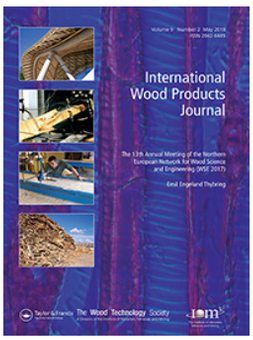

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Role of transport in wood damage mechanisms – WSE 2017 keynote lecture

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ABSTRACT

This paper is a written summary of the keynote lecture presented at the 13th Annual Meeting of the Northern European Network on Wood Science and Engineering (WSE2017). The lecture summarized work performed at the Forest Products Laboratory (Madison, WI, USA) over the past 10 years related to understanding diffusion through cell walls. The highlighted work includes advances in electrical measurements and synchrotron-based X-ray fluorescence microscopy. The paper also discusses how diffusion is related to many wood damage mechanisms. The ultimate goal of the presented research is to develop new methods of wood protection through an enhanced understanding of diffusion and its role in wood damage mechanisms.

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Introduction

Nearly all of the durability issues associated with wood involve water. When wood gets wet it swells, and when it dries it shrinks; this dimensional instability causes splitting and checking within the wood and failure of wood adhesive bonds in engineered wood products. When placed in a high humidity environment, wood is very susceptible to attack by mold fungi (Viitanen and Ojanen 2007). Finally, when the wood moisture content is high enough, fastener corrosion and fungal decay can occur, both of which can lead to structural failures (Burkholder 2004; Dunham 2013).

Over the past decade, my colleagues and I have been working to understand the fundamental root causes of these water-induced wood damage mechanisms so that new wood protection systems can be developed. We noticed that two important wood damage mechanisms, fastener corrosion and wood decay fungi, both require transport of chemicals into and through the wood cell walls. Both of these phenomena are moisture dependent and have a minimum threshold moisture content to sustain the reactions. Because the threshold moisture contents at which these wood damage mechanisms begin is well below the fibre saturation point, the damage mechanisms must involve transport through the cell wall polymers rather than through free water.

We hypothesized that transport through the cell wall was a necessary, but not sufficient, criteria for these wood damage mechanisms, and that inhibiting transport may prevent decay and corrosion (Jakes et al. 2013). This hypothesis was rooted in our earlier discovery that ionic conduction in wood was a percolation phenomenon (Zelinka et al. 2008). In this percolating system, the percolation threshold represents the moisture content at which long-range conduction can occur in the wood cell walls. It follows that the percolation threshold should be the lowest moisture content where wood damage mechanisms involving diffusion can occur. The percolation threshold was set at 16% MC as this was the first moisture content at which long-range diffusion (~3 mm) was observed in radioactive tracer measurements performed by Lin (1965). Physically, the percolation threshold represents the first moisture content at which there is a continuous pathway for diffusion; above the percolation threshold the number and connectivity of the pathways increases. However, the percolation model by itself could not give physical insight into the size, location, or structure of the pathways.

Later, shape memory experiments by Plaza et al. (2013) showed that wood exhibits a moisture-induced shape memory effect. Shape memory effects are typically associated with property changes near a glass transition (Liu et al. 2007). Plaza was able to show that in wood, the observed shape memory effects are associated with the hemicelluloses, who undergo a moisture-induced glass transition at room temperature between 60% and 80% RH (Cousins 1978; Kelley et al. 1987; Olsson and Salmén 2004). This moisture regime of hemicellulose softening corresponds with the same range of wood moisture contents where there is a rapid increase in ionic conduction (Stamm 1929; James 1963; Zelinka et al. 2008; Fredriksson et al. 2013).

This research on the moisture-induced changes to the cell wall below the fibre saturation point resulted in our current hypothesis for the relationship between cell wall moisture, diffusion, and wood damage

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mechanisms (Jakes et al. 2013; Plaza et al. 2016). We hypothesized that at a certain point, adding moisture to the cell wall will cause small domains of hemicelluloses to soften. As more moisture is added to the cell wall, the size and number of these softened regions will grow until a percolating network of softened hemicelluloses is formed within the wood (Figure 1). Furthermore, it assumed that diffusion is possible only in the softened hemicelluloses and is not possible or greatly inhibited in the glassy state. Therefore, in this theory, the percolating network of softened hemicelluloses represents a threshold for diffusion, and thus wood damage mechanisms.

