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Title: The Effect of Sill Height on Decay in Air-Seasoning Crossties

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The sustainable use of wood for rail ties requires chemical treatment to increase service life and maintain structural integrity. Treatment can only be applied after lengthy air-seasoning to reduce moisture content in wood, but seasoning leaves ties exposed to attack by decay fungi for up to a year. One factor affecting the rate of decay is proximity to soil contact. The height of sills used to raise untreated ties above ground contact while drying is mandated by national standards to be 12 inches, but there is little research examining the effects of deviating from this height on decay risk.

This study examined the effects of varying sill height on colonization by decay fungi and flexural properties of black gum and red oak ties. The objective was to relate sill height with populations of decay fungi and to changes in wood strength during air-seasoning. Sampling of blackgum railroad ties from the Koppers plant in Guthrie, Kentucky at three time points during seasoning showed that the incidence of decay fungi increased at an even rate over time with little or no difference attributed to distance of stacks above the ground. Red oak ties sampled three times over 11 months showed that red oak yielded few decay fungi for 6 months followed by a rapid increase in fungal populations. Stacks of red oak and blackgum had completely different fungal populations and colonization rates despite being stored in close proximity.

Changes in fungi colonizing ties and their effect on tie properties were not strongly related to height between 6 and 33 inches, and there was no relation to sill heights of 6, 8, or 12 inches.

Isolates of decay fungi recovered from ties were evaluated for decay potential and produced a wide range of decay capabilities. Blackgum ties were colonized most by white rot fungi, while red oak ties were colonized by both white rot and brown rot fungi. Many cultures isolated from blackgum had weak decay capabilities.

The results indicate that decreasing sill height from 12 to 6 inches had no significant effect on fungal colonization or timber properties of the two species evaluated.

Key Words: Railroad ties, crossties, blackgum, red oak, fungal colonization, air-seasoning, decay, decay out of ground-contact.

The Effect of Sill Height on Decay in Air-Seasoning Crossties

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APPROVED:

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Head of the Department of Wood Science and Engineering

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Leon Rogers, Author

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CONTRIBUTION OF AUTHORS

Jed Cappellazzi and Dr. Jeffrey Morrell provided the initial study design, and many of the methods used. They also reviewed and provided editorial support for all chapters of this thesis. Jed Cappellazzi provided substantial methodological support and frequent consultation.

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DEDICATION

This work is dedicated to my grandparents Tom and Jacque Rogers

CHAPTER 1. INTRODUCTION

Protecting wood from decay is essential if it is to be used sustainably, especially in exterior exposures. The use of wood for rail ties is dependent on effective preservative treatment, which often includes prolonged air-seasoning (Taylor *et al.* 2016). Air-seasoning is a simple and inexpensive way to reduce moisture in wood prior to treatment, but it introduces the risk of fungal attack during the seasoning period (Conners 2008; Thompson & Koch 1981; Webb *et al.* 2016). Decay fungi are a major concern in untreated wood because they cause severe strength loss in a short amount time (Curling *et al.* 2002b; a; Wilcox 1978).

Several methods have been adopted to minimize the risk of decay during seasoning (AREMA 2015; Taylor *et al.* 2013). These methods are standardized by the American Railway Engineering and Maintenance-of-way Association (AREMA) and American Wood Protection Association (AWPA).

Recommendations for proper tie seasoning include limiting seasoning time, stacking ties to allow for maximum air-flow to accelerate drying, removing vegetation from the site, using gravel drainage to minimize the pooling of water, and placing stacks of ties on preservative treated timber footings (sills) to limit potential for direct contact with the ground. These procedures are listed in Chapter 30 of the AREMA standards and standard M1 of the American Wood Protection Association (AREMA, 2010; AWPA, 2017). Of particular interest in both standards is the requirement that sills be a minimum of 12 inches in height. This requirement was designed to ensure that untreated, seasoning ties were not exposed to direct soil contact or excessive splashing, but it creates a secondary issue for seasoning yards.

Injuries have occurred at some tie plants as two ties must be used to create the necessary sill height. While most activities in seasoning yards use mechanization to minimize the risk of injury, sills are generally created by manually lifting and stacking two 6 by 8-inch or 7 by 9-inch treated timbers. The

first timber is easily placed, but the second one must be lifted into place, creating the risk of worker injury. These concerns could be addressed with shorter sill heights, but it has not been well established if deviating from the standard height alters decay risks.

There is a precedent for shorter sill heights of 7 inches in regions with very low humidity and high temperatures, but this is approved on a case-by-case basis between tie purchasers and manufacturers (Forest Products Group of the Union Pacific Railroad 2014).

Examination of prior reports suggested the standard height was somewhat arbitrary, though highly effective. Reducing the sill height could allow for the use of a single timber; however, it also brings ties closer to the ground and could increase the risk of fungal decay during seasoning (Conners, 2008; Duncan, 1965). Ground contact is known to increase decay risk by increasing exposure to fungi, insects, and moisture (Liese 1975; Meyer *et al.* 2016); however, few studies have examined the relationship between decay and distance out of ground contact (Meyer *et al.* 2016; Råberg *et al.* 2005). There are no data examining the relationship between sill height and fungal attack in this application; although fungal attack during air seasoning, or stack-burn, is well correlated with strength loss in ties (Taylor, 2013). The purpose of this work was to examine the relationship between 6, 8, and 12 inch sill heights and fungal colonization in freshly cut blackgum/tupelo (*Nyssa sylvatica* Marsh.) and red oak (*Quercus rubra* L. group) ties in a seasoning yard.

In order to assess the effects of sill height on ties, we made the following assumptions:

1. The rate of fungal colonization would be related to distance above the ground. Fungal colonization was expected to increase with proximity to ground because of combinations of increased humidity, reduced drying rates from lower wind speeds, proximity to fungal communities in the soil, and increased risk of splashing water during rain.

2. The greatest risk to tie quality related to sill height would occur within the first three layers of a stack where relative humidity levels would be highest. Layers above this were assumed to have little or no effect from ground proximity.

3. Flexural properties of beams cut from ties could be used to measure how fungi and sill height affected ties at different heights. These beams could be cut from ties after a complete air-drying period to evaluate differences during seasoning.

4. A site in Kentucky was representative of the decay risk in many tie plants in terms of climatic conditions.

5. Blackgum represented a decay susceptible species, while red oak represented a species that was more durable, but still reasonably susceptible to fungal attack. This was assumed based on prior observation and experiences from tie manufacturers.

The original intent of this study was to include community analysis from both classical culturing techniques and high throughput (Illumina, next-gen) DNA assessments. High throughput DNA samples have been collected and prepared for analysis. The culture isolate data presented here allowed assessment of succession of decay fungi over a complete air-seasoning period in different hardwoods. Ultimately; however, the Illumina sequencing data will need to be compared with the classic isolation data to develop a better understanding of the fungal flora that develops during the seasoning process.

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CHAPTER 2. LITERATURE REVIEW

Rail Ties

Rail ties, also known as crossties or sleepers, provide essential support to the intense lateral and downward forces created by trains weighing thousands of tons, rolling at speed along rails supported by ties. The concentrated force of a train's weight is distributed from over 100,000 psi at the rails to about 80 to 90 psi in the ballast under a tie (AREMA 2015; Oldknow 2017). Under such forces steel can flow like plastic, and ties must maintain the strength and flexibility to distribute this energy into a smoothly dampened ride without dangerous oscillation or shifting. Just as important is the ability to maintain gauge, or track width, as the accurate contact of train wheels is over a space smaller than a dime, or about 15 mm^2 (Oldknow 2017).

Wood has been used for almost two centuries with great success to meet these demanding conditions and its performance will likely continue to improve with ongoing research into preservation and structural engineering (Borton 1901; Webster 1992). The earliest locomotive designs from 1680 to 1804 attempted to use the stone streets then available, and were often deemed a menace to society. The first attempts at specialized track in 1804 were far more successful and utilized stone sleepers (Borton 1905). The use of granite blocks for sleepers seems to have been an emulation of early test tracks and rails used near Welsh quarries. After some experimentation, wood crossties quickly became the standard after 1830 and continue to serve as the primary rail support in the United States (Borton 1901; Gallery *et al.* 2000).

Why Wood?

Crosstie engineering utilizes the naturally anisotropic properties of wood that allow it to flex vertically under loads while providing rigid strength in lateral tension to maintain gauge. Compared to rigid stone sleepers, a wood crosstie allows a smooth, even distribution of loads along rail and ballast (Borton 1901). Compared to steel and concrete, wood offers a superior strength to weight ratio and is less susceptible to some types electromagnetic, thermal, and chemical

agents (Brischke *et al.* 2006; Thompson & Koch 1981). Wood/plastic composites and engineered wood have been assessed, but there is no material for ties that matches the strength, cost, and renewability of wood (AREMA 2015; Gallery *et al.* 2000; Webb *et al.* 2016).

The primary asset of wood as a crosstie material is that it is sustainable in an economic and environmental sense. Adequate preservative treatment produces wood that can be expected to endure longer than the required rate of replacement, all while sequestering carbon. The expected service life of a wood tie is longer than the time required to grow replacement trees, and wood ties provide opportunities for secondary products such as fuel or composite material after replacement (FPL 2010; Webster 1992). Currently, 81% percent of replaced ties are recycled for energy production, and many are utilized in other ways (Webb *et al.* 2016).

What is Wood?

Wood is essentially a matrix of multiple interlinking hydrocarbon polymers that each have unique properties and functions. These polymers, as tree components, are constructed from atmospheric carbon, water, and solar energy with other trace inputs. The primary components of all wood are cellulose, hemicellulose, and lignin.

Cellulose is a crystalline structure of glucose sugar molecules attached through interlinking hydrogen bonds into long fibrils (Schmidt & Czeschlik 2006). Cellulose is present in trees in very long chains of thousands of molecules. Fibrils can be cross polymerized through transient hydrogen bonding, lending flexibility and tensile strength to the wood matrix (Blanchette 1995; FPL 2010). Because cellulose is comprised of many identical molecular glucose units, it is susceptible to enzymatic digestion and is available as a food source to organisms ranging from bacteria to herbivorous mammals.

Hemicellulose is a heterogeneous polymer made from saccharides that may be pentose (including xylose and arabinose), hexose (mainly mannose, less

glucose and galactose) and sugar acids (Dashtban *et al.* 2010; FPL 2010; Zhang & Schilling 2017). The active sites of hemicellulose allow cross polymerization as well as chemical water holding capacity. Woods' strength results from all polymers working in conjunction, but initial losses of modulus of rupture are most directly related to degradation of hemicellulose, either by acid digestion or chemical weathering that disrupts the lignocellulose matrix (FPL 2010).

Lignin is a massive three-dimensional polymer of phenylpropane units attached to semi-random branched hydrocarbons. It comprises 20% to 30% of wood by mass and interlocks with cellulose and hemicellulose in a physical and chemically bound matrix (FPL, 2010). The chemical complexity and hydrophobicity of lignin make it very stable against enzymes and acids, but it will begin to photodegrade within hours of exposure to ultraviolet light under normal atmospheric conditions (FPL 2010).

The cell wall matrix composed of these three polymers is strong and rigid, with unique chemical properties that allow wood to differ from other plant cells in that the protoplast of older cells is usually completely absent and unnecessary. The bulk of any tree and all wood in service is composed of dead cells, and is a relatively pure polymeric substance called the lignocellulosic matrix (FPL 2010). The structure and content of lignin and hemicellulose are species dependent (Dashtban *et al.* 2010; FPL 2010).

Other wood components include extractives and metabolites. Metabolic by-products and some defensive compounds accumulate in the inner most portions of trees, delineating the heartwood. Heartwood of some wood species is more durable, and generally more difficult to pressure treat with preservatives compared to sapwood (Butcher 1968; Thompson & Koch 1981). Heartwood and sapwood extractives in the living tree may create selective pressure against many potential fungal colonizers that might otherwise be more competitive in wood after harvesting (Berry & Lombard 1978; Butcher 1968; Kiser 2009; Schmidt & Czeschlik 2006; Shigo 1967). Sapwood includes the outermost living portion of the tree and is the site of all metabolic production and storage (FPL 2010).

Compared to softwoods, the hardwoods are often more susceptible to white-rot fungi because of differences in micro-porosity, available sugar stored in parenchyma, and their lignin components (FPL 2010). Hardwoods also have a high ratio of hemicellulose to other polymers (Thompson & Koch 1981). Oaks have high levels of tannin that inhibit many decay fungi (Shigo 1967), and sapwood of oak has demonstrated variable durability out of ground contact (Eslyn *et al.* 1985).

Grading Wood for Crossties

Wood acquired for manufacturing ties must first be assessed for quality. Crossties are either "grade", or "industrial" depending on their wood characteristics (AREMA 2015). Industrial ties represent materials with defects such as knots, slope of grain or other defects that preclude their use in main line track. Industrial ties are often used in "shortlines" or turn-outs where train speeds are slower and stresses tend to be less severe. Wood cants (squared logs) are graded twice; immediately prior to stacking and seasoning, then again just before chemical treatment. Before seasoning, ties may be rejected for gross deviation from AREMA requirements in terms of grain, decay pockets, off-sized cuts, and splits. Ties can also degrade during the seasoning process due to biological degradation or by checks and splits developing as ties dry and shrink (AREMA 2015; Conners 2008). There are 10 defined defects in the American Railway Engineering and Maintenance-of-Way Association (AREMA) manual for railway engineering; wane, decay, holes, knots, shake, splits, checks, cross or spiral grain, bark seams, and manufacturing defects (AREMA 2015). As noted, ties that fail to meet grade, but are still functional may still be used as "industrial," in settings like turn outs, transfer yards, or places where trains move much slower and with less freight.

How Much Wood?

Early in crosstie development, it was recognized that wood had to be sustainably managed in both harvest and durability. In arguing for the establishment of the Forest Service Administration Act of 1897, the Chief of the

Forestry Division, Bernhard Fernow, emphasized the demand for rail ties when asserting that forests must be regulated and methods developed to preserve timber in use (Fernow 1897). His proposal was to ensure the sustainable use of wood in essential infrastructure by combinations of forest management and preservative treatment. In an earlier in-depth assessment of the state of the United States' forests and their relation to rail transportation, Fernow calculated that a fifth of the available timber in the United States had already been harvested to construct and fuel rail lines (Fernow 1887). Historic records show that by 1900, wooden rail ties were being replaced at a rate of 110,000,000 ties annually (Spartz 2009) with another 90,000,000 being laid in new miles of rail (Gallery *et al.* 2000; Webster 1992), closely matching early estimates by Fernow. The origin of forest conservation and wood research in the United States was largely due to the demands of the rail tie industry (Borton 1905; Fernow 1887, 1897; Riley *et al.* 2014).

The amount of wood needed for ties is still considerable. With over 230,000 miles of track, using an estimated 3000 ties per mile, there are some 690 million ties in service in the United States (Federal Railroad Administration 2017). Despite innovation in new tie technology and heavy investment in cement ties outside the United States, new ties in the U.S. remain about 94% wood (Sneider 2014); although there are improving wood preservation methods, the number of renewed ties remains significant with a replacement rate of around 3% or 21-25 million ties replaced annually (Federal Railroad Administration 2017; Sneider 2014). This is without consideration for new miles of track or renovation of older unused lines. In recent years, available hardwood inventories were unable to meet demand for rail ties because of weather conditions that limited harvests, and more intense seasonal damage interrupting rail maintenance (Conley 2019; RTA Economic Team 2019) as well as competition for industrial wood material from increased oil fracking (Sneider 2014). The amount of timber available for ties remains adequate, but it is sometimes difficult to match supply with demand.

Prices for new tie material and fluctuating availability have begun to limit tie production and raised concerns for the general safety and the production of a long standing renewable infrastructure (RTA Economic Team 2019). One of the most effective aspects of sustained tie production is limiting decay. Besides loss of physical material, decay also presents a safety risk if undetected. Decayed ties can completely fail to hold critical rail components in place.

As B. A. Worthington, chairman of the Pacific Coast Railway Club stated in 1901,

> *"if the ties are old, the timber dozy or decayed, or the rail under a train springs up and down, the spikes will be found lifting their heads in protest." (C.C. Borton 1901)*

Tie processing

Wood in Ground Contact

The decay of all organic material, including timber, is increased through ground contact. Untreated ties in ground contact had a negligible increase in durability from proper moisture seasoning compared to ties that were still green (freshly harvested) (Forest Products Laboratory 1931), but moisture conditioning was able to increase durability of green wood by several years when it was later used out of ground contact (Highley 1995). This shows that ground contact severely increases the decay hazard of otherwise stable wood. Ground contact exposes wood to a broad range of microorganisms in an environment with readily available moisture and the supplemental nutrition required for microbial growth (Richards & Humphrey 1939; Scheffer 1971). The best solution to increasing service life of timber is to avoid direct soil contact and keep the wood dry; however, that is not possible with railway ties. Although railway ties are generally used in well-drained ballast without direct soil contact, the conditions are still suitable for aggressive fungal attack. The most efficient way to continue using wood as a renewable base material for ties is preservative treatment (Borton 1901, 193–199).

Preservatives in Rail Ties

In the case of rail-ties, early preservative research from 1830 to 1860 primarily focused on utilizing naturally durable wood species or impregnating ties with water-based compounds such as mercuric chlorides, also called Kyanization (Borton 1905; Thompson & Koch 1981; Webster 1992). The much safer and more effective treatment with creosote was invented in 1836, but wasn't easily applied until Bethel patented an effective pressurized injection method in 1839, followed by more efficient pressure treatment techniques developed by Rüeping and Lowery in the early 1900's (Thompson & Koch 1981). Even these treatments were not widely adopted until many years after their development because untreated wood was so inexpensive and easily replaced (Borton 1905; Fernow 1887). Concerns about future timber shortages eventually led to the use of preservative treatments to prolong tie service life. While other oil-borne preservatives have been developed over the last 150 years, creosote remains the preferred tie treatment (Chakraborty 2002.; Thompson & Koch 1981; Webster 1992).

Prior to the widespread adoption of creosote treatment, and into the early 20th century, rail ties were replaced at a rate of 100 million per year with an expected service life of 5 to 10 years for untreated ties, compared to 20 to 50 years for treated ties (Borton 1905, 193–212; Forest Products Laboratory 1931; Spartz 2009; Webster 1992). Preservative treatment standards have gradually evolved over the past century under the guidance of the American Wood Protection Association (AWPA) to ensure that creosote meets minimum content standards and that wood treated with this system receives the proper loading delivered to the proper depth in a tie (AREMA 2015; AWPA 2017; Conners 2008; Webb 1998).

The contributions by Dudley to the *Report on the Relation of Railroads to Forest Supplies* illustrate that by 1887 fungi had been recently identified as the cause of decay, and that wood in service must be preserved against any fungus for safety and sustainability. At that time, he indicated a preference for zinc and

mercuric chlorides, or “Burnettizing” and “Kyanizing” to treating many wood species (Fernow 1887). By 1939, Richards had continued the line of investigation to develop a well-illustrated and concise summary of fungi and their associated decay patterns (Richards & Humphrey 1939). Her emphasis on rail tie material, especially in storage, emphasized the difficulty of detecting incipient decay and that proper seasoning before treatment was imperative. In the same publication, Humphrey established the need for sanitizing temperatures during pressure treatment with creosote (Richards & Humphrey 1939). These established procedures successfully reduced the rate of replacement to sustainable levels.

Current standards mandate that preservative penetration of red oak ties should cover at least 65% of the cross-section as measured by growth rings (Conners 2008; Mathewson *et al.* 1949; Webster 1992) and 75% of the cross section should be penetrated for mixed hardwoods including blackgum (Conners 2008). The AWPA Standards also mandate a minimum retention of 7 pounds of creosote per cubic foot of wood, though retentions can reach 12 pounds per cubic foot for some applications (AWPA 2017; Thompson & Koch 1981). Both red oak and blackgum are relatively easy to treat and pressure processing generally results in complete treatment (Forest Products Group of the Union Pacific Railroad 2019; Thompson & Koch 1981).

Pre-Treatment

Studies of untreated wood out of ground contact have often shown that wood can be naturally durable for a period of several years, and that abiotic degradation can pose as much risk as decay in some environments (Eslyn *et al.* 1985; Highley 1995; Kirker & Winandy 2014; Råberg *et al.* 2005). Conversely, studies in ties have shown that incipient decay or “stackburn” can occur within a matter of months (Taylor *et al.* 2013, 2015). These differences reflect the fact that wood in above ground tests is often sterile and dry when placed in test, while ties are wet and can contain a varied fungal flora. Some treatment methods have been developed that are compatible with wet wood, primarily waterborne boron treatments. Boron treatment of wet wood is merely temporary because it is water

soluble and will eventually leave the wood (AREMA 2015; Lebow 1996), but it can be easily applied and is sufficient to limit fungal colonization during the air drying process (Taylor *et al.* 2013). In addition to limiting invasion by decay fungi with pre-treatments using chemical salts, it may be desirable to better understand decay out of ground contact, and the factors affecting air-drying.

Seasoning

Moisture Content

No single measure of tracking moisture can fully illustrate the dynamic processes of wood drying during seasoning, especially where wood members overlap or where there is underlying material with any water holding capacity (Meyer *et al.* 2016). Moisture content (MC) is generally the limiting factor for decay of wood in service (Brischke *et al.* 2006; De Groot 1992; Käärik 1975; Shigo 1975; Zabel & Morrell 1992). Moisture content can be measured by several methods, but the most common is the oven-dry method. The oven-dry method of MC is the difference between wood mass when dry and its mass with moisture as a percent of the dry mass (FPL 2010).

Scheffer and Lindgren (1940) found that wood inhabiting fungi had a range of MC optima, and that fungal activity generally increased as moisture decreased in fresh wood until a lower threshold or optimal seasoning condition was reached. This was predicted to be a result of decreasing free water allowing more oxygen exchange in cell lumens, until a point was reached where water became a limiting factor to fungal attack. This level is generally described as 15% MC with many fungi unable to thrive below 25% MC (Scheffer & Lindgren 1940; Zabel & Morrell 1992). Many wood boring insects, which may act as vectors of fungal pathogens, are also inhibited below 15% MC (Clausen 2010; Zabel & Morrell 1992). Optimal MC for decay is generally between 40% and 70% with most species of fungi inhibited at MC above 90% (Brischke *et al.* 2006; Richards & Humphrey 1939; Zabel & Morrell 1992).

