in the decay process, for example, conditioning the wood for attack by more aggressive decay fungi (Butcher 1968, Rayner and Boddy 1988). Seven (20,6 %) of the fungi tested caused between 6% and 11% weight loss, suggesting that they were beginning to exert more influence on wood strength. While these levels seem low, it is important to remember that fungi can have profound effects on timber properties are very low weight losses (Wilcox 1978). Only four taxa caused mass losses between 21 % and 30 %, while the remaining five isolates (14,7 %) caused greater than 30 % weight loss. It is interesting to note that three of these five more aggressive isolates cause brown-rot, illustrating the potential for this group of fungi to cause substantial mass loss in wood even thought they were a minor part of the overall fungal community.

Table 3: Classification of test fungi based upon average mass losses on red oak, bigleaf maple and southern pine blocks in a soil block test.

Average Mass Loss (%)[a]				
<5	6 to 10	11 to 20	21-30	>30
A. bisporum	B. adusta	C. radians	Phanerochaete sp.	A. minuta
C. purpureum	D. granulosum	G. sessile	R. americanus	A. oleracea
Cylindrobasidium sp.	P. fuscoatra	P. flavidoalba	S. pachyodon	G. trabium (Ref)
Hypochnicium sp.	P. limonella	P. strigosozonata (conidium)	H. fimbriatus	L. cincinnatus
L. betulinus	P. squarrosa	R. casearius		R. placenta (Ref)
P. adiposa	P. strigosozonata (sans conidium)	S. complicatum		T. gibbosa
Peniophora sp.	S. commune	S. hirsutum		T. versicolor (Ref)
S. brinkmannii	S. delectans	Stereum sp. X. frustulatus		T. versicolor (wild)

[a]Table 1 for complete fungal names.

CONCLUSIONS

Decay fungi isolated from air-seasoning blackgum and red oak tended to be classified as white-rotters, reflecting the tendency for this type of decay to predominate in hardwoods out of soil contact. The isolates exhibited a range of decay capabilities, with three of the four most aggressive isolates causing brown rot decay. The results illustrate the wide range of decay capabilities of fungi present in seasoning hardwood timbers and highlight major differences in fungal propensity for attacking different wood species.

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