Later, we re-examined our proposed transport mechanism and provided testable hypothesis into whether diffusion is a result of softened hemicellulose and whether this indeed is a necessary condition for wood decay (Zelinka et al. 2016b). Currently, experiments testing these hypotheses are under way. In the remainder of this paper, results of some recent experiments that examine these linkages between diffusion and decay are presented.

Electrical measurements

One of the primary sources of the hypothesis that diffusion may be related to wood damage mechanisms was that the electrical properties of wood can be described as a percolating ionic conductor. The data used to construct the percolation theory was collected by Stamm in the 1920s on macroscopic specimens (Stamm 1929). Mathematically, the percolation model inherently treated wood as a homogenous substrate, which is patently false; a large percentage of the volume of wood is empty cell lumina. Furthermore, the cell wall material itself is not uniform as there are chemical and structural differences between the compound middle lamella and secondary cell wall. Therefore, if transport involves a percolating network of softened hemicelluloses, then we would expect that there may be differences in the percolation threshold or conductivity of these different cell wall layers.

We tested this hypothesis by developing a method to take subcellular electrical measurements on different cell wall layers using off the shelf components (Figure 2). In the latewood, we were able to measure in both the cell corner compound middle lamella (CCCML) and the secondary cell wall (S2). In the earlywood, we were only able to measure in the CCCML. The measurements were taken with a tungsten probe whose diameter was 1 μm and conducted through the thin wood section into a metallic (grounding) plate. The probe was controlled by a micromanipulator whose minimum step size was 62.5 nm and the position was observed with a stereomicroscope. The relative humidity and thus wood moisture content were controlled by building an enclosure around the microscope and continuously flowing humidified air, provided by a relative humidity generator, through the enclosure at 2 L min^{-1}. With this experimental setup, it was possible to observe the resistance of various layers of the wood cell wall as a function of relative humidity. Using equations for the resistance of a point-source (Telford et al. 1990), and sorption data collected on southern pine (Zelinka and Glass 2010), we were able to transform these measurements into conductivity as a function of moisture content.

Figure 3 shows the data collected in CCCML and the S2 layers of the latewood along with the conductivity data of Stamm which we used to construct the percolation model. From the figure, it can be seen that the S2 and CCCML layers have different behaviours as a function of moisture content. The curve for the S2 layer has the same shape as the curve of the macroscopic data of Stamm which suggests that the S2 layer may form the basis of the percolating network observed in the