Moisture contents of freshly cut timber range from 40% to well over 100% (oven dry basis) (FPL 2010; Scheffer & Lindgren 1940). Preservative treatment

requires that the MC of wood be reduced to allow penetration of oil or waterborne chemicals. Failure to adequately dry timber prior to treatment results in poor penetration and reduced performance in service (Mathewson *et al.* 1949; Richards & Humphrey 1939). Timbers can be dried using a variety of methods, but rail ties are usually subjected to a lengthy air drying process, ranging from 6 months to over a year depending on wood species and local climate (Conners 2008; Mathewson *et al.* 1949; Taylor *et al.* 2015; Webb 1998; Webb *et al.* 2016). The target MC for oak species is less than 50%, and less than 40% for other mixed hardwoods (AREMA 2015; Conners 2008). Conifer species require a MC less than 30% and different seasoning methods may be used. Mixed hardwoods dry at slightly different rates, but are generally suitable for treatment within 6 months of air seasoning. Oak ties generally take twice as long and can sometimes require longer than 18 months to reach an acceptable moisture level (Conners 2008; Mathewson *et al.* 1949). These times vary with location and climate.

Air-seasoning does not require a large capital investment, except for space to store ties, but it does introduce risk to tie quality. Wood remains susceptible to fungal attack as long as it is above the fiber saturation point. Fiber saturation is the point where wood can hold no more bound water, but has no free water in the lumen (FPL 2010). As a result, ties are inherently susceptible to fungal colonization as they air-season (Taylor *et al.* 2013). A study of sapstain fungi, clearly indicated that stacks of ties maintained an environment suitable for fungal growth throughout their inner layers despite outer ties drying much more quickly (Scheffer & Lindgren 1940). Stacked ties also insulated and protect fungi from temperature and moisture extremes.

Decay that occurs during the air-drying process is called “stackburn” and can occur continuously from the time a tree is harvested to the point at which a cross tie is preservative treated (AREMA 2015; Conners 2008; Taylor *et al.* 2015). Regardless of the term, decay reduces wood properties and decreases tie service life. The goal of the seasoning process is to reduce the MC as rapidly as possible

to minimize fungal attack without introducing drying stresses that cause defects such as excessive checking or splitting.

A number of strategies are used to enhance drying, reduce fungal colonization and reduce excessive checking. For example, incising ties at the start of air-seasoning decreases drying time, minimizes check propagation, and improves preservative treatment (AREMA 2015; Thompson & Koch 1981). Other pre-treatment processes include Boulton conditioning (driving water off with heat and vacuum pressure) or steam conditioning. These two methods are considerably more expensive than air drying, although the former process is commonly used as a final moisture conditioning process (Taylor et al. 2013; Webb et al. 2016). Steaming is more useful for drying and treating southern pine.

Stacking

The other important factor in air seasoning is how the ties are stacked to facilitate drying. Moisture can only leave the wood as vapor and ties must be placed to maximize air-flow over the wood surfaces to facilitate evaporation. While there are a variety of methods for stacking timbers to dry, most ties in North America are seasoned in a configuration called “German stacking” (Conners 2008; Taylor *et al.* 2015) or “8-by-1” in some literature (Mathewson *et al.* 1949). In German stacking, ties are stickered only on one end by a single tie (stringer, or sticker) laid across each tier, creating a zig-zag arrangement of ties with no extra material included. In essence, one tie in each layer serves as a sticker; reducing the need for extra material to create air spaces. However, this stacking system also creates a large contact point on one side of each tie and uneven airflow along the length of the tie. The other option of stacking ties flat with smaller treated stringers spaced evenly between tiers is called open stacking. This method is infrequently used because it requires the use of an excessive amount of treated wood stickers that become damaged over time and pose a disposal challenge. Ultimately, the stacking method is specified by the tie purchasing railroad company and is determined by the drying conditions and

available wood type (AREMA 2015; Forest Products Group of the Union Pacific Railroad 2019).

In addition to the stacking method for air-seasoning, several mandates exist to mitigate the risk of decay during air seasoning; including that tie yards be kept free of vegetation, debris, standing water, and decaying wood, and that stacks have adequate airflow between tiers (AREMA 2015; Conners 2008). There is also a requirement that ties are to be seasoned at least 12 inches above the ground. The required space is generally made from treated wood sills, or footings. This requirement is designed to reduce the risk of excessive condensation on the lower surfaces of air seasoning ties as well as to mitigate the risk of water splashing on the ties closer to the ground. The AREMA manual specifically states that:

> *"All stacks of seasoning material must be supported on treated or other non-decaying sills. The bottom layer of material shall be supported at least 12 inches off the ground. In warm, humid localities, more space should be provided." (AREMA 2015, secs. 30-3–31)*

There is little documentation about how deviating from this standard might alter the risk of decay and stackburn. The standard is unquestionably effective, judging by its long unaltered prescription, but an assessment of its efficacy may be useful within the tie industry, as well as any case where untreated wood is exposed out of ground contact. There is a precedent for lowered sill heights of 7" in conditions where very low humidity and high temperatures cause stacks to dry too fast, resulting in checks and splits (Forest Products Group of the Union Pacific Railroad 2014). Cases for reduced sill height are taken with other prescriptions such as end plating of all ties and different stacking arrangements, and are made on a case-by-case basis by tie purchasers.

Distance from ground

In general, the risk of biological decay should be lower out of ground contact, and few applications require prolonged storage under conditions that promote decay. As a result, the effects of distance above ground on decay rate has

been subjected to limited study beyond comparing above ground to in-ground decay rates. Studies of decking, fencing, and siding have often included more assessments of degradation by abiotic weathering over several years (Kirker & Winandy 2014; Zabel & Morrell 1992, 23–25). In one ten year study of treated decking material in and out of ground contact, researchers did not include any measure of height for samples above ground and did not separate decay from weathering for samples above ground (Crawford *et al.* 1999). A nearly identical study of similar woods and conditions utilized a height of 30.5 inches and found no decay in treated wood after 10 years. The authors suggested that slight decay in a similar Swedish study may have been the result of placing samples 10 inches closer to the ground (Lebow & Halverson 2015). Tests of natural wood durability in several configurations indicated that untreated wood can persist for several years without signs of fungal decay, and that accelerated weathering tests might be a reasonable alternative (Råberg *et al.* 2005).

Untreated, but seasoned wood persisted for 5 to 20 years without failures from decay (Highley 1995). In that study, wood was placed in overlapping cross joints that may simulate overlapping sections from stringers in tie stacks, but no mention was made of specific height other than “out of ground contact.” Types of joinery were compared, but heights were not. It is also important to note that failure indicated extremely advanced stages of decay with no measure of flexural strength, and probably does not translate to the stricter standards of rail ties.

Material testing organizations only began to regularly include assessments of height as a variable in methods for testing wood durability above ground against fungal decay at the end of the last century (De Groot 1992; Råberg *et al.* 2005). Current material testing standards have generally assigned a standard height in durability testing for wood out of ground contact, and the absence of information on test height in many older assessments make it difficult to compare data. Despite more than a century of studies comparing rates of decay in and out of ground contact (Duncan & Lombard 1965; Fernow 1887, 43–50; Hartig 1975) and the development of the Scheffer index of decay hazard for wood

exposed above ground (Carll 2009; Scheffer 1971), the USDA Forest Products Laboratory still considered predictions of decay risk based on height to be in the beginning stages (Kirker & Winandy 2014). The ingenious Scheffer index for predicting decay of wood out of ground contact has proven effective over the last half century, and has received regular updates for changing climates, but does not include a prescription for height (Carll 2009; Kirker & Winandy 2014; Scheffer 1971). In other words, how close is too close to the ground?

Few evaluations have compared decay relative to height, even as an incidental variable across different experiments. Meyer *et al* (2016) compared above ground decay testing methodologies from several other studies, and found drastic differences in decay response based on factors of wood orientation, ground type, size of test pieces, distance between test pieces, and size of end grain exposure. In general, the analysis supported the common assumption that approaching ground contact increased decay hazard. This was explained by increased incidence of splashed water, reduced wind speed (and therefore reduced drying), smaller temperature swings, and a higher relative humidity. They also found that larger untreated test members generally had a shorter service life because of moisture trapping, and more consistent internal environments as the surface-volume ratio of wood members decreases with increased size. The findings supported the premise that large stacks of incised, wet cants should be very susceptible to fungal decay compared to most other types of wood stored out of ground contact.

Fungal activity as measured by percent of defacement increased with proximity to ground in much smaller stacks of dimensional lumber over a shorter test period (Williams *et al.* 1997). No assessment of height was provided beyond "top" or "bottom" of 55-piece lumber stacks, but all chemical dip-treated and control stacks had increased fungal degradation over 24 weeks in a rough gradient of distance from ground. Their findings also presented a horizontal gradient, where outer members were much more defaced than members further inside stacks, but these results were not thoroughly discussed. These findings

may not be directly comparable because the measures of defacement were essentially cosmetic, not structural, and caused by pigmenting (or *spalting*) fungi. The chemical treatments applied were primarily sub-prophylactic levels of copper compounds; similar to treatments that have been shown to actually increase the activity of spalting fungi specifically (Robinson *et al.* 2011).

Many comparisons of decay and height have included ground contact and examined very small differences of a few inches. Butcher found that decay fungi colonized wood very differently between ground line and 2 inches above or below ground (1968). The small stakes used by Butcher also represented single units that should be assumed to permit fungal growth along their length. The wood used in these studies was also treated, creating a much more limiting environment for fungal colonization.

The scale of height differences in a stack of seasoning ties has not been well-documented for wood out of ground contact, but several things can be predicted from prior research on wood decay. Changes in fungal community should reflect changes in microclimates and MC. In the case of rail ties, there is the extra consideration of multiple sources of colonization; rail ties arrive from many different locations and could have first inoculated each other by lateral transmission. Subsequent communities are expected to have followed from below by inoculation in ground contact (mitigated by treated sills) and from above by airborne spores.

We fully expected fungal activity to increase among lower layers of stacks, but no prior estimate could be made of how fine a spatial gradient to expect, or whether a difference could be found between the few inches of difference caused by adjusting sill heights between 6 and 12 inches.

Measuring Decay

Changes in Strength and Flexibility

Changes in tie strength can be detected in a variety of methods including flexural testing, spike pull tests, or mass loss. Decay, in general, can be detected

by methods ranging from the abstract, such as increased electrical conductivity, to simple methods like pick tests (Wilcox 1978; Zabel & Morrell 1992)

The simplest method of decay detection, of course, is visual observation. Decayed wood may develop pockets, bleaching, crumbly textures, or be covered in mycelium. Strength loss extends in a variable gradient beyond the visible epicenter of decay (Taylor *et al.* 2013). Wood should be considered to have already lost most of its strength where decay is detected visually (Wilcox 1978). Decay is often observed in rail ties during grading, and passed or rejected based on intuitive assessment. The AREMA guide states:

> *"Ties with decay greater than 1-1/2 inches in diameter within the rail bearing areas will be rejected. Slight incipient decay will be allowed if the tie as a whole is of good quality. Decay is allowed outside of the rail bearing areas if the decayed area does not exceed 3 inches in diameter. Ties with decay greater than 2 inches in diameter appearing in both ends of the tie will be rejected. (AREMA 2015, sec. 3.9.1.4.2)"*

Weight loss is strongly correlated to reductions in all of the strength properties of wood, with 1% weight loss resulting in strength losses of 6% to 50%, and 10% weight losses resulting in greater than 50% strength losses measured by modulus of rupture (MOR) (Clausen 2010; Wilcox 1978). Decay resulting in 10% weight loss is often imperceptible to casual observation, but can cause significant structural losses (Clausen 2010; Curling *et al.* 2002b; Winandy *et al.* 2001; Zabel & Morrell 1992). Even thorough microscopic analysis does not detect decay below about 5% mass loss (Wilcox 1978).

Strength loss occurs much faster than mass loss caused by both brown rot and white rot fungi, though brown rot fungi generally cause greater strength loss and lower mass loss than white rot fungi at the early stages of decay. In both cases, strength loss seems to be directly associated with degradation of hemicelluloses (Curling *et al.* 2002a; b; Winandy *et al.* 2001).

The different measures of modulus of elasticity (MOE) found in shear and bending for wood may vary in their relative proportion by as much as 50 times

(Samson 1991), so it is desirable to measure each aspect (shearing and flexing) of MOE in isolation. While 4-point loading does not completely eliminate the possibility of shear stress at all points of a tested beam, it does neutralize shear stress within the expected region of failure (Brancheriau *et al.* 2002; Samson M 1991). The isolation of bending MOE must be compared carefully to MOE determined by three-point testing (center point loading), which includes a combination of MOE from bending and shear. Third point bending is a traditional standard (ASTM International 2014); however, 4 point methods are frequently encountered in international standards (Brancheriau *et al.* 2002; Yang *et al.* 2017) and equations have been developed for converting different measures of MOE in wood (Brancheriau *et al.* 2002).

MOE is generally considered one of the best measures for assessing decay-related changes in wood strength (Baar *et al.* 2015) and has been well-correlated to effects from decay, especially at early stages before weight loss or changes in rupture strength can be detected (Curling *et al.* 2002b; Winandy *et al.* 2001). Changes in MOE and MOR are some of the most sensitive physical properties for detecting decay and are only surpassed slightly by tension parallel to wood grain (Wilcox 1978).

Reductions in static MOE can indicate the effects of fungal activity in various woods in roughly half the time required to observe mass lost in classic soil bottle tests (Ma *et al.* 2017), and MOE has the benefit of being comparable between different testing formats (e.g. flexural 3-point and 4-point testing, as well as time-of-flight) with or without testing to destruction (Babiak *et al.* 2018; Davis *et al.* 2012; Råberg *et al.* 2005).

MOE and MOR are affected by de-polymerization within all parts of wood, but especially from the destruction of hemicellulose (2014; Zabel & Morrell 1992). The loss of side chains and active binding sites from hemicellulose effectively unbinds cellulose from lignin, weakening internal bonds of wood cells and microstructures, even before any other polymeric components have been metabolized by a fungus.

Fungal Decay

Degradation in wood can be measured by several means depending on what wood property is of greatest concern or what is convenient to monitor. Physical properties include strength, flexibility, or mass; other methods include microscopic analysis or chemical and spectroscopic changes (Zabel & Morrell 1992). Infrared spectroscopy has become a common and useful method for tracking relative amounts of wood-polymers with the ability to detect decay at very early stages (Vane 2003). The ability to detect and correlate degradation to any observable feature is an ongoing effort. In order to detect the risk of decay before it leads to material changes, it is useful to predict the causes of decay through environmental or biological monitoring, implicating fungi as the cause of decay before severe destruction occurs (Buller 1906; Cappellazzi *et al.* 2018; De Groot 1992; Käärik 1975; Richards & Humphrey 1939; Taylor *et al.* 2013; Vane 2003; Zabel & Morrell 1992).

In an early description of wood destroying fungi published in 1906 Reginald Buller provided some concise references to the earlier work of Theodore Hartig's 1833 book *Abhandlung über die Verwandlung der polycotyledonischen Pflanzenzelle in Pilz- und Schwamm-Gebilde, und der daraus hervorgehenden sogenannten Fäulniß des Holzes*. Hartig's seminal work on the causes of decay identified that fungi were strongly correlated to decay in wood, but stopped short of labeling them a causative agent; proposing instead that wood could spontaneously collapse and reaggregate into nodes that would make mycelium. Hartig attempted unsuccessfully to trace mycelium in wood to mushroom fruiting bodies (Buller 1906). Further studies of decay made tentative associations between fungi and lost cellular components of plant material until 1878 when the younger Robert Hartig published descriptions of the wood degrading capacity and pure culture characteristics of several decay fungi in *Die Zersetzungserscheinungen des Holzes der Nadelholzbaeume und der Eiche in forstlicher botanischer und chemischer Richtung* (Hartig 1975). This is considered to be the first of the modern decay studies. As Buller's own work

illustrated, the identification of fungi as corruptive agents allowed for accurate determination of conditions that could promote or resist decay. Early work had identified changes to wood cell polymers, but the ability to study effects of fungi on wood in isolation quickly led to descriptions of different modes of decay and an understanding of their impact on wood qualities (Buller 1906; Hartig 1975).

The ability to inhibit decay by observing and combating fungi quickly advanced so that within a few years, Humphrey and Richards had compiled lists of fungi associated with the various woods used for ties and under what conditions many of the fungi either failed or flourished (Humphrey & Fleming 1915; Richards & Humphrey 1939).

The primary study of wood pathology has often focused on diseases of living trees (Duncan & Lombard 1965; Hartig 1975; Hepting *et al.* 1971), as the loss of forests is uncountably more devastating than the loss of timber in service. This can be attested by the loss of whole forests or species to fungal pathogens. For example, in 1905, American chestnut was a common and desirable rail tie material (Borton 1905), but it was nearly unobtainable by 1930 as a result of fungal blight (Hepting, 1971), eliminating what Hepting implied to be the most useful tree in America. With relation to rail ties, Humphrey found that fungi were the cause of nearly all decay in storage as well as defects present at the time of harvest due to advanced heart rot persisting from living trees (Richards & Humphrey 1939).

Culturing/DNA analysis

Detecting fungal presence to indicate decay has become a more precise art since the early work of the Hartigs' and Buller, and continues to benefit from new advances in molecular techniques. Almost all fungal taxonomy and detection has relied on fruiting bodies or very skilled and time consuming cultural isolation techniques (Butcher 1968; Duncan & Lombard 1965; Hartig 1975; Ryvarden 1972; Wang & Zabel 1990).

Most fruiting body identification has been made by spore characteristics, as spores are produced by both macro and microscopic structures in both Ascomycota and Basidiomycota (Wang & Zabel 1990). Macroscopic fruiting bodies are more distinct and easier to observe, but are also ephemeral and climate dependent (Richards & Humphrey 1939; Wang & Zabel 1990; Zabel & Morrell 1992). The most important limitation to fruiting bodies as indicators is that they only represent the sexual phase of a single member of an entire underlying community. There is an inherent assumption that the largest and most visibly reproductive fungal presence is also the most ecologically active. Fruiting body assessment cannot capture all community associations or any fungus that does not enter a sexual phase of its life cycle in that piece of wood (Råberg *et al.* 2005). The presence of any fruiting body in wood is a likely sign of established fungal presence rather than recent colonization. In the case of rail ties, mushrooms should be considered evidence of advanced decay (Badalyan *et al.* 2011; Richards & Humphrey 1939; Taylor *et al.* 2013) with some notable exceptions such as *Schizophyllum commune* Fr. (Richards & Humphrey 1939, 35) and *Sistotrema brinkmannii* Bres., (Carey & Hull 1989). These fungi have been noted to grow prolifically among other decay fungi and to produce basidiocarps (fruiting bodies) very quickly, but have low potential to cause decay on their own.

More precise observations of fungal colonization have been made by culturing. Culturing works by capturing viable vegetative mycelium on sterile media. A pure sample of a fungus can be isolated from an initial community through selective media and multiple precise transfers to new media. Many interacting fungi can be isolated and studied from a single substrate, then preserved or applied to a new substrate for evaluating capabilities like decay or enzyme output. Cultural studies capture a reasonable sample from a community, but can be very difficult to differentiate. Cultural studies are also susceptible to artificial selection by various media and storage conditions (Carey & Hull 1989; Råberg *et al.* 2005; Wang & Zabel 1990).

In the last couple of decades, molecular techniques have ranged from restricted fragmented length polymorphism (RFLP) and Sanger sequencing to differentiate between cultures, to full genomic analysis to assess or manipulate genetic capabilities (Presley *et al.* 2018) and high throughput community identification (Bellemain *et al.* 2010). There are two advantages to modern molecular analysis. The first is speed or ease of identification by comparison among databases informed by prior fruiting body research. The second advantage has been a more objective community assessment (Råberg *et al.* 2005). In theory, DNA analysis is less subject to the artificial selection characteristic of culturing or to subjective identification parameters. Genetic analysis of a population is still subject to artifacts of non-viable tissue and amplification bias (Bellemain *et al.* 2010; Moťková & Vytřasová 2012; Zhang *et al.* 2010), but can reasonably capture an entire fungal community with proportional representation (Bellemain *et al.* 2010; Råberg *et al.* 2005; Torres-Andrade *et al.* 2019; Zhang *et al.* 2010). In reality, there is growing awareness of bias during amplification and biases among sequencing technologies (Bellemain *et al.* 2010; Kiser 2009; Ohm *et al.* 2014).

Fungi Are the Primary Cause of Wood Decay

Fungal populations can serve as a proxy measure for wood degradation because they are the primary agents of decay (Clausen 2010; FPL 2010; Zabel & Morrell 1992). Other organisms damage wood, but none completely degrade all the polymers found in wood, living tissues, and even many synthetic materials. Decay fungi are generally classified as *White, Brown*, or *Soft* rot; white-rot and brown-rot being the most destructive in most environments.

As stated in chapter 30 of The Manual for Railway Engineering:

> *"Decay is the disintegration of the wood substance due to the action of wood destroying fungi. "Blue stain" is not decay and is permissible in any wood. (AREMA 2015)"*

Buller used chemical analysis of fruiting bodies and decayed wood to determine that carbon from wood was almost entirely converted to gas through fungal respiration (Buller 1906). A range of oxygen levels from 10% to 20% in wood are

suitable for fungal degradation (Schmidt & Czeschlik 2006, 58–61), and fungi are able to survive in very low oxygen environments below 1% (Scheffer & Livingston 1937).

Other forms of decomposition by weathering, physical loading, or other abiotic factors are more easily accounted for in the engineering and planning of tie service life, while decay is an irregular and unaccountably destructive process.

How Wood is Degraded

Intact biopolymers of wood are too large to be absorbed directly by hyphae so they must first be degraded by extracellular enzymes and acids into primary constituents such as phenylpropane from lignin and glucose from cellulose. Smaller, cleaved molecular units are taken up by hyphae and digested intracellularly into metabolic energy and fungal biomass (Schmidt & Czeschlik 2006). Understanding the nature of a depolymerization is important for associating fungal communities to the risk of decay.

Whether a strain of fungi causes white or brown rot can be assessed by several different means. The first and simplest is to identify the pattern of decay that a strain is most associated with, but association is not the same as causation. Early decay research was based on discovery of fruiting bodies (mushrooms or conks) on decayed wood (Buller 1906; Hartig 1894, 1975; Taylor *et al.* 2013), yet the most prolifically fruiting cultures may not be the same organisms that caused damage (Boddy 2000; Käärik 1975; Rajala *et al.* 2011; Shigo 1967). Decay is a dynamic and cumulative process with various participants changing over time through community turnover (Boddy 2000; Hiscox *et al.* 2018). The next method of determining decay type is identifying enzymatic output. This is usually based on extractions from decayed wood in monoculture decay tests (De Groot *et al.* 1998; Peng *et al.* 2018; Presley *et al.* 2018), but can also be determined by detecting genetic loci for enzyme synthesis (Ohm *et al.* 2014; Peng *et al.* 2018; Zhang & Schilling 2017). Many fungi that are not conventionally considered decay fungi possess at least some genetic potential for wood degrading enzyme

production (Jiménez *et al.* 1991; Kang *et al.* 2010; Peng *et al.* 2018; Riley *et al.* 2014).