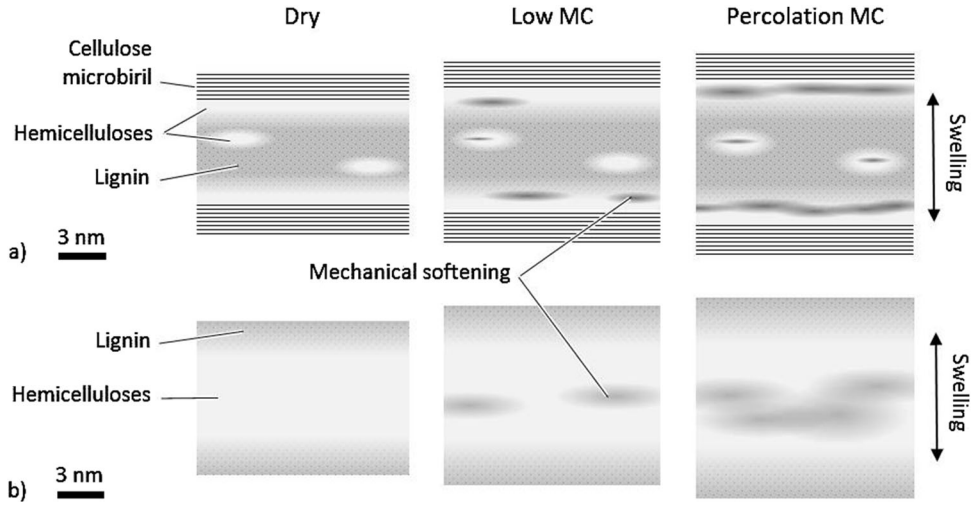

Figure 1. Schematic illustration of a percolating network of softened hemicelluloses in the S2 layer (a) and middle lamella (b) of the wood cell wall. Illustration by Dr. Joseph Jakes, first published in Jakes et al. (2013). Recent measurements using small angle neutron scattering (SANS) suggest that diffusion may also be possible within the microfibrils (Plaza et al. 2016).

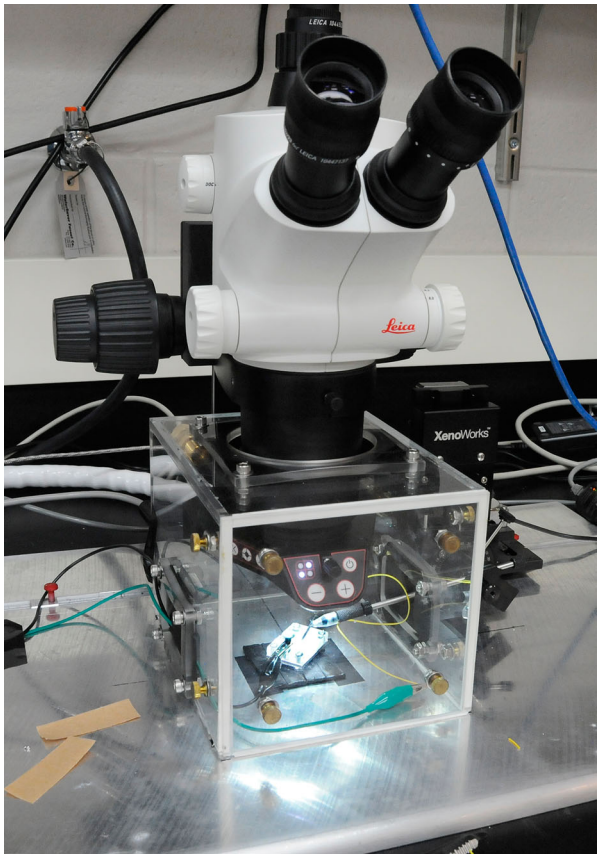

Figure 2. Apparatus of measuring the electrical properties of different cell wall layers consisting of a stereomicroscope, enclosure, RH generator (not shown), and a micromanipulator with a tungsten probe. Photo taken by Steve Schmieding (FPL), not previously published.

macroscopic data. The CCCML has a less rapid increase in conductivity with moisture content when compared to the S2 layer. However, when examined as a function of RH (not shown), the CCCML data exhibit a sharp increase in conduction between 71% and 78% RH. This sharp increase may be a manifestation of a percolation-type phenomenon in the CCCML.

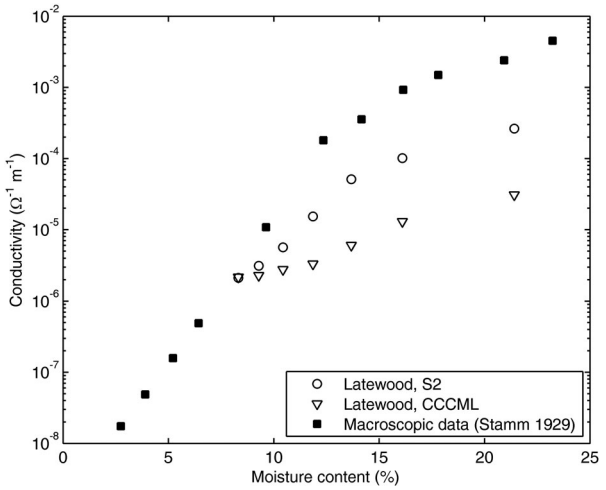

Figure 3. Key results from the subcellular electrical measurements study showing how the conductivity of the S2 layer and the cell corner compound middle lamella (CCCML) compare against the macroscopic data of Stamm. Figure first published in Zelinka et al. (2016a).

Overall, the subcellular measurements of electrical conduction are consistent with the established percolation theory. However, these data show that conductivity is not homogeneous across cell wall layers. From the proof of concept measurements in Figure 3, it is not fully clear how these differences in conduction are related to differences in cell wall structure and chemistry. However, this technique shows promise as a tool for understanding the cell wall and cell wall modifications. Future work with this technique could explore the effects of wood anatomical features, such as reaction wood, as well as chemical and thermal modifications.

X-ray fluorescence microscopy

In addition to using electrical measurements as a way of probing the ionic mobility and transport in wood, we have also used synchrotron-based X-ray fluorescence microscopy (XFM) to study transport more directly. With this technique, it is possible to measure trace levels of elements with sub-micron spatial resolution. Experiments were performed at beamlines 2-ID-E, 2-ID-D, and 8-BM at the Advanced Photon Source (APS) at Argonne National Laboratory (Argonne, Illinois, USA). The APS light source supplies more than 10^9 photons per second to the beamline where they are then run through a monochromator, and then focused with a zone plate, and order sorting aperture (OSA) before striking the sample. Fluorescence photons are then captured with a detector and the fluorescence spectra are recorded and quantified. By raster scanning the sample, a spatially resolved map of the elements can be reconstructed. We utilized the MAPS software package of Vogt to construct the quantitative elemental maps of the elements and analyse the data (Vogt 2003). The remainder of the paper presents several different XFM experiments we have performed that give insight into wood damage mechanisms.

Diffusion threshold

Percolation theory suggests that there is a threshold moisture content below which ions cannot move in wood, and we have further hypothesized that this threshold is related to moisture-induced wood damage mechanisms. However, beyond electrical measurements, there is sparse evidence for a diffusion threshold in wood. The dissertation of Lin contains the most direct observation of a diffusion threshold (Lin 1965). Lin studied the movement of radioactive tracer ions under an electric field in macroscopic wood samples with a spatial resolution of 3 mm and found a net

migration of charge at 'approximately 16% moisture content', although no mention of the temperature and RH conditions were given, nor actual gravimetric moisture contents. Given the potential importance of the diffusion threshold, we decided to use the high spatial and concentration resolution of XFM to track the movement of implanted ions as a function of relative humidity.

The experiments were performed at beamline 2-ID-E using 2 µm thick sections of *Pinus taeda* (Jakes et al. 2014; Zelinka et al. 2015). Furthermore, the experiments utilized a custom-built chamber that was placed into the beam to control the relative humidity. Sharp ion fronts were implanted on the wood sections with a micropipette positioned by a micromanipulator on an inverted microscope. A short pressure pulse was given with a microinjector which resulted in a small droplet of the salt solution being deposited on the surface of the wood which dried nearly instantaneously because of the heat produced by the lights on the microscope and the thinness of the wood section.

Figure 4 shows example data collected for potassium chloride, with the potassium map on the top and the chloride map on the bottom. The elemental map on the far left is that of zinc, which is a trace element in the wood cell walls with a higher concentration in the middle lamella (Saka and Goring 1983), which makes it possible to visualize the cell wall structure of the wood. The image labelled 'dry' was taken under a purge of dry nitrogen and shows the ion front at the beginning of the experiment. The remainder of the images are labelled with a relative humidity; before each of these images were taken, the 2 µm thick sections were brought to the RH of interest for 10 minutes and then taken back to dry conditions for imaging. The image taken at 60% RH is indistinguishable from the image taken under dry conditions suggesting that there was no diffusion below this RH. However, slight changes can be observed in the images collected at 60% RH and 67% RH. Therefore, the diffusion threshold for potassium chloride must be between 60% and 67% RH. Furthermore, above 67%, the diffusion occurs more rapidly. The image taken after the sample was held at 78% RH for 10 minutes shows that the ion front had diffused out of the field of view.