These associations imply that any fungal metabolism inside dead wood material is directly contributing to decay. This contrasts with non-decay fungi like moulds that do not utilize the cell wall polymers, but thrive under similar conditions and may act as indicators of a climate conducive to decay fungi (Scheffer & Lindgren 1940; Zabel & Morrell 1992).

Soft Rot Fungi

Soft-rot fungi are an integral part of decay succession and occupy many specialized niches, but are generally tertiary colonizers and very slow to cause decay (Richter & Glaeser 2015; Scheffer & Lindgren 1940; Shigo 1967). Their especially slow rate of degradation and mild enzyme output allow them to continue to cause decay in almost anaerobic environments, even when completely submerged under water (Schmidt & Czeschlik 2006). Soft rots tend to degrade angiosperms more readily than gymnosperms because of the higher hemicellulose content in hardwoods (Thompson & Koch 1981) and some soft rots have demonstrated greater lignin degradation potential than brown rots (Eslyn *et al.* 1985; Wang & Zabel 1990).

Brown Rot

Brown-rot fungi cause a characteristic brown hue from altered, but unassimilated lignin, and a cubicle pattern of decomposition. Their enzymes and oxalic acid target the wood carbohydrates (cellulose & hemicellulose) and penetrate crystalline portions of cellulose microfibrils, causing a very high rate of strength loss in correlation to weight loss. Maximum weight loss due to brown-rot ranges from 60% to 70% (Zabel, 1992). Brown-rot fungi cannot usually degrade pure cellulose, but degrade cellulose and hemi-cellulose very quickly under more natural conditions of mixed biopolymers (Blanchette 1995; Gilbertson 1980). The decay trajectory of brown rot increases in rate as incubation time increases (Tanesaka *et al.* 1993). The longer a brown rot fungus is active in the wood, the more destructive it becomes.

White Rot

White-rots degrade lignin and cellulose with a combination of enzymes including lignin peroxidases that leave a characteristic white punky patch or open decay pocket. As decay progresses, pocket-rot patterns appear from the chemical progression of sequestered pure cellulose. After cellulose is used to fuel rapid mycelial growth, they may be followed by a white calcareous lining of the cavities (Otjen & Blanchette 1984) or by white mycelial mats. White rot fungi produce extracellular phenol oxidases and can process all wood biopolymers as an energy source (Blanchette 1984; Dashtban *et al.* 2010; Fukasawa *et al.* 2011). White rot fungi degrade cellulose more slowly than brown rot because peroxidase enzymes depolymerize long chain sugars (including cellulose and starch) only at the ends; brown rot enzymes cause depolymerization throughout long chain sugar polymers (Schwarze *et al.* 2000, 22). White rot tends to affect wood strength more slowly than brown rot, and at a more constant rate (Tanesaka *et al.* 1993). Compared to brown-rot, the weight loss from white-rotters is slower, but more complete, ultimately reaching over 90% (Zabel, 1992). A white rot fungus can generally be differentiated in culture by color changes in gallic or tannic acid supplemented growth medium (Gilbertson 1980; Wang & Zabel 1990; Zabel & Morrell 1992).

Fungal Communities

Colonization

"...organisms must be understood both as individuals and as part of an ecosystem." - (Shigo 1967)

It is generally thought that airborne spores are the primary vector of colonization, though fungi can spread laterally very quickly when moisture and temperature levels are high (Råberg *et al.* 2005). Fungi also spread vegetatively through dispersal of their fragmented thallus. From a perspective of decay and structural losses in wood, initial fungal colonization seems to be dominated by populations already present within or immediately adjacent to the wood

members in question. (Boddy & Hiscox 2017; Hiscox *et al.* 2016; Råberg *et al.* 2005)

Primary colonization of wood out of ground contact may be a very slow process. In comparisons of wood out of ground contact, wood artificially inoculated prior to testing had signs of decay and fungal attack within a few months, while exposed pieces subjected only to environmental sources of fungi had much lower rates of fungal attack, even after three years (Meyer *et al.* 2016). Natural or *in situ* methods of colonization require very long establishment periods. It remains to be seen if fungi present in freshly cut rail ties are analogous to prior inoculation, or if they require similarly long periods to re-establish after the tree dies; but it is reasonable to expect greater deviations in wood properties among ties containing viable decay fungi at the time of initial stacking.

Succession

Decay is not a singular event or the product of a single fungal presence, but a cumulative process representing the activities of an entire community of microorganisms and abiotic damage. The exact details of enzymatic degradation outside of sterile monoculture monitoring has been difficult to deduce. The last century of studies has shown that fungal decay is regulated by a complex succession of many microorganisms; ascomycetes, and bacteria may all modify the degradation potential of basidiomycetes (Gilbertson 1980; Käärik 1975; Oliver *et al.* 2010; Rajala *et al.* 2011; Shigo 1975).

There is a strong temporal component to polymer degradation by fungi that is complex and environmentally regulated (Boddy 2000; Hiscox *et al.* 2018; O'Leary *et al.* 2018). This temporal component may be a significant factor in growth rate and interspecies competition during colonization of wood. In other words, the levels of local decay may regulate fungal behavior, and this may be a determining mechanism of order of succession.

The decay potential of colonizing brown rot fungi may be higher at early stages vs that of the more evenly active white-rots (Wilcox 1978; Zhang &

Schilling 2017). Wood is also a selective substrate that provides different polysaccharides and polymer ratios depending on the species. The wide difference in community composition and colonization rates between two immediately adjacent hardwoods can be explained by lignin-specific enzyme activity (Anh *et al.* 2007; Blanchette 1984; Dashtban *et al.* 2010; Kang *et al.* 2010; Otjen & Blanchette 1984). Differences in lignin are also responsible for different wood preferences of white-rots and brown rots (Kang *et al.* 2010; Peng *et al.* 2018).

Some white-rot fungi in isolated lab conditions have been known to completely degrade lignin with only peripheral enzymatic reduction of polysaccharides (Blanchette 1984). But sampling of decayed wood of forests *in situ* indicates more even reduction of all polymers with the hemicellulose xylan frequently being converted most readily by white-rot fungi (Schmidt & Czeschlik 2006; Vane 2003). A possible explanation for this is that more diverse substrates cause upregulation of more diverse enzymatic genes on an as-needed basis (Presley *et al.* 2018; Schmidt & Czeschlik 2006; Wymelenberg *et al.* 2011; Zhang & Schilling 2017). The complex enzymatic resources necessary to degrade wood are generally conserved and adjusted in rapid response to local availability of many interacting resources. Over 3000 lignolytic enzymes have been described from white-rot fungi, but studies of the model organism *Phanerochaete chrysosporium* Burds. have shown that almost all enzymes are inhibited or induced through multiple interactions with the local substrate and other enzymes (Schmidt & Czeschlik 2006; Wymelenberg *et al.* 2011). Many of the enzymes must be produced in a sequence to degrade lignin or they would interfere with each other (Liese 1975). Many fungi may then be dependent on prior succession to thrive (Anh *et al.* 2007; O'Leary *et al.* 2018).

The general chain of microbial succession in wood described by Butcher started with bacteria and moulds, followed by soft rot fungi, basidiomycetes, then final dominance by Ascomycete Pleosporaceae (then called Tubercullariaceae) (Butcher 1968). This progression was observed below, at, and above ground (with

2 to 4 inches between sample points) with variations in turnover rate attributed to ground contact; possibly by insulating moisture and temperature to create a more stable environment. Within the general progression, the fungal community compositions could be very different even over a few inches. Butcher also determined that many fungi demonstrated competitive advantages in established communities as late comers, while others were most resilient only if they were the primary colonizer. Torres-Andrade used multiple wood substrates and molecular comparisons against culturing data to demonstrate that wood type and season were also determining factors of the fungal community. Torres-Andrade found similar late stage populations and determined that community diversity followed annual boom and bust cycles (Torres-Andrade *et al.* 2019). The dominant populations of communities in both studies were not the most capable of decaying wood, and there were community differences over gradients as small as 2 to 4 inches of wood.

Little beyond the general trend of community dominance has been determined except that there is often a succession prior to basidiomycete colonization of wood, and the rate of wood decay generally increases when succession occurs. The primary colonizers of otherwise sound wood have been extremely diverse, or almost identical in vastly disparate sites (Gilbertson 1980; Levy 1975). The complexity of the initial community is affected by a complex of competing and synergistic roles, and more advanced molecular techniques have not yet clearly improved our understanding of this system. Shigo's studies of succession of decay fungi revealed that prior infection and sequence of colonization were determining factors in eventual decay (Shigo 1967). Succession plays an important role as well as revealing that the fungi present in decaying wood at any point were not necessarily responsible for, or sometimes even capable of causing decay (Butcher 1968).

Within the secondary (and more ecologically productive) decay community; however, the various roles and relationships of basidiomycetes have been slightly more predictable, and community complexity generally decreases

decay potential. When fungi with similar enzymatic output interact in the same substrate, their metabolism is redirected to defensive products and behaviors, generally sacrificing rate of decay for other competitive advantages (Boddy 2000; Hiscox *et al.* 2016, 2017; Laird & Schamp 2006). There are also many combinations of higher fungi that co-exist or even provide synergistic support (Hiscox *et al.* 2017), though these interactions are not the general trend.

Increased community diversity often decreases rates of decomposition; however, succession and community turnover are related to increased resource acquisition by more competitive fungi, and subsequently increased decay in wood (O'Leary *et al.* 2018). The presence of a few fungi in long term stable positions may indicate competitive inhibition of decay, while single species dominance, either individually or in series, may indicate more severe wood decay. Previous studies suggest that diversity indices would not predict decay hazard without some knowledge of community turnover, and there may be certain key species combinations or successions that cause more severe decay than any single presence (Butcher 1968; Fukasawa *et al.* 2011; Levy 1975; Oliver *et al.* 2010).

In summary, we expected the risk of decay in rail ties to increase as ties were stored closer to the ground, but there are many intersecting factors that prevent predicting changes in decay rates. The ramifications of a change in sill height could improve worker safety by allowing easier tie manipulation, but must be compared to any decrease in durability or changes that would reduce the effectiveness of preservative treatment. The greatest changes related to sill height should result from the response of fungal communities in wood; different populations and different fungal species cause different rates of decay. We expected each wood type to support a different assortment of fungi, and for community succession to progress differently in each wood type. The initial colonization of rail ties may be very slow, or may be dominated by members in competition that cannot yet cause noticeable decay. Understanding decay from both a biological and a physical perspective requires accurate assessment of

fungal communities as they progress and a comparison of the effects of these fungi on physical properties of rail ties after air seasoning.

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CHAPTER 3. MATERIALS AND METHODS

Study Site

This study incorporated classic mycological isolation, decay testing, genomic analysis, and strength tests by 4-pt bending to assess the effect of stack sill height on quality of red oak (*Quercus rubra* L. group) and blackgum (*Nyssa sylvatica* Marsh.) ties exposed in a seasoning yard in Guthrie, Kentucky (red arrows on map; Fig. 1). The tie yard processes approximately 0.6 to 0.9 million ties per year by air seasoning followed by creosote treatment. The site is in AWPA (American Wood Protection Association) Decay Category 4 (Kirker et al. 2017), and is representative of many tie-seasoning sites in the region (See Fig. 1).

Ninety-eight freshly cut red oak ties and an equal number of blackgum ties were obtained from local suppliers. The ties were visually graded to ensure that they met the requirements for a graded tie according to AREMA (American Railway Engineering and Maintenance-of-Way Association) and RTA (Rail Tie Association) (AREMA 2015). Treatments in the form of sticker height were

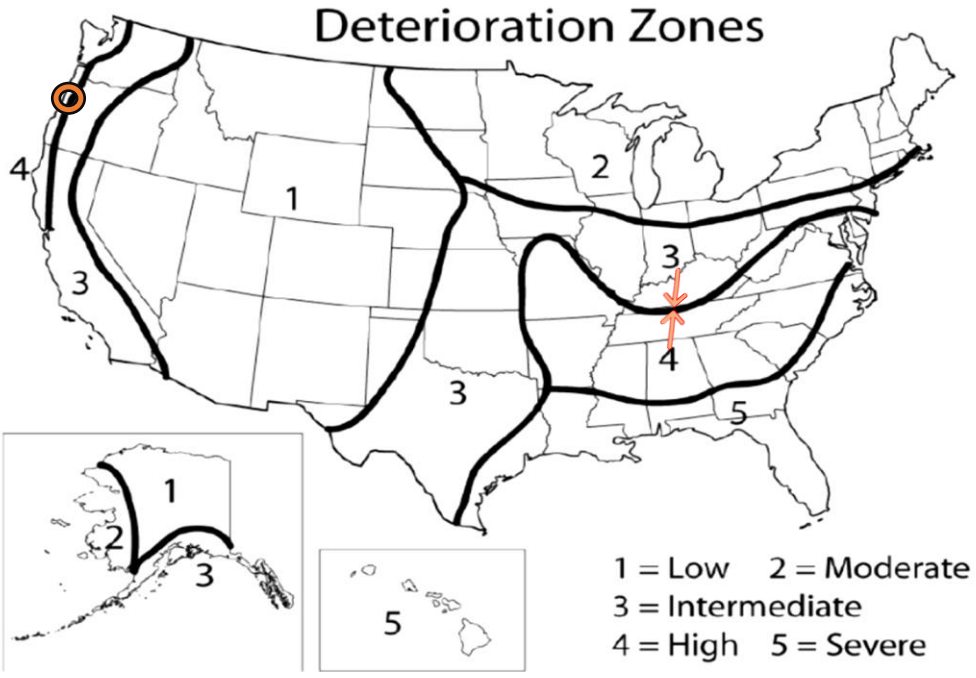

Figure 1. The current revised AWPA Decay Hazard Map (Kirker et al., 2017). Numbers indicate increasing decay hazard. Guthie KY, is indicated by red arrows. Corvallis, Oregon is circled.

applied as either 6, 8, or 12 inch sills. Cultures of basidiomycete decay fungi were isolated from cores extracted from ties at three sampling periods for each tie species. Fungal populations were also determined by high-throughput sequencing of retained portions of the same core samples for analysis and discussion at a later date. The effects of seasoning on material properties were assessed on 4-pt bending of small clear beams cut from 196 ties. Twenty of the 196 ties were used to determine a pre-seasoning baseline of flexural properties without treatment effects.

Grading, air seasoning, and moisture tracking

Freshly cut blackgum/tupelo (*Nyssa sylvatica* Marsh.) and red oak (*Quercus rubra* L. and similar species in section *Lobatae*) ties (178 mm x 229 mm x 2.59 m) (7 inch by 9 inch by 8.5 feet long) were visually graded according to RTA and AREMA guidelines (AREMA 2015; Conners 2008; Webb *et al.* 2016).

Wood species were verified by microscopic and anatomical assessments of several samples. The diagnostic characteristics of *N. sylvatica* were large scalariform plates within vessel elements (Fig. 2) and spiral interlocking grain with microscopic diffuse porous vessels and parenchyma (Hoadley 1999). Red oak had characteristically large aggregate rays and lacked tyloses in large ring-porous vessels (FPL 2010).

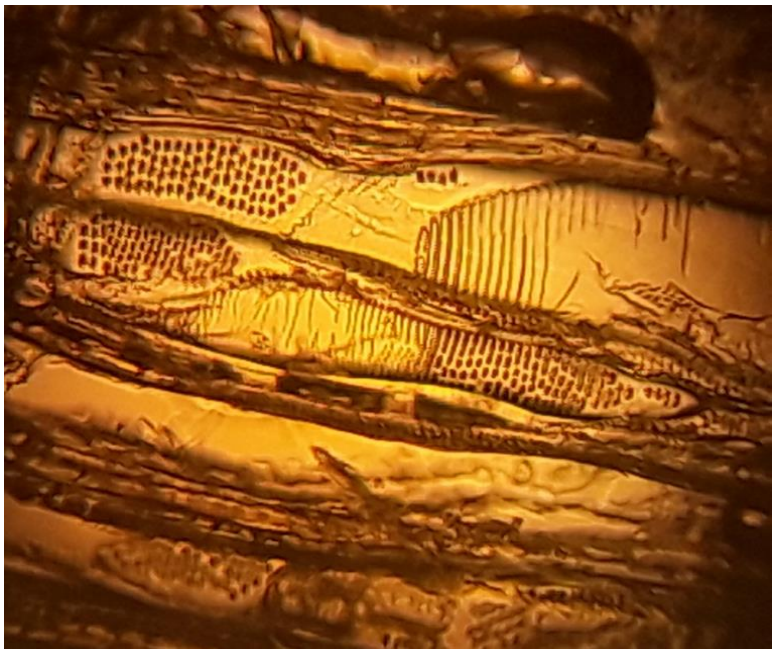

Figure 2 – The scalariform plate and alternate pitting of blackgum vessels. Magnification 100 x.

Coring

The ties were laid out, tagged for later identification, and increment cores were removed from the center of one wide face and locations 12 to 18 inches from

each end on the same face. The increment cores were placed into plastic drinking straws, labeled with the tie number and location, and stapled shut to minimize entry by other fungi. Increment core holes were plugged with tight-fitting, creosoted, wood dowels. Core sampling from blackgum ties was performed in July 2017, September 2017, and February 2018. Oak ties were sampled in July 2017, January 2018, and May 2018.

The first two samplings for each species were performed at the treatment facility. The ties were then shipped to Oregon State University where the final samples were taken.

Moisture Content

Final sampling times were based on MC (oven dry weight basis) determinations by Koppers' personnel. For MC determination, a separate collection of increment cores was removed, weighed, oven-dried to equilibrium at 104 C, and re-weighed. Mass differences were used to calculate MC to ensure that moisture levels were suitable for treatment.

Moisture content is determined by the following equation:

$$MC\% = \frac{(Wet\ Weight) - (Oven\ Dry\ Weight)}{(Oven\ Dry\ Weight)}$$

Upon arrival at OSU, a non-cultured set of core samples was taken from 16 oak ties representing all stack heights. These were used to determine MC before beam cutting and conditioning. The average MC was 29.2% (+/- 1.0%). Blackgum ties were determined to be between 25% and 30% MC upon arrival. Shipping may have affected MC.

Stacking Procedure

The remaining 88 ties of each species were placed into normal German seasoning stacks (Taylor *et al.* 2015) on creosote treated sills that left ties in bottom tiers at 6, 8, or 12 inches (15.24, 20.32, 30.48 cm) off the ground. The ties were placed in courses of 5 test ties between 2 non-test ties to avoid edge effects. Only the first 3 layers above the treated sticker received test ties (Fig. 3). Non-test ties were then placed on top of the first three rows to create a full stack containing approximately 204 ties. Two stacks of each species were set at each sticker height, creating 12 stacks in total. Six HOBO U23 Pro v2 Temperature/Relative Humidity Data Loggers (Onset Computer Corporation, Bourne, MA) were attached to the underside of ties in the first and third course of one stack at each sticker height. Data were recovered during each core sampling.

Figure 3 - Air seasoning rail ties in German Stack configuration. Sampled courses (tiers) are labeled 1, 2, 3; and conventional 12" sill height is indicated. Reduced sill heights were arranged by using a single treated rail tie footing, where two are shown here.

The data loggers were moved to new locations in the stacks at each sampling time to assess temperature and relative humidity at as many locations as possible.

The gum stacks were disassembled and sampled after 3 months of air-seasoning using the same increment core procedures described above, while the oak ties were not sampled until 6 months of seasoning in recognition that this species requires a longer seasoning period (AREMA 2015; AWPA 2017; Conners 2008). Ties were returned to the same position in a stack after sampling. The gum ties were removed from the test after 6 months when they had reached a MC suitable for treatment. These ties were transported to Oregon where a third set of increment cores were removed for fungal sampling. The ties were then cut to produce small clear beams for strength testing. The red oak ties were sampled after 6 months and then shipped to Oregon after 11 months of seasoning.

Fungal Culturing

Each increment core was removed from the straw and surface-flamed to minimize the presence of contaminating fungi. Several small pieces were removed and frozen for extraction of total fungal DNA for high-throughput ("Next-Gen") sequencing via the Illumina MiSeq platform (Illumina Inc. San Diego, CA). The remainder of each core was placed on benomyl [methyl1-(butylcarbamoyl)-2-benzimidazolecarbamate] amended 1% malt-extract agar for selective isolation of decay fungi. Benomyl retards the growth of fast-growing, non-basidiomycetes (Carey & Hull 1989; Richter & Glaeser 2015). This is useful as nearly all of the most potent wood decay fungi are basidiomycetes (Duncan & Lombard 1965; Richter & Glaeser 2015; Schwarze *et al.* 2000; Zabel & Morrell 1992). The plated cores were examined for evidence of fungal growth over a 1-month period and basidiomycete fungi growing from the wood were sub-cultured for later identification. Potential decay fungi were segregated by their growth characteristics as well as the presence of hyphal clamp connections that are generally indicative of basidiomycetes (Wang & Zabel 1990; Zabel & Morrell 1992). Cultures could generally be sorted by textural appearance, color, and patterns in expansion of mycelium. Pure sub-culture isolates were subjected to

conservative DNA identification where multiple isolates of each morphogroup were sequenced. Fungal DNA was extracted from pure-culture vegetative mycelium using the cetyltrimethylammonium bromide (CTAB) protocol (Gardes & Bruns 1993) with minor modification. Extracted DNA was amplified by polymerase chain reaction methods described below prior to submission for sequencing.

PCR of Isolates

Polymerase chain reaction (PCR) was performed in a Bio-Rad PTC-100 thermal cycler (Life Science Research, Hercules, CA, USA). The internal transcribed spacer (ITS) region of the fungal rRNA gene was amplified using the fungal specific forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes & Bruns 1993) and the general eukaryotic reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). PCR amplification was carried out in 20 µl reactions with the following reagents: template DNA (1.0 µl), dNTP's (2.5 mM, 2.4 µl), ITS1F primer (50 µM, 0.2 µl), ITS4 primer (50 µM, 0.2 µl), 5x Promega Hot Start GoTaq® colorless flexi buffer (4 µl), $MgCl_2$ (25 mM, 2.4 µl), dH_2O (7.38 µl), BSA (1mg/ml, 3.2 µl), and Hot Start GoTaq® (5 units/µl, 0.06 µl). PCR conditions involved initial denaturation at 95 °C for 2 min, 30 PCR cycles of denaturation at 94 °C for 30 seconds; annealing at 50 °C for 60 seconds; extension at 72 °C for 90 seconds, and a final extension at 72 °C for 10 minutes. Positive and negative controls were used in every set of PCR samples to check target band amplification and purity of the reagent mixture. Amplicons were viewed on a 1.5% agarose gel stained with the Nucleic Acid Staining Solution RedSafe™ to identify a single, target fungal band of approximately 500 to 700 basepairs. Contamination by ascomycetes was sometimes circumvented by use of ITS4-B primers (CAGGAGACTTGTACACGGTCCAG) to selectively amplify basidiomycetes from ascomycete-contaminated culture isolations (Kirker *et al.* 2014; Kiser 2009; Prewitt *et al.* 2008).

Amplified DNA from each unique morphogroup was cleaned with the Exo-Sap™ PCR product cleanup kit (Affymetrix, Santa Clara, CA) and submitted to

the Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR) for Sanger sequencing on an Applied Biosystems 3730 capillary sequence machine (Life Technologies, Grand Island, NY). Fungal ITS sequences were manually compared, edited, and fully processed using Geneious Pro v11.0.5 (Biomatters, Auckland, New Zealand). Sequence alignment at 97% similarity was performed using MAFFT (Katoh 2002) to ensure molecular species were differentiated. Sequences from morphogroups that were ≥ 97% similar were considered to be the same molecular type and were combined as the same fungal species. The GenBank (Benson *et al.* 2012) MegaBlast search feature for highly similar sequences was used to assign taxonomies to fungal species. Final taxonomic identities were compared to the curated UNITE database (Abarenkov *et al.* 2010). Taxonomies were assigned based on overall BLAST consensus if the majority of the fungal ITS1 and ITS2 regions aligned with a GenBank sequence as well as the following criteria: (1) 97-100% minimum identity for species-level; (2) 95-96% identity for genus-level; and (3) 90-94% identity for family-level.

Sequence results were compared to known morphological characteristics of reported species for some isolates to minimize the risk of gross errors or mislabeling. Several identification manuals for microscopic features and growth patterns of decay fungi were used together to compare changing nomenclature, and to compare varying morphologies that were not historically differentiable prior to molecular techniques. Early experience differentiating basidiomycetes from other fungi in culture was improved by assessing hyaline to white mycelium, and clamp connections (Wang & Zabel 1990). The online database of mycological nomenclature *MycoBank.org* was used to assign current names to identified isolates (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands & Botanische Staatssammlung München, München, Germany).

Soil Block Decay Test

Isolates were further evaluated for their ability to decay wood by using soil block decay tests following procedures described in AWPA Standard E10 (AWPA, 2017). A representative pure culture isolate of each genus or species was selected

at the finest taxonomic level available. Multiple isolates were selected for comparison where species complexes were found, or where single species presented very different morphologies. For example, *Schizophyllum commune* (Fr.) and *Radulodon sp* were very abundant so some effort was made to find varying morphologies to compare. Two distinct forms of *Punctularia strigosozonata* (Schwein.) with and without conidia that may also have had different degradation capabilities were evaluated.

In total, 38 isolates were selected for decay tests. In addition to fungi isolated from ties, *Trametes versicolor* (L.:Fr.) Pilat (Isolate MAD 697), *Rhodonia placenta* (Fr.) Niemelä, (Isolate MAD 698), and *Gloeophyllum trabeum* (Pers.) Murrill (Isolate MAD 617) were included as these fungi are widely used in laboratory tests and their decay capabilities have been well characterized (AWPA 2016; De Groot *et al.* 1998). Decay chambers with no fungi were also included as a control. Of the 47 unique basidiomycete species isolated, 9 were lost in storage from a failed refrigeration unit, or by contamination. The 38 isolates from the ties represented 34 species in 26 genera. (Table 1)

A soil block decay test was adapted from AWPA Standard E10 (AWPA 2016) and from similar methods outlined by Worrall *et. al.* (1997) to test relative decay potential of several fungi. The fungi were evaluated for their ability to degrade 19 mm cubes (blocks) of red oak (*Quercus sp.)*, southern yellow pine (*Pinus sp.)*, and bigleaf maple (*Acer macrophyllum)*. Each isolate was evaluated on 8 blocks per wood species (1104 blocks in total). Bottles were inoculated with clean pure isolates recovered from tie cores by several iterations of sterile tissue transfers.

The chambers (bottles) were incubated for 16 weeks at 22 °C (72 °F). Mass loss was determined by comparison of final oven-dry weight to initial dry weight. The use of hardwood and softwood test blocks was intended to differentiate varying modes of decay, as softwoods are generally less susceptible to white-rot, although this distinction can be overcome when wood is in soil contact (De Groot

et al. 1998). The comparison of oak and maple was intended to assess fungal decay capabilities against woods with slightly different durability.

Screw top bottles (500 ml) were half-filled with 250 -300 ml of soil and provided with a feeder strip (28 mm x 34 mm x 3 mm thick) of western hemlock (*Tsuga heterophylla*) or alder (*Alnus rubra* Bong.), with hardwoods and softwoods assigned respectively to receive hardwood or softwood test blocks (Fig. 4). The soil mix used was a combination of even parts blended compost and Willamette River sandy loam. Sandy loam was originally 40% sand, 40% silt, and 20% clay, and the compost blend was a combination of several types of manure and composted fir bark comparable to OMRI (Organic Materials Review Institute) listed manure/compost mixes. All components were believed to be free of pesticides or synthetic additives. The final mixture was then sieved through #6 (3.36 mm opening) mesh. Final acidity was approximately pH 6.4.

Bottles were then brought to water holding capacity with 25 ml distilled water, and sterilized for 75 minutes at 121 °C (250 °F) under 103-130 kPa (15 -20 psi). After cooling in HEPA (high-efficiency particulate air) filtered laminar air flow, bottles were inoculated with two 5 mm (0.2 in) diameter agar plugs of each test fungus placed at the soil-wood interface of feeder strips, capped, labeled, and incubated at 22 °C (72 °F) for 3 weeks. Bottles were regularly checked for contamination or failure of the fungi to colonize.

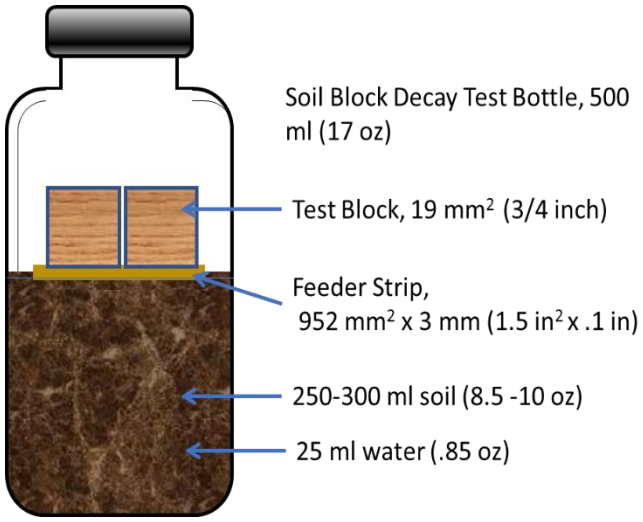

Figure 4 - Example of a soil bottle decay test. Two test blocks are applied per bottle after inoculation with test fungi and incubating for 3 weeks. Blocks are removed and weighed after 16 weeks.

Test blocks were oven dried for 72 hours at 50 °C (122 °F), weighed (nearest 0.001 g), and wetted by submersion in de-ionized water under mild

vacuum for 30 minutes and being allowed to sit submerged another 60 minutes. Packets of blocks were then autoclaved for 75 minutes at 115 °C (239 °F) under 103-130 kPa (15 -20 psi).

Bottles were incubated for three weeks to allow colonization of the feeder strip, then all bottles received two wood test blocks. Bottles were capped, and incubated at 28 °C (72 °F) for 16 weeks. At the end of the incubation period, the blocks were cleaned of outside hyphal mass, and weighed (wet mass). All blocks were then placed in 50 °C (122 °F) oven for a minimum of 72 hours, and re-weighed (oven dry mass post-inoculation).

Bottles with obvious contamination were noted, as were any bottles that appeared to lack initial colonization or had become desiccated. Extremely low weight losses were a potential cause for eliminating outliers.

Table 1 - Isolates evaluated for their decay potential in soil block tests.

ID#	OTU	ID#	OTU
K2	*Chondrostereum purpureum*	K1	*Bjerkandera adusta*
K3	*Coprinellus radians*	K6	*Ganoderma sessile*
K4	*Cylindrobasidium sp.*	K7	*Gloeocystidiellum* sp.
K5	*Filobasidium sp.*	K8	*Gloeostereum incarnatum*
K12	*Phanerochaete sp.*	K9	*Hypochnicium sp.*
K13	*Phlebia fuscoatra*	K11	*Lenzites betulinus*
K14	*Phlebiopsis flavidoalba*	K16	*Pholiota (adiposa?)*
K15	*Pholiota squarrosa*	K21	*Radulodon casearius*
K17	*Pholiota (limonella/adiposa)*	K25	*Spongipellis delectans*
K18	*Punctularia strigosozonata* (with arthroconidia)	K27	*Stereum gauspapatum*
K19	*Punctularia strigosozonata*	K28	*Stereum complicatum*
K20	*Radulodon americanus*	K30	*Tinctoporellus epimiltinus*
K22	*Schizophyllum commune*	K41	*Epicoccum sorghinum*
K23	*Sistotrema brinkmannii*	K43	*Antrodia minuta*
K24	*Spongipellis pachyodon*	K44	*Dichostereum*
K26	*Stereum hirsutum*	K45	*Hydnopolyporus*
K29	*Stereum spp.*	K46	*Peniophora*
K31	*Trametes gibbosa*	K47	*Phlebia subserialis*
K32	*Trametes versicolor* (from blackgum)	K48	*Trametes versicolor* (from oak)
K33	*Xylobolus frustrulatus*	K37b	*Trametes versicolor* (Lab standard: Madison 697)
K35	*Antrodia oleracea*	K38b	*Postia placenta* (Lab standard: MAD 698)
K36	*Laetiporus cincinnatus*	K39b	*Gloeophylum trabium* (Lab standard: MAD 617)
K40b	Control (No Fungi)		

Cutting Small Clear Beams

Ties were tested for strength by 4 point bending of small clear beams cut from each tie according to ASTM D143-14 (ASTM International 2014).

Beams were cut to 25.5 mm x 25.5 mm x at least 41 mm in length so that they would be as close to 25 by 25 mm after drying. Beams were assessed for grain and defects according to ASTM D143-14. Signs of decay deemed to have been present such as heartrot in the living tree, as well as insect galleries and open decay pockets were rejected from testing, but beams with visual signs of incipient decay such as mycelial growth or melanin deposits ("zone lines") characteristic of white-rot decay, as well as any fungal pigmenting (*spalting*) from other fungi were included in the tests. Visual indicators of fungal growth were retained in testing to capture the potential variations in strength caused by decay fungi. There were no obvious decay pockets or decayed regions noted that could not be attributed to decay present at the time the tree was felled. The most common causes to reject small beams were knots or grain defects.

Two different sampling schemes were implemented for blackgum and oak ties. Blackgum ties were sampled with the intention of correlating intra-tie strength variations to core-sample positions. Deviations in strength could potentially be related to distance from the presence of a decay fungus.

Blackgum ties were initially sampled at a rate of 12 beams per tie. Four planks were cut across the narrow end of each blackgum tie (32 mm thick by 178 mm wide/1.28 in by 7.12 in). The remainder of each tie was retained in case additional beams were required. The end-most 280 mm (11 in.) of each plank as well as the top incised plank was discarded. The remaining plank portions were cut into 406 mm (16 in.) long segments labeled A, B, C, D, or E with segment-A matched to core-A. All incised outer portions were discarded, then 12 beams were cut from the 15 available segments. All un-used portions were labeled and retained for potential re-sampling. The beams were cut to 25 mm (1.0 in) squares with length of 406 mm (16.24 in). This resulted in 1056 small clear beams, labeled according to depth and position within ties. (Fig. 5)

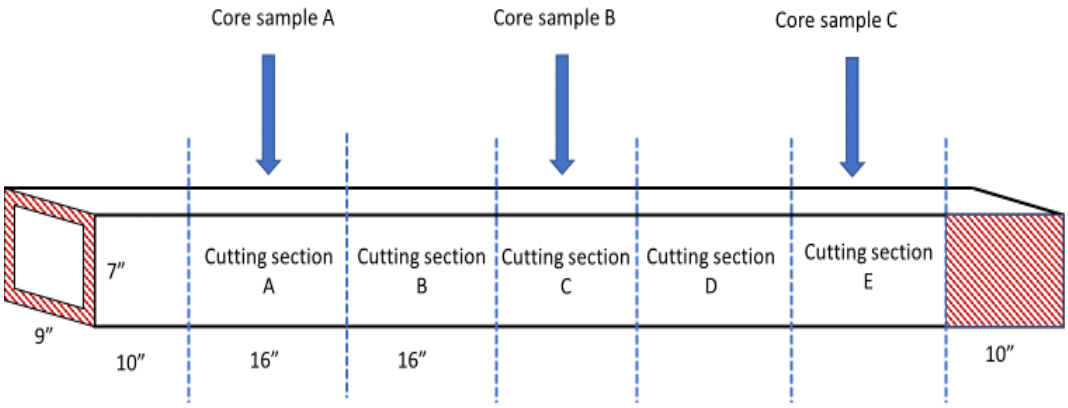

Figure 5 - Cutting and sampling locations from a rail tie. Core samples were taken from the wide face. All outer surfaces and endmost 10 inches were excluded from beam cutting. Blackgum beam samples were taken from all sections A-E, Oak beams were taken only from section-A

While the blackgum beams provided a wealth of test data, there were no correlations of fungal community or flexural properties related to intra-tie positioning. Since the isolation frequencies from the oak ties were much lower than those from the blackgum ties, and because initial tests suggested that the wood properties were less variable, the beam cutting pattern for oak ties was reduced.

Beams were cut from all red oak ties, but for simplicity, only the comparisons of top and bottom stack layers and heights are presented for analysis. Beams of red oak ties were cut from tie ends closest to core-C positions (or south-westerly facing ends of stacks). All beams were cut from the area 280 mm to 775 mm (11.2 to 31.0 in) from the end, and from a depth of 50 mm to 130 mm (2.0 to 5.2 in) on the narrow 178 mm (7 inch) face. The endmost 280 mm 11.2 in) and all incised outer portions were avoided to minimize edge effects; this section had the greatest checking, weathering, and solar exposure. As a rule, ties were cut with pith on center. This resulted in essentially all beams being cut from heartwood, but avoiding the most juvenile wood at the pith in as much as the orientation of heartwood allowed. Ties sampled at the beginning of the air-drying period to establish baseline measurements were an exception to this sampling

method. The first ten oak ties were processed and sampled in the same manner as blackgum.

Conditioning Small Clear Beams for Testing

Beams were conditioned to constant weight at 65% MC and 20 °C (65 °F) after cutting as described by ASTM 4933-16 ((ASTM International 2016). Blackgum beams reached 12% MC in an ASTM standard conditioning room and oak beams reached 15% MC. Equilibrium was determined by tracking mass on a weekly basis until beams reached stable a weight. MC was verified by oven drying random samples at 50 °C (122 F) for 72 hours for comparison to oven-dry mass after testing.

Bending Test

The effects of air-seasoning conditions on wood properties were assessed using 4-point loading. Ten ties of each species were shipped to OSU without seasoning. Thirty beams were cut from each tie. The initial tests on non-air-seasoned ties were used to establish intra-tie variability and the degree of homogeneity between the original increment core sampling positions. Beams cut from blackgum ties tended to deform and twist as they seasoned and 71 blackgum beams were ultimately rejected for improper sizing or shrinking.

At the end of the seasoning period for each species, the remaining 88 ties of each species were shipped to OSU where additional beams were cut from ties of each species that had been air-seasoned to determine the effects of seasoning conditions on timber properties.

Strength and flexibility, expressed as Modulus of Rupture (MOR) and Modulus of Elasticity (MOE), were determined according to ASTM Standard D143-14 (ASTM, 2017). Because the effects from decay may not be homogenous throughout wood members, we chose to apply a constant bending moment, free of shear, in the widest sample span possible; and altered the test for use with 4-point bending. We selected the “secondary test method” of ASTM D143-14 to test beams with a 14:1 span to depth ratio, and overall size of 25 by 25 by 400 mm

(1.0 by 1.0 by 16.0 in) long to maximize the chance of having all clear wood in each beam.

The beams were conditioned to stable weight at 65% relative humidity and 23 °C (73.4 F) before being tested on an Instron 5982 Universal Testing Machine (Instron, Illinois Tool Works, Norwood, MA,) with downward force applied at two points dividing the entire span into 11.85 cm (4.66 inches) thirds, and 25 mm (1.0 in) of overhang beyond each side of the testing apparatus. The suggested loading rate from ASTM D143-14 of 1.3 mm (0.05 in)/minute (ASTM International 2014) was adjusted in accordance to the findings of Forest Products Laboratory so that beams achieved ultimate stress in around 5 minutes (Gerhards 1977). All blackgum beams were tested at a loading rate of 4 mm (0.16 in)/minute, and all oak beams were tested at 3 mm (0.12 in)/minute. These test speeds are similar to other loading rates used for small wood beams (Babiak *et al.* 2018; Davis *et al.* 2012). The suggested rate of failure within one minute during 4-point testing of dimensional lumber by ASTM D4761-18 was unsuitable for the small beams used (ASTM International 2018). All beam specimens were loaded with the pith side as the

Figure 6 - A small clear beam under 4-point loading with Instron Universal Testing Machine.

bearing surface (i.e. the sample side from closest to the core of a tie should face the load head).

Load and deflection were continuously recorded up to the point of beam failure, and these data were used to calculate MOE (modulus of elasticity) and MOR (modulus of rupture) in pounds per square inch. Results were compared with previous reports for each species and tie grading standards, (ASTM International 2017; FPL 2010; Webb *et al.* 2016) as well as baseline tests

MOR for 4-point bending was calculated as:	MOE (E) was calculated from the linear most portion of the stress/strain curve by:
$\delta = \frac{FL}{bd^2}$	$[\frac{23KL^3}{108bd^3}]$ or equivalently [E=σ/ε]
Where:	Where:
F is the load, or force applied	**K** is the slope of the proportional limit
L is the span (14 inches)	**σ** is the stress (force per area)
b is the width (1 inch)	**ε** is the strain (deflection) determined by $\varepsilon = \frac{\Delta l}{l}$ (change in length per original length from load head)
d is the depth (1 inch)	

Table 2 – Equations for MOR and MOE in 4 point bending

conducted on ties of the same batch that were not exposed to air-seasoning conditions. MOE figures were calculated as flexural modulus and may be presented as modulus of elasticity, Young's modulus, or tensile modulus of elasticity with the caveat assumption that tensile and flexural moduli are equivalent. In all cases, MOE was determined using the most linear portion of the load deflection curve.

A total of 676 small beams cut from 88 air seasoned blackgum ties were tested to failure. Averages for MOR and MOE of beams were compiled per tie.

A total of 300 oak beams cut from 60 air seasoned ties were sampled and tested to failure. Five beams were tested from each tie. Ties from the first and third tiers were tested, avoiding 28 ties from the second tier of all stacks. This facilitated direct comparison of strength measures in the bottom tiers across all treatments as well effects from height within each stack. Additional beams from all oak ties were conditioned and retained in case results warranted further testing.

Analysis

The data were subjected to community analysis by ANOVA and Tukey's test of means for modulus of rupture (α=0.05). We also compared treatments for abundance of isolates by MRPP (multi-response permutation procedure), ISA (indicator species analysis), and outlier analysis using PC-ORD software version 7 (Wild Blueberry Media, Corvallis, OR). Pairwise comparisons were not corrected for multiple comparisons and we expected some type-I errors given the number of comparisons made (Mielke *et al.* 1976; Neyman *et al.* 1933; Parchami *et al.* 2008).

Flexural properties were ultimately calculated as an average of all beams from each tie. The matter of weighted averages per tie was resolved by considering all ties as complete and equal sample units. Relative abundance and isolate counts of fungi were similarly combined from cores in each tie.

Analysis of Variance

Empty sample units (i.e. ties with no fungal isolates) precluded the use of Bray-Curtis to measure the dissimilarities of fungal communities, so a method based on Pythagorean distances between observations was more applicable. Permutational multivariate analysis of variance (PerMANOVA) and multi-response permutation procedure (MRPP) both allowed the simple application of Euclidean distance among species and sample units, while avoiding any

assumptions of normality (Biondini et al. 1991; McCune et al. 2002). MRPP and PerMANOVA yielded similar results, and MRPP was ultimately selected as more applicable with potentially unbalanced group sizes.

MRPP

MRPP (multi response permutation procedure) is a nonparametric technique that allows comparison of groups by distance (dissimilarity) in multiple variables at the same time (Biondini *et al.* 1991). MRPP provides a p-value to evaluate differences, as well as an A-value of chance corrected within group agreement, i.e. the size of effect between two or more groups. A-values range from 1 (items are identical within groups) to negative values (groups are less different than expected by chance) with A = 0 indicating groups are as heterogenous as expected by chance (McCune *et al.* 2002).

Indicator Species Analyses

Indicator species analysis (ISA) compares species abundance and frequency against an assigned grouping variable for sample units. ISA provides a single value combining both the frequency and fidelity of each species to each group, followed by a Monte Carlo test of significance (McCune *et al.* 2002). Whereas MRPP determines if a difference exists between groups, ISA identifies what in the community data is causing the difference.

The comparisons made are for all OTUs against assigned groups. Groups are naturally present in sample units (e.g. tier, stack, height) or based on synthetic categories. We also assigned groups based on standard deviations of MOR and MOE from the sample average; a species association would indicate a fungus was related to a certain range of MOR or MOE. Because grouping by synthetic variables is entirely subjective, we checked for trends of indicator values even if they were not calculated to be significant for a single group; that is, to see if a species could be associated with a range of groups. We did not attempt to reclassify OTUs into community groups based on cluster analysis.

Because ISA requires distinct groups, we assigned categorical grouping variables to physical properties. Our initial grouping applied very small groups to minimize distorting data, and larger groups were used if there was evidence for a trend. ISA with 4999 randomizations was used to identify fungal OTUs related to modulus of rupture (MOR) in 27 and 11 groups for blackgum and 11 and 3 groups for oak. ISA with 4999 randomizations based on modulus of elasticity (MOE) was made on 30 and 11 groups for blackgum, and 5 and 3 groups for oak .

Groups based on strength scores were assigned by determining how many standard deviations (rounded to the nearest 10th decimal place) from the sample average each tie was in its MOR, then multiplying by 10 to provide integer groups. Any tie left in a singular category received a score rounded to the next nearest grouping to ensure group means could be calculated that would include all ties. The same procedure was done for MOE values.