In addition to potassium chloride, measurements were also performed on copper sulphate and zinc sulphate in both the longitudinal and transverse planes. The RH threshold for diffusion ranged from 60% RH for potassium (Figure 4) to as high as 90% RH for Zn in the longitudinal direction. Interestingly, the range of relative humidities over which we see the first movement of ions is the same range of relative humidities over which the softening of hemicelluloses and a rapid increase in ionic conduction occur (Stamm 1929; James 1963; Cousins 1978; Kelley et al. 1987; Olsson and Salmén 2004; Zelinka et al. 2008; Fredriksson et al. 2013). The XFM measurements are therefore consistent with our hypothesis that diffusion is controlled by a percolating network of softened hemicelluloses. While these measurements are consistent with the hypothesis, they are not definitive proof of causation and therefore more research in this area is needed. However, these measurements show that XFM can be used as a tool to explore diffusion in wood and may be especially useful for examining how different wood modifications affect diffusion.

Fungal decay

We have also performed proof of concept measurements that show how XFM can be a tool to better understand wood decay. It is known from bulk measurements that decayed wood has higher concentrations of Ca, Mn, K, Fe, and Al (Blanchette 1984; Green et al. 1991; Jellison et al. 1992, 1997; Daniel and Bergman 1997; Ostrofsky et al. 1997). For brown rot fungi, it is believed that these ions play a central

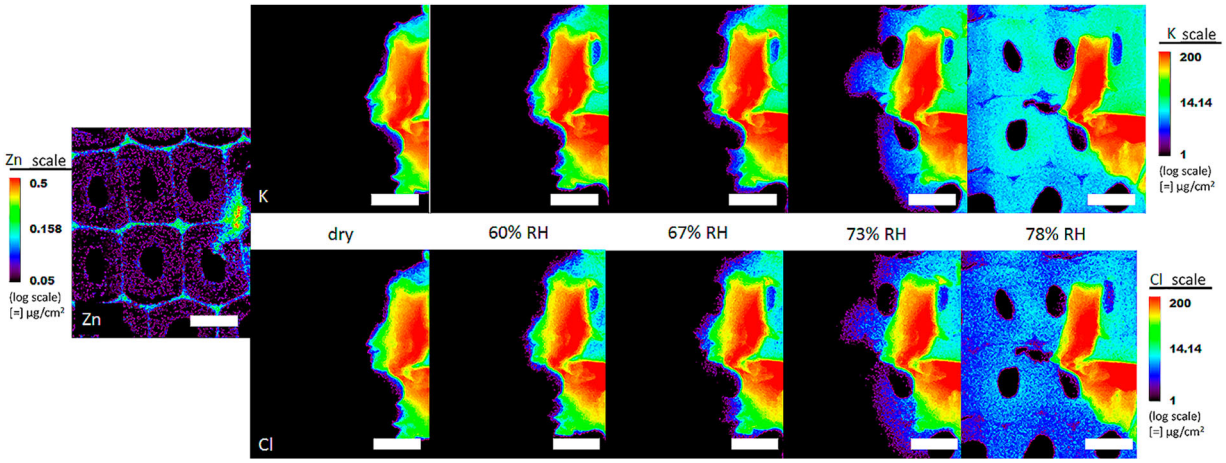

Figure 4. Example results from XFM experiment showing the threshold for diffusion of KCl in the transverse direction of a section of southern pine. The image on the far left is the elemental map and shows the cell wall structure. The top row contains images for potassium and the bottom row Cl (Zelinka et al. 2015).

role in the non-enzymatic breakdown of the cell walls (Arantes and Goodell 2014; Cragg et al. 2015). Despite the important role that metal ions play in the breakdown of wood through decay processes, relatively little is known about their spatial distribution during the decay process. Furthermore, the bulk measurements cannot ascertain whether the ions are contained intracellularly within the fungal hyphae or deposited into the cell wall. We utilized XFM to image the concentration and distribution of these ions for the first time (Kirker et al. 2017).