Indicator species analysis of the initial groups indicated that fewer larger groups would capture community effects better so scores were reduced by binning values into groups labeled -5 to 5, with 0 centered on the sample average for blackgum. Red oak ties presented much lower variance than blackgum ties, so there were fewer groups of MOR and MOE. For red oak ties, we assigned 3 groups of “below,” “at”, or “above” average based on divisions at 1.5 standard deviations.

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CHAPTER 4. RESULTS

Environmental Monitoring

There was a measurement failure in the sensors for the first three months of exposure, resulting in measurements for only one week after stacking. The data from the next nine months of sampling were more complete.

Sensors were placed in blackgum stacks at 6”, 8”, 19”, and 27” above the ground for the second monitoring period (September 2017 to January 2018). Two additional sensors were placed in oak stacks at 12” and 15” above the ground. Daily average temperature and relative humidity during this period were stable between sensors. Average standard deviations were less than 0.5 °F (0.3 °C) and

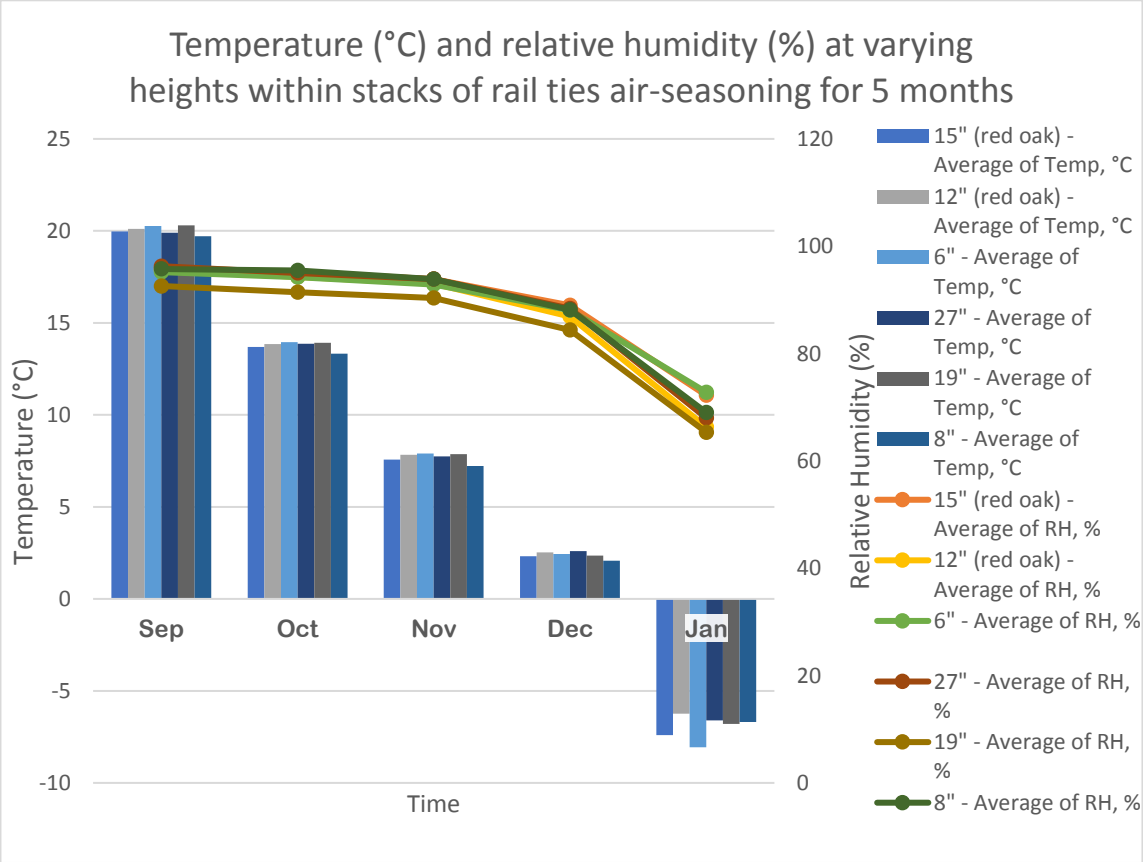

Figure 7 - Relative humidity and temperature sensor data for heights among bottom tiers of air-drying rail ties. Measurements were taken every 30 min for 116 days. January data represents only a 3-day period, but demonstrates the maximum variation captured.

there was less than 1.5% difference in relative humidity (RH) between the tiers of blackgum stacks.

The greatest difference in three day averages was detected in January between ties in bottom tiers; 6” heights in blackgum and 12” heights in oak. The RH differed by 6.4% and temperatures differed by 3.0 °F (2.2 °C). Variations in temperature and RH were not explained by height, suggesting that fungal colonization would not be influenced by the relatively small RH differences related to stack distance from the ground.

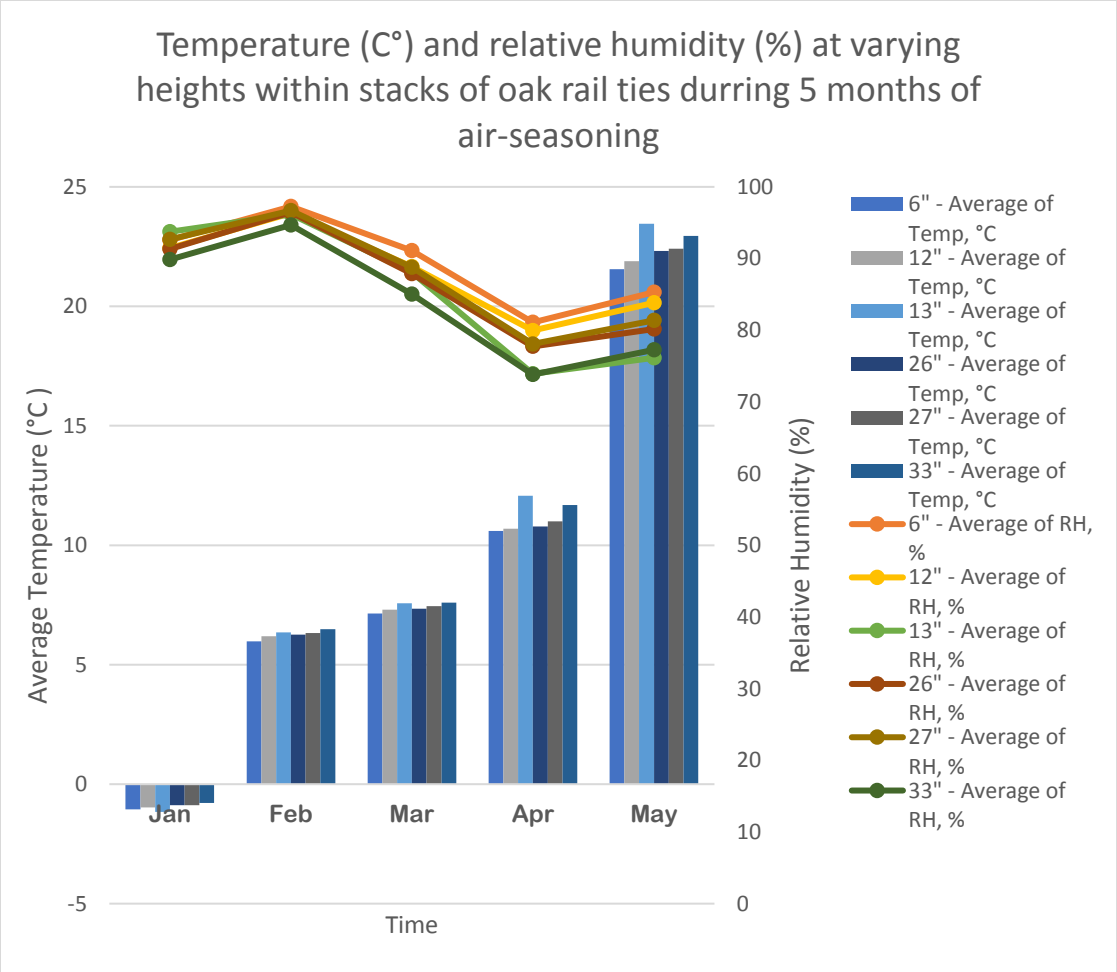

Figure 8 - Relative humidity and temperatures at different distances above the ground in stacks of red oak ties

From January to May of 2018 sensors were repositioned in stacks of red oak at heights of 6”, 12”, 13” 26”, 27” and 33” to capture the widest variation in stack conditions.

The average differences between sensors were similar to prior measurements. Variations in monthly average temperatures reached 3.8 °F (2 °C), while there was a difference of 6% RH between bottom heights and 33” in April 2018 (maximum difference among sensors). Sensors at 27” above ground recorded conditions similar to those found 6” and 12” above ground, and sensors at 13” above ground more closely matched readings from 33”, with the highest average temperature recorded at 13”. As expected, temperatures increased in the stacks during the spring. Relative humidity in stacks was over 90% during the cooler months, then declined to around 70% as temperatures increased. Elevated RH’s would be a concern for fungal colonization; however, high RH was associated with cooler temperatures. The results indicate that the stacks presented a stable moisture and temperature environment that should be conducive to fungal colonization. There appeared to be no differences in temperature or RH with the use of different sill heights. Anecdotally, we experienced much greater variability in temperature and humidity from different aspects of a tie stack than from any variation in the bottom tie conditions. It is important to note that the winter period of the study was exceptionally cold for the region, with temperatures dropping to 12 °F (-11 °C) from an average of 23 °F (-5 °C) (AccuWeather.com 2019). This may have impacted the rates of colonization or community turnover.

Moisture Content of Ties on Arrival to OSU

Moisture contents of red oak ties on arrival in Oregon were verified by removing increment cores 16 inches (400 mm) inwards from the ends of 16 ties that were originally 6, 8, 12, 20, or 26 inches above the ground (15.24, 20.32, 30.48, 50.8, or 66.04 cm). The cores were weighed, oven-dried and reweighed to determine MC. The oak ties were at 29.2% MC (+/- 1.1%) upon arrival.

Measurements were not made on blackgum ties immediately after arrival to Corvallis, Oregon, but beams cut from the ties had MC's ranging from 15% to 30% before conditioning.

Fungal Isolations by Stack Position

Community Changes with Height for Blackgum

Outlier analysis of fungal abundance and community composition ("species space") indicated that blackgum ties numbered 1519 and 1550 were potential outliers. They had 12 and 10 isolates, respectively, recorded from all sampling. These were the highest isolate counts of all ties. Beams from these ties had MOE and MOR values that were close to the averages from all ties sampled so there was no support for removing them from the results.

Isolates for Blackgum

A total of 319 isolates of potential decay fungi were recovered from 98 blackgum ties including 88 ties exposed to air-seasoning over 6 months (Fig. 9). The isolates included up to 37 species from 25 genera including 2 incidences of an unknown basidiomycete. Some isolates could only be identified to genus so isolates were analyzed in 25 groups. Ascomycetes and non-saprobes were not considered in culture analysis. The most common basidiomycete isolates from all sampling times were *Radulodon* (16.9%) followed by *Spongipellis* (11.9%), *Stereum* (9.7%), and *Bjerkandera* (9.4%). Three isolates were recovered from non-air seasoned ties; one each

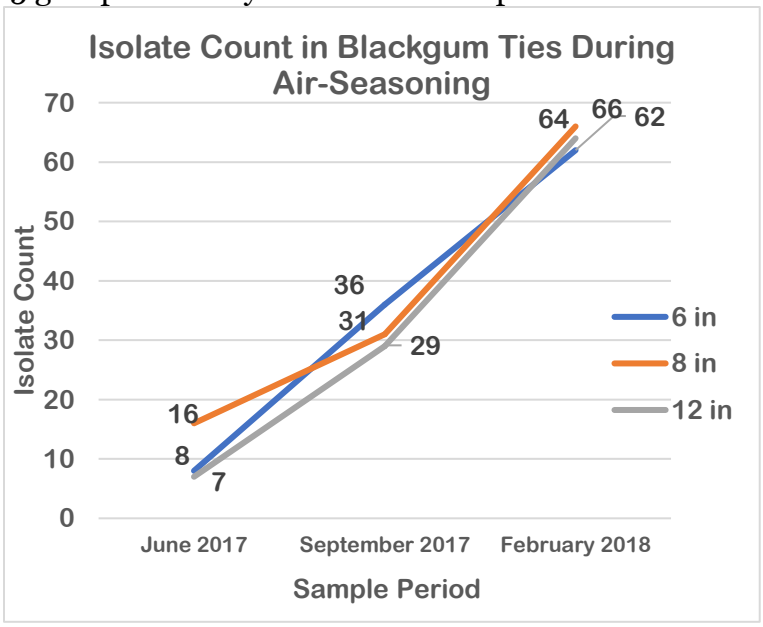

Figure 9 - Numbers of fungi isolated from black gum ties air seasoned for 6 months at different distances above the ground.

of *Lenzites, Radulodon*, *Spongipellus*. Thirty-one decay fungi were isolated from the ties upon delivery to the plant, increasing to 96 isolates recovered at 3 months, and 192 isolates recovered after 6 months of air-seasoning. Fungal isolates were randomly, but unevenly distributed across sill heights; stacks on 8" sills yielded twice as many isolates as stacks on either 6" or 12" sills. Isolation frequencies became more evenly distributed over time, and isolation frequencies were almost identical between sill heights after 6 months of seasoning.

The initial decay fungi community in blackgum ties consisted of 8 species, and was dominated by *Radulodon americanus* (32%), and *Bjerkandera adusta* (19.4%), with some *Stereum complicatum* (9.7%) and *Spongipellis delectans*

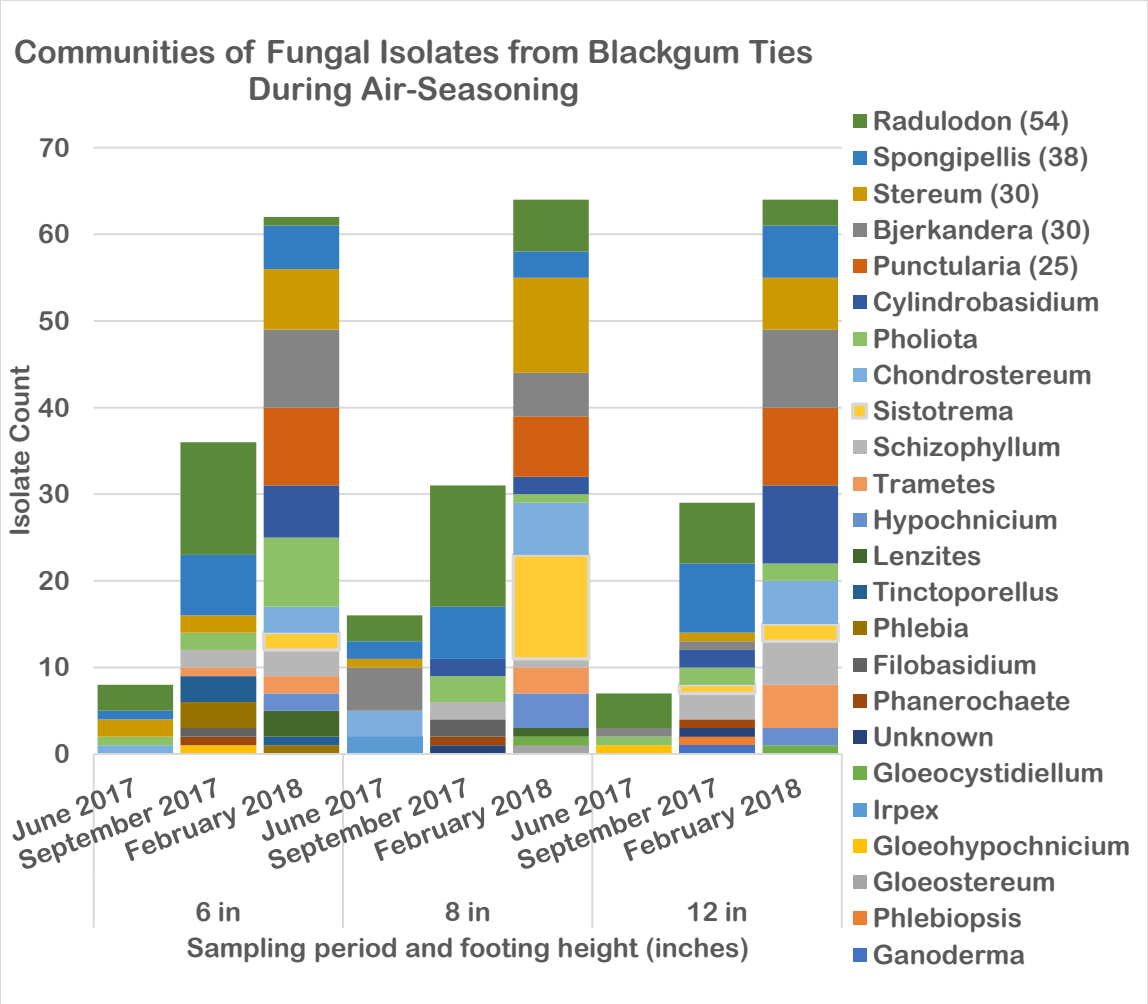

Figure 10 - Isolation frequency of basidiomycetes from black gum ties seasoned in stacks at different sill heights over a 6- month period.

(9.7%). Initial sampling produced the only incidence of *Irpex lacteus* (6.5%) (Fig. 10).

Sampling after 3 months of air seasoning yielded 17 species and was dominated by *R. americanus* (35.4%), *S. delectans* (21.9%) and *Schizophyllum commune* (7.3%). Five culture types were unique to 3-month sampling; *Filobasidium*, *Ganoderma*, *Phanerochaete*, *Phlebiopsis*, and an unknown basidiomycete. *Filobasidium* is the sexual teleomorph of pathogenic *Cryptococcus* fungi and is commonly detected in decayed wood (Jiménez *et al.* 1991; Kwon-Chung 1976; Lazéra *et al.* 1996). It is often overlooked as a decay fungus; however, *Filobasidium* has demonstrated activity against hemicellulose (Jiménez *et al.* 1991). It may also affect decay hazards by attacking other fungi, as the entire related family are potential mycophages (Mueller *et al.* 2004, 360).

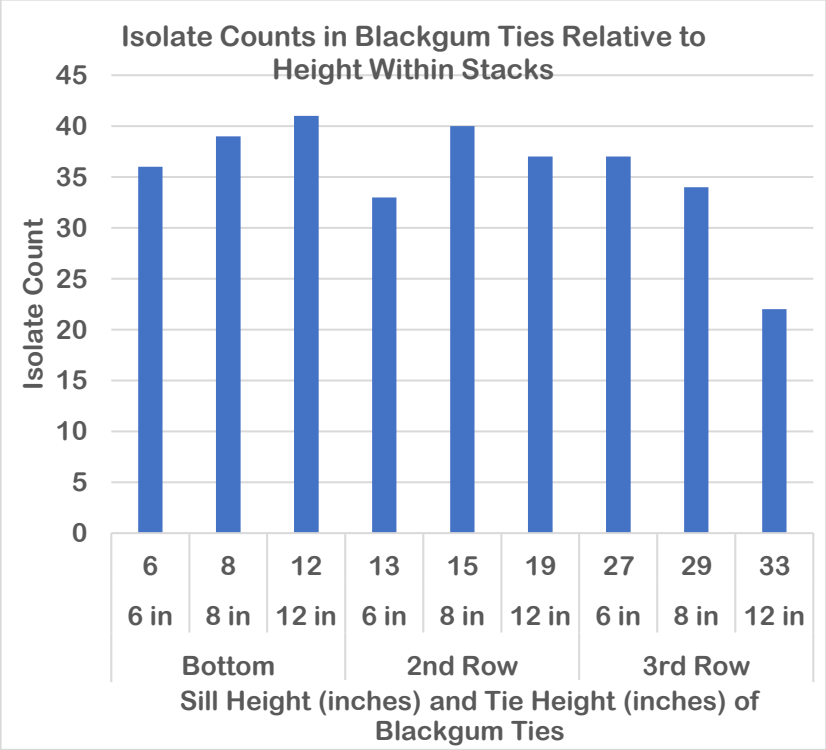

Figure 11 - Isolate frequencies in blackgum ties seasoned at different distances above the ground on 6, 8 or 12 inch sills.

Final sampling at 6 months yielded 18 species dominated by *Punctularia strigosozonata* (13%), *Stereum spp.* (13%), *B. adusta* (12%), *Cylindrobasidium sp.* (8.9%), and *Sistotrema brinkmannii* (8.3%). There were 6 culture types

unique to final sampling; *Punctularia*, *Lenzites*, *Hypochnicium*, *Gleostereum*, *Gloeocystidiellum*, and *Coprinellus*.

Isolate counts increased steadily and evenly at most heights across sampling times. There was an exception in ties seasoned 33" above ground. These ties were most distant from the ground, and had the fewest fungi in the 2nd and 3rd sample periods. Bottom tiers of the same stacks (stacks with 12" sills) yielded the most decay fungi in the final sample. Ties grouped by tiers or layers showed a possible trend of decreasing isolate counts with height, regardless of sill height (See Fig. 11) . However, pairwise comparisons from MRPP analysis between the fungal community compositions of height groups ("species space") showed that there were no significant differences in fungal communities between any bottom tiers, or between bottom tiers and uppermost 33" heights. .

Comparison of Isolates in Bottom Tiers for Blackgum

MRPP analysis of 25 OTUs combined from all sampling periods showed that there were no statistical differences between the bottom tier groups despite ties at 12" having approximately 65% more isolate counts than either of the other sill heights. Bottom tiers on 12" sills started with no fungi isolated at time of delivery, but had the highest isolate count (26 isolations) across combined sample periods.

Ties on the bottom tier of stacks seasoned on 6", 8", or 12" sills had isolate counts of 13, 15, 23 in the final sample period; and 18, 17, and 28 isolates when all sample periods were combined.

Pairwise comparison of ties organized by height showed no significant differences in total isolate counts. However, pairwise analysis of the final sampling period with baseline comparisons removed did indicate evidence for differences between ties at 8" and 12" heights (MRPP A= 0.102, p = 0.042), and between ties at 12" and 33" heights (MRPP A = 0.135, p = 0.025) with ties at 12" height containing the most fungi in both comparisons.

The p-values were not corrected for multiple comparisons, but the results indicated no increased fungal activity from reduced sills, and that any protection from fungi imparted by increased height was only observed in ties seasoned at least 30 inches off the ground. Fungal populations across lower sill heights yielded no increase compared to 12" sill heights, and in many cases lower sills actually yielded fewer fungi.

Comparison of Isolates in Whole Stacks for Blackgum

Isolate counts from ties in the bottom three tiers appeared to decrease slightly with height. Bottom, middle and top tiers contained 36.4%, 34.2%, and 29.4%, respectively, of the 319 total isolates. Pairwise comparisons indicated these differences were too small to be considered significant. There were also no significant differences in isolate counts or comparisons of fungal community compositions across layers. Other comparisons indicated that the fungal community was almost identical between sill heights, often with greater variability between stacks at the same sill height than between stacks at differing sill heights. Ties with 12" sills had the greatest number of isolates over 6 months of seasoning.