The measurements were performed at beamline 2-ID-E at the APS and utilized 2 µm thick sections of *Pinus taeda*. To image the fungal growth, the sections were first mounted in Kapton™ (DuPont, Wilmington, Delaware) frames with a hole (approximately 1 mm) in which the section was mounted. These frames were then placed on top of feeder strips in a modified AWPA E-10 soil block test (Anon 2012) with *Serpula lacrymans*. After the exposure period, the feeder strips were removed from the bottle. The Kapton frames were carefully separated from the hyphal mat using a scalpel and stored under desiccant until they could be imaged at the same beamline as the diffusion experiments using similar settings.

Figure 5 presents representative data from the experiment. The wood section after exposure can be seen in Figure 5(a). The decay had progressed enough to cause splitting of the wood section; the red box in Figure 5(a) represents the field of view shown in Figure

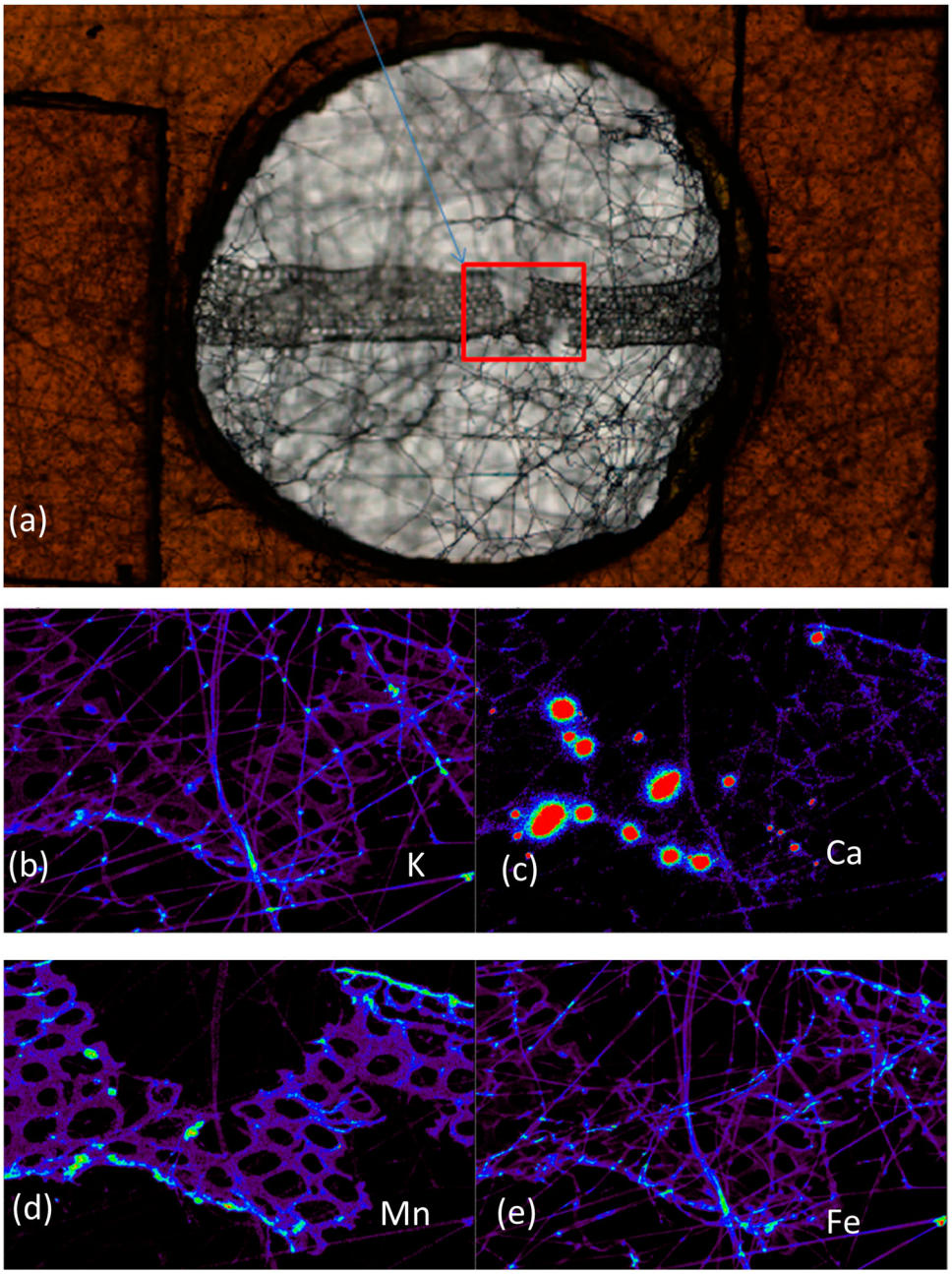

Figure 5. Example results from XFM experiments examining *S. lacrymans* colonizing a thin section of wood. (a) Optical microscopy (b–e) elemental maps of K, Ca, Mn, and Fe, respectively.