Community Changes with Height for Red Oak

Outlier analysis in species space indicated that three ties were possible outliers (tie numbers: 1782, 1752, 1716). Flexural properties of these ties were normal, but they did have the highest number of isolates. Removing these samples did not improve the analysis and they were retained for overall assessment.

Comparisons of average MOR and MOE of beams from each tie indicated that the MOR of the lowest performing tie (#1726) was 1.96 standard deviations from the group average not including baseline samples. We considered this the threshold for being an outlier. MOE for this tie was 1.84 standard deviations below sample average. No isolates were recovered from the tie and we found no other reason to reject it as an outlier. Investigation of MOR associations with the tie removed improved statistical relationships slightly between isolates and

strength, but not enough to support statistical differences. Ultimately all flexural data were retained for analysis.

Fungal Isolates for Red Oak

We recovered 113 isolates of decay fungi from 88 rail ties sampled three times over 11 months (Fig. 12). An additional 10 ties were sampled for baseline testing, but did not yield any decay fungi. The 113 isolates represented 19 identified species and an additional 3 types that could only be identified to genera. All isolates could be represented by 14 genera so we condensed groups of isolates into 14 operational taxonomic units (OTUs).

Combined isolate counts were dominated by the brown-rot *Antrodia* OTU (42.6%) representing at least three species (*A. minuta, A. oleracea* and *A. serialis)*. The next most common fungus was the white-rot *Xylobolus frustulatus* (31.3%), commonly known as the "ceramic fungus" (Fig. 14).

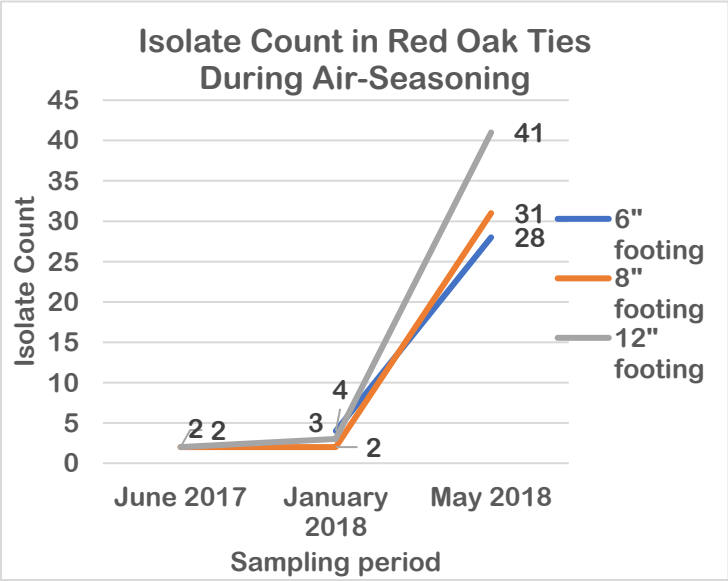

Figure 12 – Fungi increased rapidly in oak ties after 6 months of air drying.

Initial sampling of freshly cut ties yielded almost no fungi, possibly reflecting selection and grading efforts, but may also suggests that freshly cut oak provides a very limiting environment for fungal colonization. Only 4 isolates of *Xylobolus* (100%) were recovered from 3 ties at the start of the test.

Isolate frequency after 6 months of seasoning had more than doubled to 9 decay fungi recovered from 7 rail ties. The decay community was almost half *Antrodia* (44%) followed by *Xylobolus* (22%), *Phlebia* (11%), and *Leatiporus*

(11%). *Xylobolus* recoveries had decreased from the initial levels and this fungus was recovered from only one rail tie in both the initial and second sampling.

Fungal isolations increased after 11 months of seasoning, as did the frequency of all previously isolated fungi except *Laetiporus*, which was not recovered. The final community of decay fungi included 13 OTUs, and mostly consisted of *Antrodia* (44%), and *Xylobolus* (29%), followed by *Phlebia* (6%), *Stereum* (4%), and *Ganoderma* (4%).

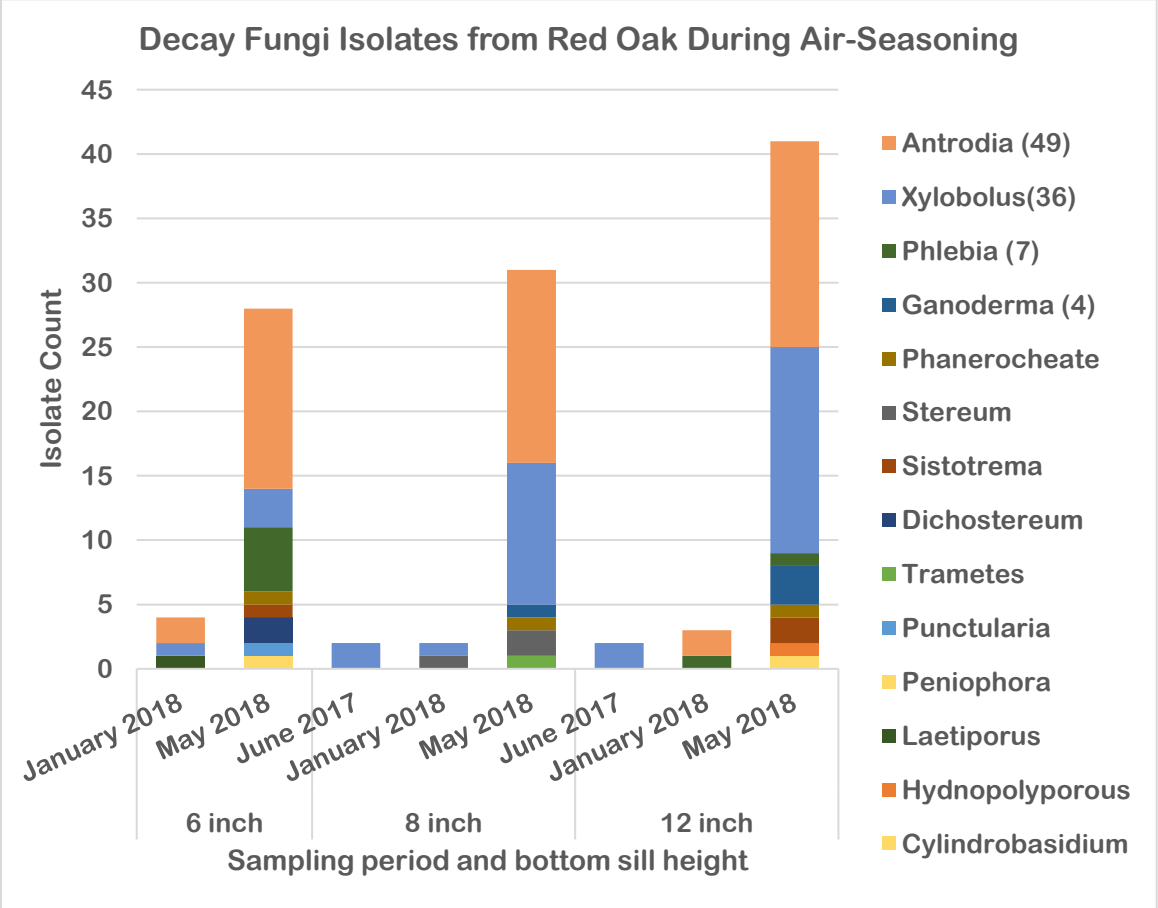

Figure 14 - Isolate counts from red oak ties air seasoned for 0 to 11 months on 6, 8 or 12 inch sills. No isolates were recovered from stacks on 6" sills in the initial sample period.

Species Associations With Stack Conditions for Red Oak

Decay fungi were evenly distributed across heights in the first two sampling periods, but their frequency increased in the final sampling along a gradient suggesting increasing fungal populations with increasing height. Fewer fungi were isolated from ties closer to the ground, while the highest height tested had the most isolates. A simple one-tailed t-test of isolate counts at each height showed that the highest height had significantly more decay fungi than the average of 12.5 isolates ($p < 0.000$). This was the opposite what we expected and was due largely to a sharp increase in isolation of *Xylobolus* at the uppermost heights. When isolate counts from 11 months were organized by height within sill treatment there appeared to be a distinct linear relationship between isolation frequency and increasing height ($r = 0.916$) (Fig. 15). Organizing isolate counts by height independent of sill height reduced the correlation ($r = 0.475$) (Fig. 16). This difference prompted careful

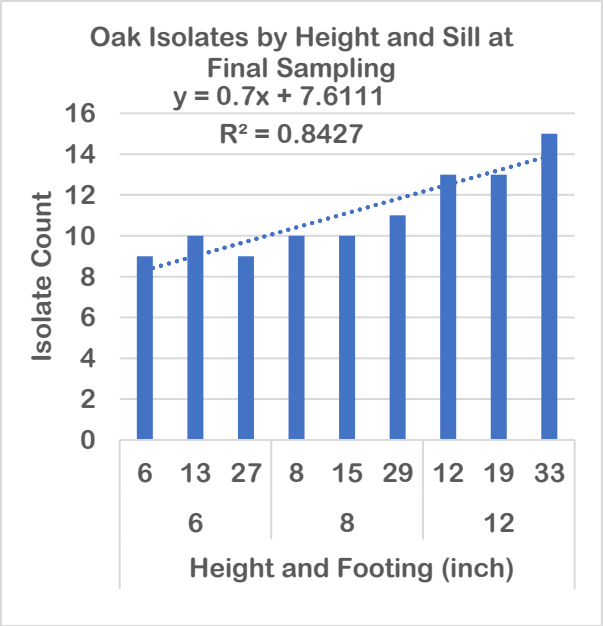

Figure 15 – Fungal isolate counts were linearly correlated with height when ties were arranged by height and sill. Isolate counts are represented by y, and x represents a combined factor of height and sill treatment.

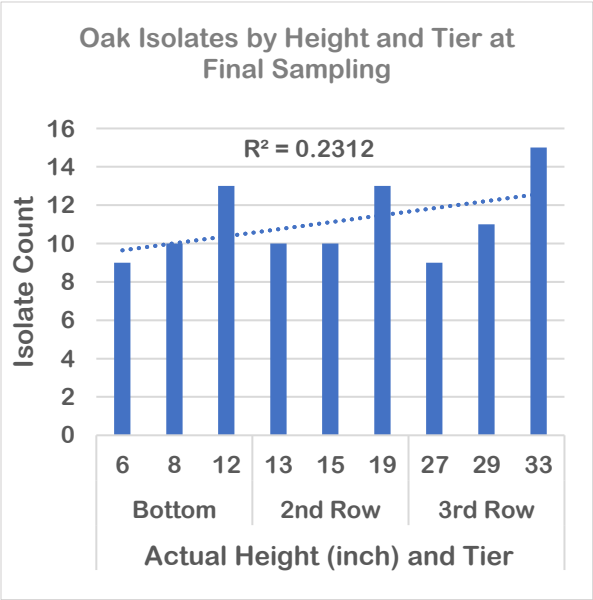

Figure 16 - Isolate counts from all sample periods were not well correlated to height when sill height was not considered.

examination of differences between individual stacks within each sill type and distributions of *Xylobolus*.

Two way cluster analysis indicated that there were two general community groups; one dominated by *Antrodia, Sistotrema,* and *Stereum* and the other dominated by *Xylobolus*, and *Phlebia*, and included *Phanerochaete*. (Fig. 24). There were several instances of community overlap and the entire isolate record may have been too sparse to capture meaningful relationships. This reflects the fact that red oak appeared to resist fungal colonization for at least a few months after being exposed in the air-seasoning yard.

Indicator Species of Tie Positioning for Red Oak

ISA on 9 height groups of 97 ties provided evidence that *Xylobolus* was associated with upper most height of 33" (ISA; 20% perfect association, p = 0.029), which prompted further analysis by more general height groups. No other species demonstrated strong indicator values at any height. ISA of the same ties categorized by 3 layer groups (putting the top height tested of all stacks into a single group, and all bottom tiers into a single group) provided no evidence that *Xylobolus* (or any other fungus) was associated with increased height in general. The increased incidence of *Xylobolus* at 33" did not appear to be an effect of height, but probably reflected a chance localized distribution. Making the same analysis for ties from individual stacks of each sill height seemed to support this premise. *Xylobolus* was most closely associated with a single stack on a 12" sill (ISA; 17% perfect association, p = 0.054). The strongest associations detected were not trends between sill or height, but more randomly assigned positions between stacks. This is important counter-evidence to comparisons of isolate counts against height that would otherwise indicate increased fungal activity with increased distance above ground.

Communities Compared by Tie Position for Red Oak

MRPP of fungal communities grouped by either height or layer showed no evidence that fungal communities differed between heights, and that communities were more similar than expected by chance. The 33" height yielded

the most fungal isolates overall (6%), and the 27" height yielded the fewest isolates (2.8%). Both positions were in topmost tiers.

Effect of Fungi on MOR and MOE

Data transformations for Blackgum

Because ISA requires *a priori* grouping for comparison, ties were assigned strength groups of MOR and MOE. Different group levels were developed based on standard deviations of strength scores, ranging from 27 groups to 30 groups each for MOR and MOE. Groups based on strength scores were assigned by determining how many standard deviations (rounded to the nearest 10th decimal place) the average MOR of each tie was from the sample average, then multiplying by 10 to provide non-decimal grouping. Any tie left in a singular category received a score rounded to the next nearest grouping. The same procedure was performed for MOE to form 30 groups.

MRPP on 11 groups ranked by MOE provided evidence that ties with the lowest MOE had different fungal communities than those with the highest MOE ($A = 0.106$, $p = 0.008$).

Indicator Species for MOE of Blackgum

ISA on 30 MOE groups suggested that *Filobasidium* was associated with slightly above average MOE's (59% perfect indicator, $p = 0.012$, ISA), but within half of one standard deviation of average MOE for all ties tested. No other fungus presented indicator values as strong. ISA on 11 MOE groups reduced the indicator value of *Filobasidium,* but presented some evidence that MOE's 1 to 1.5 standard deviations above sample average were associated with the unknown basidiomycete (26.2% perfect indicator, $p = 0.027$ ISA), *Gloeohypochnicium* (25.6% indicator value, $p = 0.035$) and maybe with *Schizophyllum commune* (19.1% indicator value, $p = 0.079$ ISA). As noted, *Filobasidium* and *Schizophyllum* are both known to have weak decay activity and may have excluded fungi with stronger decay potentials. *Radulodon* was generally more closely associated with MOE groups below average in ISA of 30 and 11 groups,

but not to any single MOE group. Larger or smaller binning methods would have presented very different evidence and these cases were very dependent on how ties were grouped into categories.

Indicator Species for MOR of Blackgum

ISA on 27 MOR groups provided different associations than MOE groups. There was some evidence ($p = 0.025$) that *Punctularia* was associated with MOR values of 1 standard deviation below sample average. *Chondrostereum* was most associated with ties of exactly average MOR ($p = 0.041$). ISA on 11 MOR groups provided no evidence for any fungi associated with particular deviations in MOR.

Among the OTUs assessed was a novel OTU compiled from total isolate counts, indicating total fungal presence. This was meant to assess the assumption that decay continues as the fungal community changes. The OTU of total isolate counts was very slightly more indicative of MOE and MOR scores below average and most associated with the lowest ranked MOE and MOR groups in all ISA, but the differences were not significant.

The lack of association between individual species and deviations in MOR and MOE is not surprising because the presence of any fungus is not a guarantee of decay, especially in variable environments and under competition from other fungi.

Pairwise comparisons and MRPP analysis of isolates against more derived flexural properties or novel groups presented occasional statistical significance, but no consistent trends of biological significance were detected.

In lieu of finding reduced MOE throughout entire ties, we attempted to check for increased variation in flexural properties resulting from fungal colonization. Incipient decay may result in increased variability before causing changes throughout an entire tie. Comparisons of fungal communities grouped by rank adjusted coefficients of variation in MOE (MOE-CV) indicated there were fungal community differences between groups, but the differences were not structurally significant. For example, the two most different fungal communities

grouped by MOE-CV had only 1.1% difference in MOE variability and were approximately 10% above and below the average MOE of all ties (A = 0.214; p = 0.010, MRPP). The difference detected was not related to functional change in variation of MOE or MOR from fungal communities. This indicates that different fungal communities in blackgum ties under similar conditions can be expected to cause similar rates of decay.

Data Transformations for Red Oak

Ties were assigned to groups of MOR and MOE based on standard deviations from the average (sample population including baseline ties). Eleven groups were assigned by determining how many standard deviations (rounded to the nearest 10th decimal place) the data from a given tie were from the sample average for all ties, then multiplying by 10 to provide non-decimal grouping. Any tie left in a singular category received a score rounded to the next nearest grouping. The same procedure was done for MOE values to form 5 groups. Three larger groups were also tested based on scores above, within, or below 1.5 standard deviations for MOR and MOE.

Flexural properties were recorded for a reduced sample set of 42 ties (9 baseline ties and 33 ties from stacks). Comparisons of flexural properties were made from this subset of 42 ties including baseline samples, and all other species assessments, such as OTUs by height, were made from 88 air-seasoned ties and baseline samples. All ties in the reduced set were from the topmost and bottom tiers from all stacks. This selection was made to capture the greatest differences in height as well as representing all sill heights; the breadth of isolates was also well represented though no ties were tested where *Ganoderma, Punctularia, Peniophora,* or *Trametes* had been isolated. Most of these OTUs represented less than 1% of overall isolates, while *Ganoderma* represented 3.5% of overall isolates.

Indicator Species for MOR & MOE of Red Oak

Indicator species analysis and multi-response permutation procedures on 42 ties did not indicate any significant associations between fungi and classes of MOR or MOE in oak ties.

ISA of 42 oak ties organized by MOR groups did not present any strong evidence for correlations of single OTUs to particular levels of strength loss, but lower MOR's were most associated with the total number of isolates (ISA; 30% perfect indication, $p = 0.093$), and with isolates of *Antrodia* (ISA; 22% perfect indication, $p = 0.251$). The highest MOR values, over 1.5 standard deviations from the sample average, were most strongly associated with *Sistotrema*, but none of the associations were statistically significant.

Indicator species analysis on 5 and 3 MOE groups did not find any correlation of between any OTU and MOE, nor was there an association between total number of isolates in a tie and its MOE.

Re-running ISA using only ties with decay fungi present (with all zero isolate totals removed, 22 ties retained), resulted in even less community difference between the 3 MOR groups, and less difference between the 3 MOE groups. The two tentatively proposed community groups found in clustering analysis, as well as ties with no fungal presence detected, were both distributed through all flexural properties of ties. These results suggest there were no real associations between individual isolates and reduced MOR or MOE.

Communities Compared by MOR & MOE for Red Oak

MRPP analysis of fungal communities among strength groups found little difference between groups, (MRPP on 5 groups; $A = -0.049$, $p = 0.786$). A single pairwise difference was detected between oak ties grouped by MOR of 1 and 2 standard deviations below average, out of groups that ranged from as many 2 standard deviations above or below average. We concluded that the community of decay fungi was not related to particular changes in MOE or MOR, and that the highest and lowest strength ties had similar fungal species and numbers of

isolates. Though ties seasoned 33” above ground had the most isolates overall, the subset tested had the greatest number in ties seasoned 12” above ground (23.8% of isolates in subset). There was no correlation between fungi isolated and MOR or MOE from the subset’s fungal community.

Flexural Properties by Stack Positions

Even though the fungal communities did not differ between heights or across sill treatments, they might exhibit different rates of decay based on height if the distance from the ground created microclimate gradients. Comparisons of tie positions to their flexural properties of MOR and MOE were similar and in some cases, such as MRPP, could be compared simultaneously. In other analyses, MOR and MOE were assessed individually. MOR and MOE were more closely correlated in oak (r = 0.8790) than in blackgum (r = 0.6598) (Fig. 25 & 26).

Baseline MOR & MOE for Blackgum

Seventy one of the 300 beams cut from ties not exposed to air-seasoning were rejected for improper sizing or methodological problems. Baseline data incorporated results from 8 ties. MOR’s of blackgum beams that were not air-seasoned averaged 11633.58 psi, while MOE averaged 1.59 x 10^6 psi. The 95% confidence intervals for MOR at 12% MC without exposure to air-drying were between 10610 to 12657 psi, while those for MOE were between 1.458 and 1.729 million psi. These values are higher than previously reported in the Wood Handbook (9600 psi MOR and 1.2 million psi MOE) (FPL 2010) as well as those in ASTM Standard D2555-17A (7040 psi MOR and 1.03 million psi MOE) (ASTM International 2017).

Post-Seasoning MOR & MOE for Blackgum

MOR and MOE values of blackgum ties were lower than baseline measures for all heights after air-seasoning, but remained above published figures for small clear beams (See Table 3).

Tier (sample size)	Isolate Count	MOE (psi x 10^6i)	MOR (psi)
Bottom (30)	115	1.41	10098
2nd (30)	108	1.51	11001
3rd (28)	93	1.58	11560
Baseline (10)	3	1.59	11634
Grand Total	319	1.50	10935
FPL		1.2	9600

Table 3 - Isolate count, MOE, and MOR for blackgum ties grouped by tier.

The simultaneous comparison of MOE and MOR among ties sorted by height showed some differences. Analyses combining MOR and MOE for the layers showed they had different flexural properties overall (MRPP; A = 0.024, p = 0.031). Pairwise comparisons produced strong evidence that top and bottom layers were the most different in simultaneous MOE and MOR (MRPP; A = 0.045, p = 0.008). There

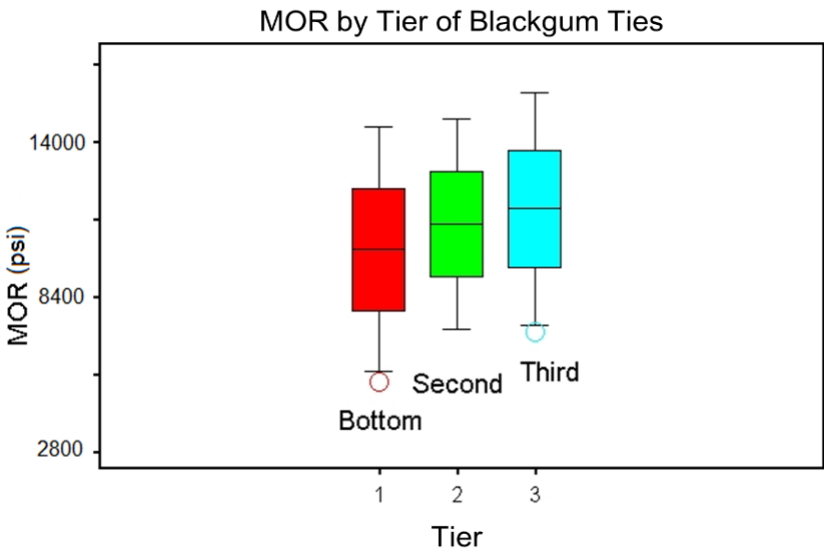

Figure 16 - Average MOR of the first three tiers of blackgum ties irrespective of sill height. Boxes represent quartile differences centered on the mean with error bars extended to 1.96 standard deviations.

was a slight trend of decreasing MOR and MOE with proximity to ground (Fig. 20).

Intuitively, the risk of decay during seasoning should decrease with distance above the ground, but this trend was not observed in comparisons of matched layers of different sill heights that differed in height by a few inches. Pairwise comparisons of tiers across all stacks suggested that there was less decay over 6" or 8" sills than in equivalent tiers over 12" sills. Average MOR decreased between heights of one foot and three feet above the ground, but there was no predictable difference from sill height. Interestingly, ties from the bottom layers in stacks with a 12" sills had the lowest MOR of all height groups (9409 psi) (See Table 4). The difference between sill heights were not greater than predicted by chance (MRPP; A = -0.010, p = 0.904).

Comparison of MOR & MOE to Tie Positions for Blackgum

The trend in flexural properties related to layers was not observed between differences in sill height so we investigated the trend relative to actual height. Grouping by height provided a finer gradient than grouping by layers and drastically reduced the apparent differences. The difference between heights was insignificant, representing the higher degree of overlap than in layer groupings (MRPP; A = 0.006, p = 0.361). Otherwise, there were some significant pairwise comparisons.

Ties seasoned 12" above ground had lower compiled flexural properties than those seasoned at 29" (MRPP; A = 0.102 p = 0.026), and lower than baseline measures (MRPP; A = 0.010, p = 0.039). Flexural properties of ties from 12" heights were also lower than ties at the 33" height, but the differences were smaller (MRPP; A = 0.073, p = 0.055).

Ties seasoned 6" above ground had lower properties than ties seasoned at 29" (MRPP; A = 0.072, p = 0.050), and baseline ties (MRPP; A = 0.077, p = 0.054). MOR or MOE were reduced with decreasing heights in stacks, but not between heights in sills (Fig. 21 & 22, Table 4).

Table 4 – Average MOR and MOE of blackgum ties organized by height and sill-height. Ties were seasoned for 6 months in Kentucky. Averages per height are from tiers in two stacks.

Comparison of MOE and MOR by Height in Blackgum						
Sill Height (Inches)	Height (inches)	Tier	Average MOE (psi x 10^6)	Standard Deviation MOE (psi x 10^6)	Average MOR (psi)	Standard Deviation MOR
6”	6”	Bottom	1.46	.250	9879	2173
	13”	2nd	1.54	.272	10912	2201
	27”	3rd	1.51	.183	11089	2204
		6” Total	**1.50**	**.233**	**10611**	**2182**
8”	8”	Bottom	1.46	.236	11006	2241
	15”	2nd	1.42	.184	10877	1413
	29”	3rd	1.57	.167	11734	1688
		8” Total	**1.48**	**.202**	**11206**	**1792**
12”	12”	Bottom	1.32	.220	9409	2106
	19”	2nd	1.57	.171	11215	2148
	33”	3rd	1.65	.217	11839	25312
		12” Total	**1.51**	**.242**	**10786**	**2416**
Grand Mean			**1.50**	**.224**		**2132**

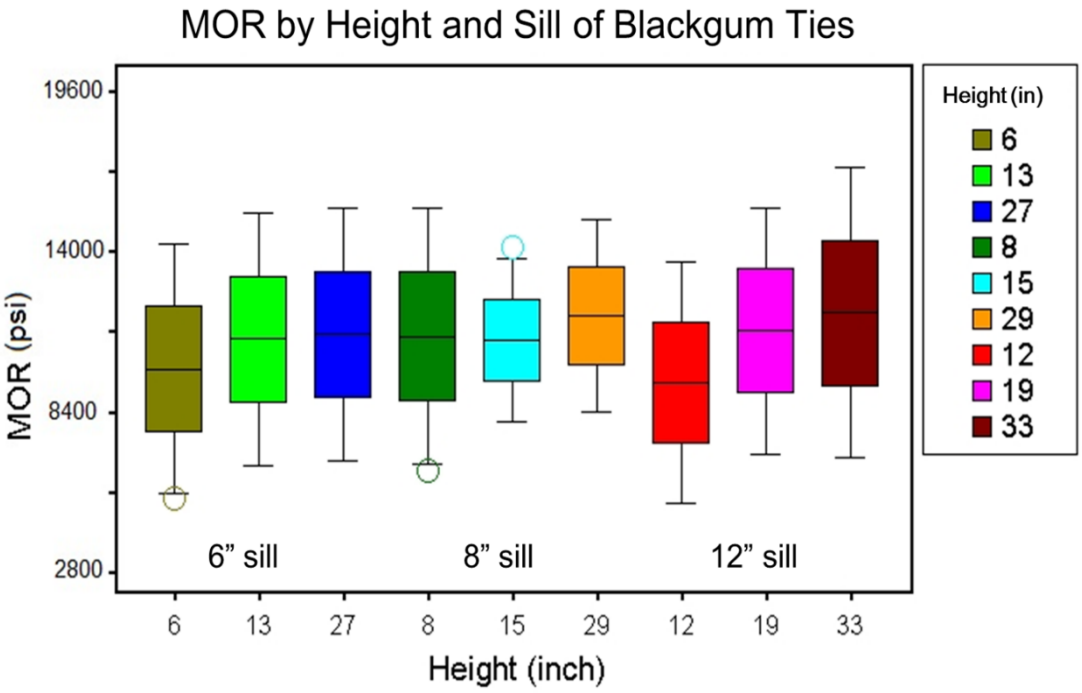

Figure 18 - MOR for black gum ties at different distances from the ground in stacks on 6, 8, or 12 inch sills. Boxes represent quartile differences centered on the mean with error bars extended to 1.96 standard deviations.

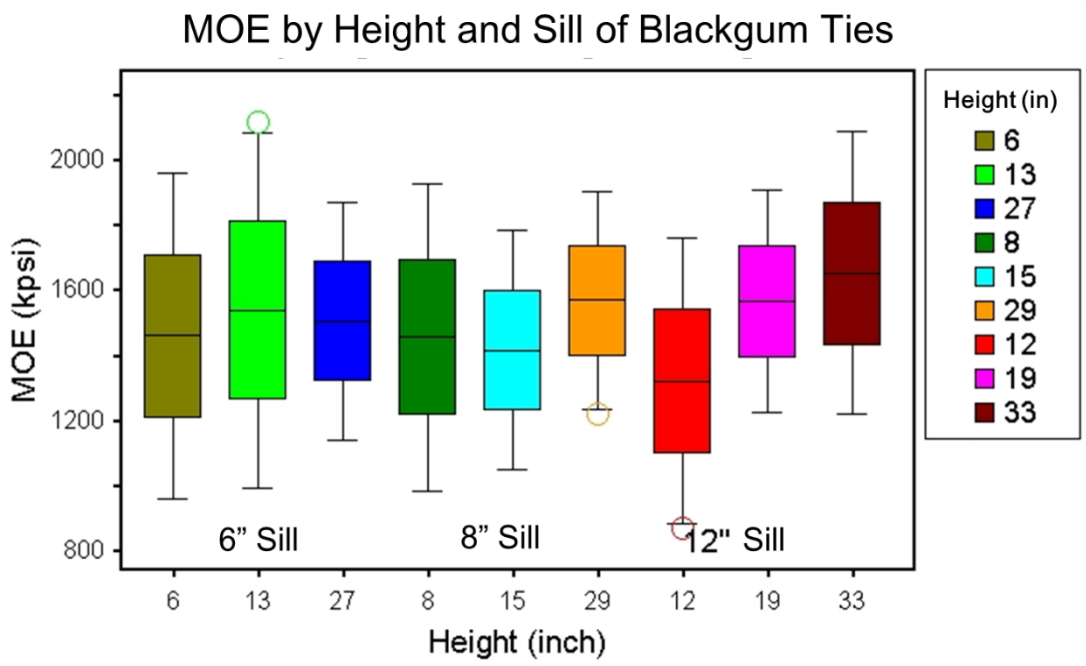

Figure 17 - MOE of black gum ties seasoned at different distances from the ground on 6, 8 or 12 inch sills. Boxes represent quartile differences centered on the mean with error bars extended to 1.96 standard deviations.

Baseline MOR & MOE for Red Oak

Preliminary testing of 315 oak beams from 9 ties showed that ties not exposed to air-drying had homogenous MOE and MOR values with a coefficient of variation of approximately 16% for both MOE and MOR. An average of 35 beams were sampled per tie. The 95% confidence intervals for MOR of red oak ties at 12-15% MC without exposure to air-drying was between 10,835 and 12,269 psi, while that for MOE was between 1.59 and 1.79 million psi. The results are close to the MOR range of 10,900 to 14,300 psi and MOE range of 1.49 to 1.82 million psi for Southern red oak or Northern red oak, respectively (FPL 2010).

Table 5 - Red Oak strength figures at 12% MC, from FPL 2011 5-3

Red Oak Groups	MOR (psi)	MOE (psi x 10^6)
Red black	13900	1.640
Southern Red	10900	1.49
Northern Red	14300	1.82

Post-Seasoning MOR & MOE for Red Oak

Samples cut from air-seasoned ties were taken from a more confined zone on the tie, which likely reduced the variation. No predictable differences were found in MOE or MOR of seasoned ties between different layers, heights, or stack sills compared across treatments, or when compared to baseline testing.

Comparison of MOR & MOE to Tie Positions for Red Oak

MRPP of height groups in dimensions for flexural properties from 42 ties provided no evidence of differences in MOR or MOE based on height (Fig. 23 & 24). The greatest difference in oak tie strength was between the high MOR (12,410 psi) from ties at the 29” height in stacks with 8-inch sill, and the low MOR (10,845 psi) from ties at the 33” height in stacks with 12” sills. The highest and lowest average MOR values between groups were both in topmost tiers, indicating that relative height was not a significant factor.

Table 6 - MOR, MOE, and isolate counts relative to height of a subset of red oak ties tested to failure. Isolate counts are from a subset of 42 ties, and are distributed differently than all isolates from all oak ties.

Height (in)	Height (cm)	MOR (psi)	MOE (psi x 10^6)	Isolate Count
6”	(15.24 cm)	11523	1.66	6
8”	(20.3 cm)	11121	1.53	8
12”	(30.48 cm)	11478	1.61	10
Bottom Average		***11374***	***1.60***	***24***
27”	(68.58 cm)	11962	1.68	8
29”	(73.66 cm)	12410	1.84	4
33”	(83.82 cm)	10845	1.56	6
Top Average		***11739***	***1.69***	***18***

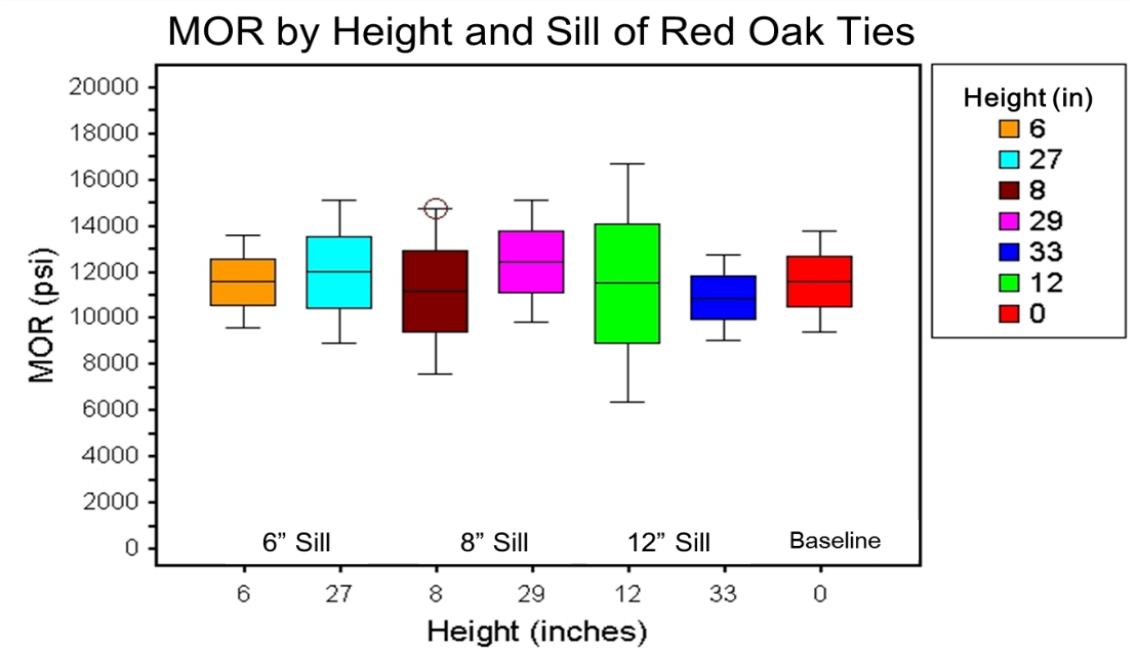

Figure 20 - MOR of oak ties seasoned at various distances above ground on 6, 8, or 12 inch sills. Boxes represent quartile differences centered on the mean with error bars extended to 1.96 standard deviations. Zero-height indicates baseline tests without air-seasoning.

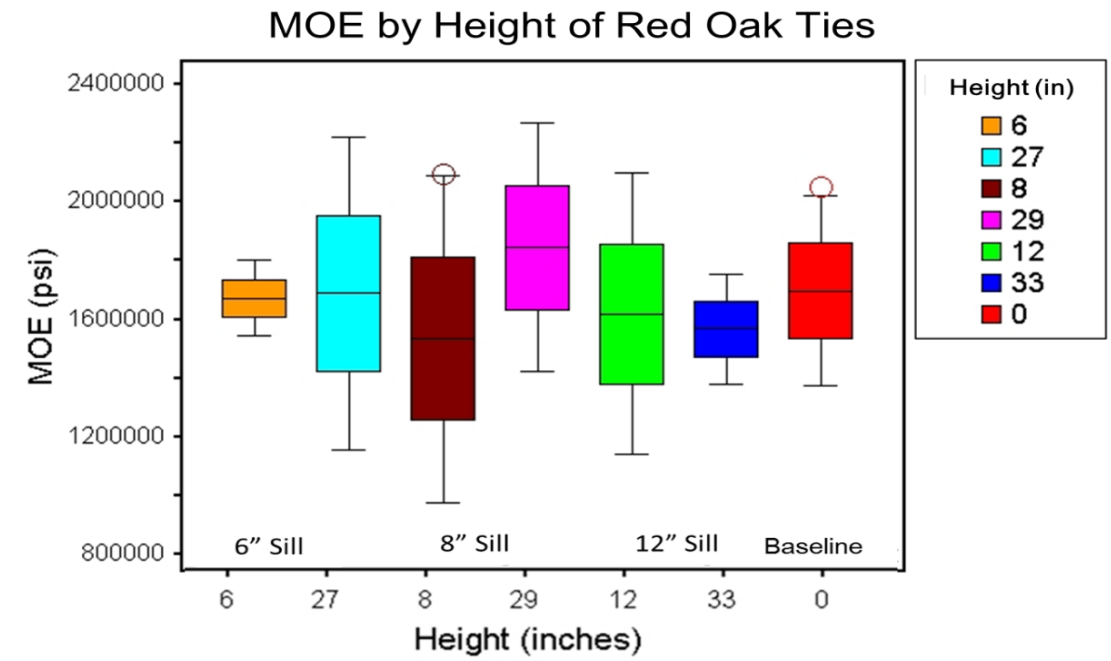

Figure 19 - MOE of oak ties seasoned at various distances above ground on 6, 8, or 12 inch sills. Boxes represent quartile differences centered on the mean with error bars extended to 1.96 standard deviations. Zero-height indicates baseline tests without air-seasoning.

Decay Capabilities of Isolates

The decay capabilities of the fungi isolated from the ties were assessed in a soil block test using 21 isolates along with three reference decay fungi (Table 7). Mass losses ranged from 1.4% for pine exposed to *Pholiota limonella* to 76.3% for maple exposed to *Trametes versicolor*.

As predicted, brown rot fungi caused the greatest mass loss on pine blocks. Interestingly, they also caused some of the highest mass losses in both hardwoods. *Laetiporus cincinnatus* and *Antrodia oleracea* caused 59.7% and 36.0% mass loss in pine, respectively. These decay rates were high compared to the AWPA standard brown-rot fungi; *Rhodonia placenta* (18.6% average) and *Gloeophyllum trabeum* (35.6% average). The brown rot isolates from ties also caused the highest mass losses in oak blocks. The greatest mass loss in oak was from *L. cincinnatus* (64.8%). *Antrodia oleracea* (40.3%) caused oak mass losses similar to the AWPA test fungi.

Almost a quarter (23.8%) of the isolates from blackgum did not cause large weight losses on any of the woods tested. *Chondrostereum, Cylindrobasidium, Pholiota,* and *Sistotrema* all failed to cause weight losses greater than 5%.

Representative cultures of *Pholiota limonella/adiposa* caused less decay in pine blocks (1.4%) than control bottles with no fungi (2.3%), and essentially no decay in maple (0.45%). Meanwhile, *Pholiota squarrosa* cultures caused 8.16% percent mass loss in both maple and pine blocks.

Some varying morphotypes were not identified by molecular differences, but had different decay capabilities. *Punctularia strigosozonata* with arthroconidia caused about twice as much mass loss in all wood types as *P. strigosozonata* without arthroconidia (Table 7).

These results represent half of the decay fungi identified over the entire study. Of 47 cultures recovered, 9 were lost in storage and another 17 isolates remain in testing at the time of this publication.

Decay Test result

Table 7 – The ability of decay fungi isolated from blackgum or red oak railway ties to cause decay on maple, red oak or pine sapwood blocks in a soil block decay test.

Fungus	Mass Loss (%)[a]						
	Maple		Red Oak		Pine		Average
Antrodia oleracea	50.23	(4.47)	40.25	(4.5)	35.97	(6.53)	**42.15**
Chondrostereum purpureum	1.75	(0.16)	2.70	(0.18)	2.47	(0.23)	**2.31**
Coprinellus radians	23.94	(5.06)	16.95	(9.83)	3.14	(0.46)	**14.68**
Cylindrobasidium sp.	2.02	(0.90)	2.89	(0.68)	2.57	(0.17)	**2.49**
Laetiporus cincinnatus	57.63	(3.30)	64.79	(1.81)	59.65	(4.31)	**60.97**
Phanerochaete sp.	45.15	(12.93)	23.52	(9.59)	10.23	(4.21)	**26.30**
Phlebia fuscoatra	10.43	(6.96)	4.25	(0.5)	5.92	(4.99)	**6.87**
Phlebiopsis flavidoalba	16.01	(3.84)	10.76	(3.06)	12.33	(2.15)	**13.03**
Pholiota (limonella/adiposa)	0.45	(0.31)	2.36	(0.18)	1.36	(0.14)	**1.39**
Pholiota squarrosa	8.16	(1.76)	2.87	(0.23)	8.16	(1.77)	**6.40**
Punctularia strigosozonata	21.10	(4.18)	3.11	(0.32)	6.28	(1.57)	**10.16**
Punctularia strigosozonata (arthroconidia)	44.94	(11.62)	5.65	(1.33)	9.34	(2.63)	**19.98**
Radulodon americanus	35.95	(10.97)	25.67	(12.37)	9.64	(4.51)	**23.76**
Schizophyllum commune	8.99	(7.91)	4.73	(0.59)	3.02	(0.3)	**5.58**
Sistotrema brinkmannii	4.37	(7.04)	2.99	(0.47)	1.74	(0.24)	**3.03**
Spongipellis pachyodon	41.50	(5.89)	27.16	(5.9)	21.25	(9.24)	**29.97**
Stereum hirsutum	27.38	(5.88)	13.99	(5.79)	14.07	(3.04)	**18.48**
Stereum spp.	22.25	(6.18)	11.31	(7.24)	7.68	(3.25)	**13.75**
Trametes gibbosa	54.16	(10.56)	14.82	(6.71)	21.44	(9.61)	**30.14**
Trametes versicolor	76.34	(10.57)	40.65	(24.8)	23.48	(11.88)	**46.83**
Xylobolus frustrulatus	19.20	(6.08)	24.07	(3.96)	3.37	(5.31)	**15.55**
Gloeophylum trabeum (MAD 617)	70.76	(5.70)	49.85	(29.71)	35.63	(7.92)	**52.08**
Rhodonia placenta (MAD 698)	54.13	(6.41)	41.82	(5.9)	18.56	(5.67)	**38.17**
Trametes versicolor (MAD 697)	74.38	(12.42)	16.07	(13.36)	18.14	(8.1)	**36.20**
Control (No Fungi)	-0.40	(0.49)	1.22	(0.87)	2.30	(0.34)	**1.04**

[a]Values represent means of 5 blocks per fungus per wood species. Figure in parentheses represent one standard deviation.