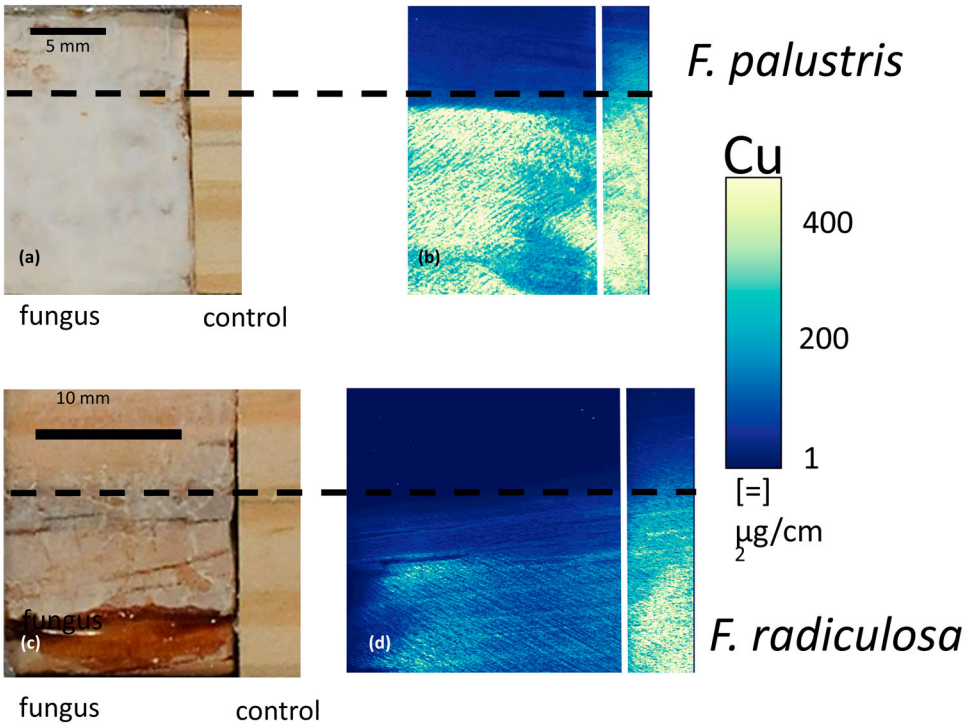

Figure 6. Photographs (a,c) and XFM maps of copper concentration (b,d) from *Fomitopsis palustris* (a,b) and *Fibroporia radiculosa* (c, d). The solid white line on the XFM map separates the control sample from the sample exposed to fungus. The black dotted line approximates the portion of the sample dipped in copper at the start of the experiment. (Image not previously published, similar to images to be published in Zelinka et al. (under consideration)).

5(b–f). Figure 5(b–e) shows the intensities of K, Ca, Mn, and Fe, respectively, and utilize a colour map going from deep blue (low concentration) to red (high concentrations). In addition to the cell walls, which are visible in the maps of K, Mn, and Fe, hyphae are also visible, especially in the K and Fe maps. The Ca map also has an interesting pattern; the Ca appears in small regions of very high concentrations. One interesting aspect is that the majority of the Ca in the image appears to be located adjacent to the hyphae where there is cell wall material. Optical microscopy in the same region (not shown) has confirmed these spots are calcium oxalate crystals.

While these preliminary measurements give us some insights into the spatial distribution of ions in brown rot decay, they are obviously very limited and include only one time point and one species of fungi. Hopefully, further work using this technique can better elucidate the role of ions in the wood decay process.

Copper-tolerant fungi

Ions are not just important for fungal metabolism but also play a key role in wood protection; nearly all commercial waterborne wood preservatives contain copper as one of the main fungicidal components (Lebow 2004, 2010). However, certain fungi are copper-tolerant and have the ability to colonize and break down copper-treated wood. While copper-tolerant fungi have been studied for over 30 years, (Murphy and Levy 1983) understanding mechanisms of copper tolerance is still an active area of investigation (Hastrup et al. 2005; Schilling and Inda 2011; Tang et al. 2013, 2016; Ohno et al. 2016). Current understanding of copper tolerance involves either the sequestration of copper into copper oxalate crystals (Schilling and Inda 2011) or intracellular removal of copper through ATPase pumps, especially in the fungus *Fibroporia radiculosa* (Tang et al. 2013; Ohno et al. 2016). However, these theories were developed based upon gene expression and wet chemical methods that do not allow for direct visualization of fungal–copper interactions. We again utilized XFM to examine these fungal–copper interactions (Zelinka et al. under consideration).

In addition to the sub-micron scale XFM, the APS also has a complementary beamline (8BM) where much larger elemental maps can be obtained on a beam with a larger spot size and lower spatial resolution. We utilized this large field-of-view beamline to visualize what happened as the fungus approached a front of deposited copper in the wood. In this experiment, blocks of wood were partially dipped into a solution of copper sulphate. The blocks were then exposed in petri dishes above malt agar extract with a monoculture of fungus; end-matched partner blocks were placed in uninoculated petri dishes as a control. Samples were then imaged after either 8 or 9 weeks of exposure to the fungus.

Figure 6 shows example results from two copper-tolerant fungi: *Fomitopsis palustris* and *Fibroporia radiculosa.* In each subfigure, the image on the far left is a

photograph of the wood that was imaged, the left side was exposed to the fungus and the right side is the end-matched control. Next to the photograph of the sample are the maps of the copper concentration. Other elemental maps (K, Ca, Mn, Fe, Zn) were also collected but are not shown.

From the photographs, it is clear that both fungi were able to colonize the entire block as evidenced by the hyphal growth on the surface even though approximately 50% of the block was treated with a copper solution; the sample exposed to *F. radiculosa* had several cracks and the dark line on the bottom of the sample is where epoxy was needed to hold the sample together for imaging. The area of the treatment can be seen by examining the right side of copper maps which show the unexposed control. The controls show that both blocks were dipped to approximately the same depth in the same treatment solution and have high amounts of copper. However, there were large differences in how these fungi interacted with the copper. In the sample exposed to *Fibroporia*, there was a large reduction in the copper concentration when compared to its end-matched control. Conversely, in *Fomitopsis*, the copper concentration is indistinguishable from the control. This image suggests that there may be differences in the mechanisms of copper tolerance in these two fungi.

Similar to previous studies presented in this paper, this examination of copper tolerance with XFM is not exhaustive. However, this preliminary investigation shows that it can be possible to use XFM to differentiate ion profiles from different fungi. In the future, it may be possible to combine this technique with gene expression or other techniques used to study copper tolerance to give a fuller picture of the wood decay fungi.

Concluding remarks

This paper presents work I have been involved with during the past decade with my colleagues at the Forest Products Laboratory. During this time, we have developed new tools for probing how water changes wood and how those changes lead to wood damage mechanisms. Clearly much more work needs to be done, but it is my sincere hope that these tools can be adopted by other research groups worldwide. Hopefully, through our collaborative efforts, we can jointly use these tools to explore wood damage mechanisms and develop new wood protection strategies.

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This keynote talk would not be possible without many collaborators and co-authors the author has had on the works presented in this paper, especially, Drs Joseph Jakes, Samuel V. Glass, Grant T. Kirker. The machine shop at Forest Products Laboratory was essential for completing several of these studies, and notably constructed the *in situ* relative humidity chamber for beamline 2-ID-E.

Disclosure statement

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