FIGURES 27-29

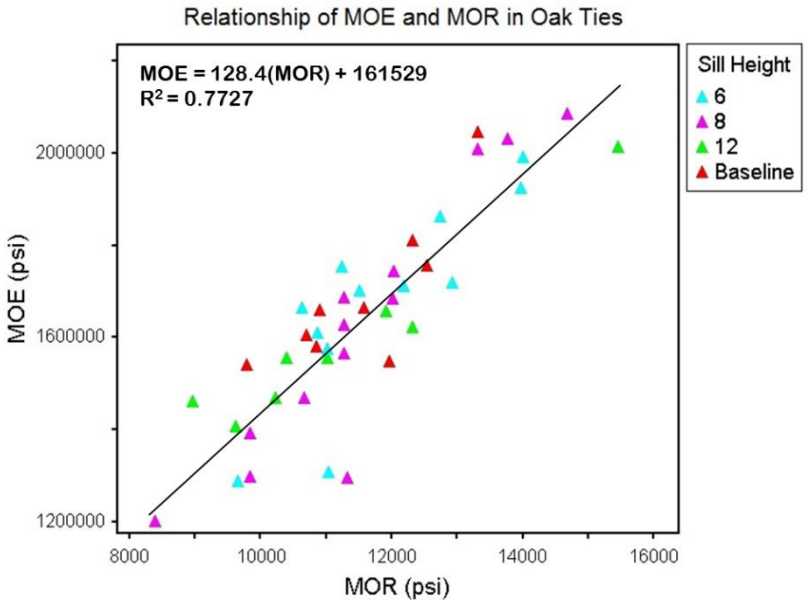

Figure 21 – Relationship between MOR and MOE in beams cut from red oak ties seasoned various distances above ground on 6, 8 or 12 inch sills. $r^2 = 0.77$

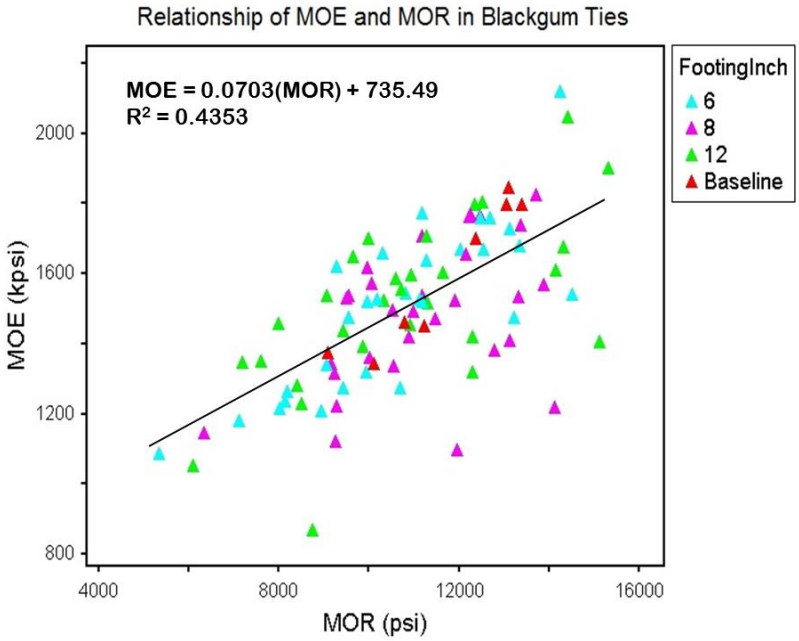

Figure 22 – Relationship between MOR and MOE in beams cut from blackgum ties seasoned various distances above ground on 6, 8 or 12 inch sills. , $r^2 = 0.44$

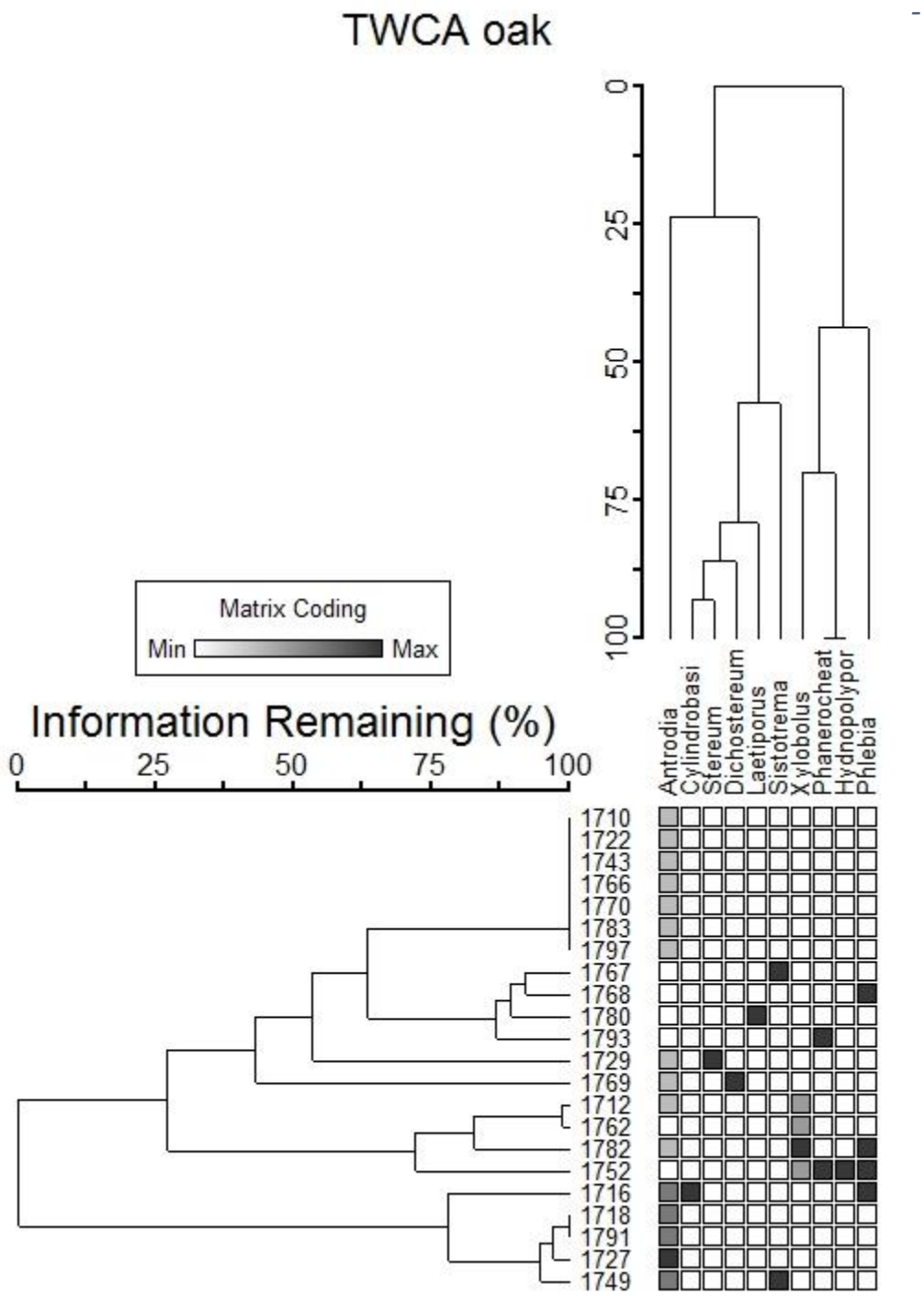

Figure 23 – Evidence of community differences among ties as shown by a two-way cluster dendrogram of oak isolates and ties. All ties with zero isolates were removed. Two communities were dominated in abundance each by Antrodia and Xylobolus. Blackgum ties did not have identifiable community differences.

CHAPTER 5. DISCUSSION

Blackgum

Blackgum ties seasoned at the 12" height yielded the most fungi at the final sampling period and the most fungi overall. Beams cut from these ties had the lowest MOR. There was a very slight relationship between relative height (tiers) and both reduced fungal isolations and higher MOR, which could not be supported statistically except in comparisons of topmost and bottommost tiers. The results failed to demonstrate a link between increased decay hazard (produced by bringing ties closer to the ground) and increased fungal colonization or reduced wood properties. This suggests that decay is not occurring more slowly in communities with lower species richness. Other studies on succession have shown that fungi in competition cause less decay because they must direct metabolism into combative behaviors (Boddy 2000; Hiscox *et al.* 2017).

Ties in the bottom tiers were expected to be exposed to the highest moisture regimes and show the greatest effects of sill height on fungal colonization. Isolation frequencies among ties in bottom tiers did not differ based on sill height; however, height groups overall may have shown some differences in the number of fungal isolates.

Even though isolations were lowest in upper heights of blackgum stacks, the difference could not be supported statistically and MRPP found no difference in community composition. We concluded that the fungal community in blackgum had the same constituents at all levels, and that there may have been some factor limiting colonization at the very uppermost height tested. Our environmental data failed to capture any obvious differences in RH or temperature that could explain any difference.

Because decay was evident, though minor, in ties exposed at all heights below 33", the risk of decay should be considered to be equal in this seasoning zone. This was shown by a reduction in MOR and MOE at all heights compared to baseline. There was a slightly higher risk of reduced flexural properties at the

bottom of stacks compared to third tiers, but no difference in either fungal community or physical properties between ties seasoned on 6", 8", and 12" sills.

Red Oak

Oak ties yielded very few fungal isolates and the community differed from that found on black gum. A larger community could probably be found in ties seasoned for a longer period and this would improve statistical associations to reductions in MOR and MOE. Decay, as evidenced by declines in flexural properties, did not appear to have reached detectable levels in any of the bottom three tiers during air-seasoning.

The data from oak ties failed to indicate changes in flexural properties or increased fungal activity related to sill heights. Isolate counts alone might suggest that just the opposite effect had occurred; however, we determined that fungal activity was concentrated randomly throughout stacks, and that all fungal communities presented similar decay hazards under actual stacking conditions. While no single isolate was related to any change in flexural properties, all isolates contributed cumulatively to decreased MOR as observed using ISA with total isolate counts. Oak ties were resistant to fungal colonization for at least the first 6 months of air seasoning, but fungal populations increased rapidly in the spring.

Previous studies concluded that beginning air-drying in winter when fungal metabolism is limited by cold will limit fungal colonization and wood degradation (Richards & Humphrey 1939; Scheffer & Lindgren 1940).

Comparison of Red Oak and Blackgum Isolates

The stacks of oak and blackgum were separated by a single row of non-test stacks. As a result, all ties should be exposed to the same airborne spores and hyphal fragments. We had predicted that later stage colonization would be from identical sources, but that succession would progress very differently.

The genera *Cylindrobasidium, Ganoderma, Phanerocheate, Phlebia, Punctularia, Sistotrema, Stereum,* and *Trametes* occurred in both wood species.

These shared OTUs represented 17.7% of oak isolates and 30.1% of blackgum isolates. *Ganoderma,* and *Phlebia* occurred more frequently in oak, the frequency of *Phanerocheate* was similar in both species although proportionally higher in oak, and all others occurred much more frequently (by count and proportion) in blackgum. Blackgum tends to be far more susceptible to fungal decay and the isolation data support this premise. The minor degree of community overlap suggests strong selective pressures from wood type.

General

The presence of decay fungi in ties at the start of seasoning reflects a combination of the heart-rot fungi that were in the live trees and remained active in the freshly cut ties (Boddy & Rayner 1983; Richards & Humphrey 1939; Schmidt & Czeschlik 2006); fungi that may have invaded the wood between felling and sampling (Schwarze et al. 2000); or latent propagules present in sapwood, but held inactive by a tree's metabolism (Kiser, 2009; Parfitt et al. 2010). The results indicate that many fungi are present at the start of air-seasoning, placing added importance on the need to process ties rapidly after arrival so they can begin to dry to levels that will inhibit the growth of fungi already present and limit the entry of additional fungi.

Fungal populations may change throughout stacks without necessarily initiating decay. Competition and environmental modulation can both modify how fungi degrade wood, but understanding the fungal communities helps delineate colonization patterns that may indicate the risk of decay. The fungal population should not be taken as an independent measure of decay. Similar populations do not imply decay rates will be the same.

Prior research on fungal degradation of wood shows that severe losses in strength occur even before noticeable mass loss within a period of only a few weeks (Winandy *et al.* 2001; Zabel & Morrell 1992). Even accounting for the fact that real environmental conditions are not as optimal for fungal growth as a laboratory incubator, we would expect severe losses of MOR or MOE in some ties that had active fungal growth over a period of several months to a year. This did

not seem to be the case, and while the tested decay potential of each isolate can certainly be used to assess decay risk and identify decay mitigation strategies, it does not form a complete picture. Interspecies competition, variable environments, changing MC, and temperature are all determining factors in decay community response. It is also important to consider that these results reflect a well-maintained tie yard free of vegetation, rotting wood, or standing water. The Koppers site is well graveled and organized to facilitate rapid drying following AREMA guidelines. Any incidence of advanced decay would have been an exception to normal operations.

There was no evidence of any difference in decay rates between ties seasoned at distances of 6 and 29 inches off the ground, and only minor evidence of protection from decay at heights above this. The conditions in large stacks of wet wood likely dampen changes in environmental conditions because of the large timber mass and available MC. As a result, minor changes in height do not directly result in changes in microclimate that might occur for smaller wood samples that were more directly exposed to the environment.

The fungal community across stack conditions was less random than expected by chance. The potential community structure, coupled with the lack of differences in fungal isolation frequency or community composition among sill heights may be evidence for a structured community by deterministic succession. This warrants further investigation. The correlation between species across sample times would indicate not just groups within a community, but might indicate organized community lineages, although this must be stated with some caution since this test represents a limited ecological sample. These relationships could guide investigations using multispecies decay tests to help understand when decay will be inhibited or promoted by species interactions. Fungal species were not a useful indicator for decay because decay was minimal, but longer exposure would likely increase community progression and lead to more substantial decay rates. Tracking the progression from natural colonization beyond incipient decay may increase our ability to predict decay more accurately.

Isolates

Punctularia

Many isolates of *Punctularia strigosozonata* were not initially counted as a decay fungus because they presented morphological characteristics that differed from typical basidiomycetes. The rust colored conidia (arthroconidia) produced by this fungus were initially considered a contaminant that had overcome benomyl media selectivity. *Punctularia strigosozonata* is in the order Corticiales, which does not contain many decay fungi, and the decay capability of this fungus is not well recorded in literature (Floudas *et al.* 2012; Mgbeahuruike *et al.* 2013). Two distinct morphotypes were isolated; with and without conidia (arthroconidia). These two types remained distinct after transfers and demonstrated different growth characteristics. Both morphotypes were ultimately counted and included in our decay tests, where they retained distinct morphologies throughout the 16-week test. Both morphotypes were white-rot fungi. The strain that regularly produced arthroconidia caused twice as much mass loss in all wood types compared to the isolate without arthroconidia.

Punctularia strigosozonata was detected only in the final sampling of gum, and along with a single isolate from the final sampling oak. It was most abundant on ties that had no prior decay fungi detected, indicating it may be a poor competitor against other fungi, but seemed to excel at rapid surface expansion.

Coprinellus

Inky-cap mushrooms in the genus *Coprinus* and *Coprinellus* can decay wood, especially hardwoods, but are generally not of economic significance. They are not often identified causing degradation of wood in service, though their enzymatic capabilities and saprotrophic habits indicate that they are potential white-rot fungi (Badalyan *et al.* 2011; Oliver 2008; Oliver *et al.* 2010). Anecdotally, we have observed similar mushrooms growing on wood inside residences. Previous studies of media preference and enzyme production have demonstrated a minor variation in the laccase production of *Coprinellus*

compared to other white rot fungi, which prevents digestion of arabinose and produces highly selective sugar affinities (Oliver 2008). Inky-cap fungi have been shown to degrade already decayed hardwoods, yet completely fail to reduce more sound wood (Oliver *et al.* 2010). The ability of *Coprinellus* to decay wood is thought to be entirely dependent on prior degradation by other agents.

In our tests, we recovered a single isolate of *Coprinellus radians* and suspected that it was indicative of advanced succession of decay communities; arriving only after hemicellulose or another polymer was sufficiently degraded by other more decay-competent fungi. However, the isolate proved to be capable of decay when exposed to sound wood in our soil-bottle decay test.

Figure 24 - Photo showing extreme deflection of a small clear beam from rail tie 1600 compared to all other beams.

While it is difficult to draw inference from a single isolate, the results are indicative of some type of prior fungal conditioning that allowed *Coprinellus* colonization. The bending data would seem to support this idea, as the single tie with culturable *Coprinellus* also sustained abnormally high displacement in bending (Fig. 28). Small clear beams from the tie 1600 experienced more than 2.2 inches (55 mm) of deflection without rupturing. Average deflection was approximately ½ an inch (12 mm) for beams from all other ties. The MOE's of beams from tie 1600 were otherwise not unusual.

This drastic change in flexibility is more in line with soft-rot activity, and was not associated with reduced MOR or any other visible changes in the wood. Average MOE for beams cut from the tie with *Coprinellus* was 1.84 standard

deviations below the sample average. Other ties had similar MOE's, but no other tie sustained a similar amount of displacement.

Sistotrema brinkmannii

Sistotrema brinkmannii was a common isolate across gum stacks (5.3%) and appeared in oak ties (2.7%), but was poorly correlated with mass loss or losses in flexural properties (3% average mass loss) (ISA 16.7% indicator of "above average" MOE of red oak, $p = 0.151$) . The species may be variable in its decay potential. Previous reports suggested that *S. brinkmannii* did not have decay potential, but was so commonly associated with decayed wood that Carey and Hull (1989) developed selective measures against culturing it in decay studies. Wang & Zabel isolated *S. brinkmannii* from preservative treated utility poles. Our decay tests also indicated that this fungus had only minimal decay potential with a preference for hardwood.

Schizophyllum commune

Schizophyllum commune has worldwide distribution on dead wood, but is considered to have very limited decay potential (Richards & Humphrey 1939). Our results show that it caused minor decay under optimal conditions, resulting in 5.58% average mass loss across wood types over 16 weeks.

Schizophyllum commune is much better genetically equipped to degrade pectin than other biopolymers (Ohm *et al.* 2010); which may be a form of co-degradation with other fungi as pectin has been reported to be a significant barrier to enzyme degradation of cellulose and lignin (Peng *et al.* 2018).

Other Fungal Taxa

Filobasidium was not tested in decay bottles, and was not a common isolate. As noted, it may be mycophagous and was most associated with undecayed blackgum ties with above average MOE.

Seven isolates failed to cause at least 5% mass loss in at least one wood type in the soil block tests. Four of these isolates failed to cause 5% mass loss in any of the woods tested (Table 8). The presence of fungi with limited potential to decay wood such as *S. brinkmannii* and *S. commune* raises questions about their ecological roles. Possibilities include serving to pre-condition wood for more aggressive decay fungi (Hiscox *et al.* 2016), inhibiting colonization by other fungi (Boddy 2000), or serving as tertiary colonizers indicating that the wood substrate has somehow been modified (Oliver *et al.* 2010).

Radulodon was recovered 54 times, all from blackgum ties; 48 of these isolates were identified by ITS-gene identification as *R. americanus* and another six were identified as *R.casearius*. The latter identity has been documented very rarely and could warrant further identification (Nakasone 2001; Ryvarden 1972), but we could not use basidiospore characteristics because fruiting bodies were not available (Nakasone 2001). Molecular identification is still in development. The potential for damaged and varying DNA fragments, and the use of open-source database compilation create a potential for misidentification. However, the overall frequency of *Radulodon* identifications suggests that this identification is correct. Our decay test and isolate counts showed that *Radulodon* had good decay potential and was abundant, yet it was not significantly associated with reduced flexural properties during air-seasoning.

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CHAPTER 6. CONCLUSION

Relative humidity and temperature variations in stacks were slight, and were more associated with aspects of stack placement than with small changes in height.

Fungal colonization differed between timber species, reflecting the inherent susceptibility of each species to fungal attack.

Blackgum

The number of isolates in blackgum ties was poorly correlated to increasing decay, but fungal communities were dominated by fungi that can cause severe decay under optimal conditions.

Decay fungi were present in blackgum before ties were stacked, and fungal communities increased in both size and diversity over the course of air-seasoning. Increases in the fungal community were essentially equal at all heights and all sill levels; except for the uppermost height tested, where there was a smaller increase in fungi compared to 12” heights. There was no evidence that fungal frequency increased with decreasing sill height across all stacks. Interestingly, fungal isolations were sometimes higher from ties seasoned on 12” sills than from ties seasoned at lower sill heights.

While there was a trend towards decreasing strength with proximity to the ground, the effect was common in all stacks and did not appear to be related to sill height. Interestingly, ties seasoned on 12 inch sills yielded the most fungal isolations and had the lowest MOR and MOE values of all heights.

The members of the fungal community were consistent at all heights and were organized less randomly than expected by chance, but there were community differences related to different levels of strength loss within height groups. The strongest community indicators were from fungi that had associations with above-average MOR and MOE results. While most fungi contributed to decay, some might have inhibited decay.

Even though the fungal community was consistent at all levels, and we did not capture a difference in microclimate, the amount of decay was greatest in ties closer to the ground. The effect of height on reduced MOR and MOE was evident over the span of a few feet but not in the difference of a few inches between sill heights. We interpret this as indicating that similar amounts of fungi produced different rates of decay in response to microclimate differences that were not well detected. Ties seasoned on 12" sills yielded the most decay fungi as well as the lowest MOR and MOE. They were the only group with strength values lower than the estimated range of baseline measurements.

Red Oak

Red oak contained very few fungi before stacking. The number of decay fungi remained low for at least 6 months then increased rapidly. There was no evidence that reducing sill height resulted in an increase in fungal isolations. Once again, ties seasoned on 12 inch sills yielded the most decay fungi, but in positive relation to height. This may be the result of localized community expansion rather than any general effect from height.

Strength loss in red oak ties was more associated with the total number of isolates than any particular fungus, and no difference in community composition could be found among different MOR or MOE levels. Strength loss was not correlated to height or any difference in stack conditions, and there was no increase in decay or fungal community related to reduced sill heights below 12".

While ties of both timber species were colonized by a range of decay fungi, there was no evidence that sill height had any effect on either the frequency of colonization by decay fungi or residual strength of the ties at the end of seasoning.

Appendix -List of Fungi

Fungi	**Authority**
Acanthophysium bisporus	(Boidin & Lanq.)
Antrodia minuta	(Spirin.)
Antrodia oleracea	(R.W. Davidson & Lombard)
Bjerkandera adusta	(Willd.)
Chondrostereum purpureum	(Pers.)
Coprinellus radians	(Desm.)
Cylindrobasidium sp.	
Dichostereum sp.	
Epicoccum sorghinum	(Sacc.)
Filobasidium sp.	
Ganoderma sessile	Murrill
Gloeocystidiellum sp.	
Gloeophylum trabium (Lab standard: MAD 617 or similar)	(Pers.)
Gloeostereum incarnatum	(S. Ito & S. Imai)
Hydnopolyporus sp.	
Hypochnicium sp.	
Laetiporus cincinnatus	(Morgan)
Lenzites betulinus	(L.)
Peniophora sp.	
Phanerochaete chrysosporium	(Burds.)
Phlebia fuscoatra	(Fr.)
Phlebia subserialis	(Bourdot & Galzin)
Phlebiopsis flavidoalba	(Cooke)
Pholiota adiposa	(Batsch)
Pholiota limonella	(Peck)
Pholiota squarrosa	(Oeder)

Postia placenta (Lab standard: MAD 698 or similar)	(Fr.)
Punctularia strigosozonata	(Schwein.)
Radulodon americanus	(Ryvarden)
Radulodon casearius	(Morgan)
Schizophyllum commune	(Fr.)
Sistotrema brinkmannii	(Bres.)
Spongipellis delectans	(Peck)
Spongipellis pachyodon	(Pers.)
Stereum complicatum	(Fr.)
Stereum gauspapatum	(Fr.)
Stereum hirsutum	(Willd.)
Tinctoporellus epimiltinus	(Berk. & Broome)
Trametes versicolor (Lab standard: Madison 697 or similar)	(L.)
Trametes gibbosa	(Pers.)
Trametes versicolor	(L.)
Xylobolus frustulatus	(Pers.)