

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1

Biology

**Untreated and copper-treated wood soaked in sodium oxalate:
Effects of decay by copper-tolerant and copper-sensitive fungi**

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Paper prepared for the IRG48 Scientific Conference on Wood Protection
Ghent, Belgium
4-8 June 2017

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ABSTRACT

Copper is widely used as the primary component in wood protectants because it demonstrates a broad range of biocidal properties. However, a key concern with using copper in wood preservative formulations is the possibility for brown-rot basidiomycetes to resist the toxic effect. Many brown-rot basidiomycetes have evolved mechanisms, like the production and accumulation of oxalate, which helps these fungi to tolerate copper-treated wood by detoxifying copper. The purpose of this study was to determine if untreated wood and copper-treated wood soaked in sodium oxalate influenced the rate of decay by brown-rot basidiomycete fungi. Both untreated and 1.2 % ammoniacal copper citrate-treated test blocks were subjected to an additional sodium oxalate treatment via two soaking methods (ten minute and two hour). Test blocks were exposed to two *Fibroporia radiculosa* isolates (FP-90848-T and L-9414-SP) and one isolate of *Gloeophyllum trabeum* isolate (MAD 617) and evaluated for weight loss at four and eight weeks. Decay was between 40-43% weight loss at week eight for *F. radiculosa* L-9414-SP when untreated blocks were soaked with sodium oxalate. *F. radiculosa* L-9414-SP demonstrated decay of 38% at week eight when copper citrate-treated blocks were soaked with sodium oxalate. *F. radiculosa* FP-90848-T decay was much lower for untreated blocks soaked with sodium oxalate (12-13%) and only slightly higher on copper-treated blocks soaked with sodium oxalate (19%) by week eight. *G. trabeum* MAD 617 decay was between 40-46% when untreated blocks were soaked with sodium oxalate. *G. trabeum* was unable to successfully decay the copper citrate-treated blocks soaked with sodium oxalate (0.5%) by week eight. The copper-tolerant and copper-intolerant test fungi used in this study demonstrated no major increase in decay when untreated and copper-treated wood was amended with oxalate.

Keywords: oxalate, copper-tolerance, brown-rot decay

1. INTRODUCTION

The porous nature of wood makes it easily susceptible to biological attack by a wide range of biological agents (i.e. bacteria, fungi, insects, etc.), resulting in severe deterioration of wood-based materials. Because of the high biodegradability of wood products, preventative measures to protect the wood have evolved. Today, copper is the primary biocide used in wood protectants for residential applications because it exhibits algacide, bactericide, fungicide, insecticide, and moldicide properties (Freeman and McIntyre 2008). Copper possesses efficacy against a wide variety of wood-inhabiting organisms including the decay fungi. However, the use of copper as the primary form of protection can pose certain problems including tolerance, corrosivity, and aquatic toxicity.

Some of the most destructive organisms to wood in-service are the decay fungi, which initiate wood cell degradation by depolymerizing the structural components (lignin, cellulose, and hemicelluloses) found within the wood cell wall. Brown-rot fungi can employ enzymatic or non-

enzymatic (i.e. low molecular weight mediators) mechanisms to remove cellulose and hemicellulose components (Nilsson 2009; Goodell et al. 2003). Some brown-rot fungi are stimulated by their environment. Specifically, copper-tolerant brown-rot fungi thrive in environments with high concentrations of copper due to their ability to detoxify the copper ions found in copper-treated wood (Gadd 1993; DeGroot and Woodward 1999; Pohleven et al. 1999; Hall 2002; Humar et al. 2002; Green and Clausen 2003; Hastrup et al. 2005; Freeman and McIntyre 2008). The presence of copper stimulates a rapid production of low molecular weight mediators, like oxalate, and copper is inactivated by the excess oxalate (Green and Clausen 2005).

Although, oxalate has been shown to function in a number of unique ways in brown-rot fungal decay, its importance is still not entirely known. Researchers have shown brown-rot fungi to decrease wood pH via oxalate production, which is necessary to facilitate initial colonization and to maintain the function of extracellular proteins used during non-enzymatic decay (Green et al. 1991; Humar et al. 2001; Goodell 2003; Clausen and Green 2003; Green and Clausen 2003, 2005). Specifically, in copper-tolerant brown-rot fungi, it has been hypothesized that the production and accumulation of oxalate are crucial to the decay mechanism (Murphy and Levy 1983; Sutter et al. 1983; Daniel 1994; Leithoff et al. 1995; Pohleven et al. 2002; Green and Clausen, 2003, 2005; Hastrup et al. 2006; Freeman and McIntyre 2008; Arango et al. 2009; Schilling and Inda 2011; Ohno et al. 2015). Several theories involving the significance of oxalate have been suggested including managing pH during fungal establishment (Bech-Anderson 1987; Green et al. 1991; Shimada et al. 1994; Clausen and Green 2003; Goodell 2003;), regulating acid hydrolysis of cellulose (Schwarze 2007), facilitating iron reduction in non-enzymatic decay (Schmidt et al. 1981; Arantes et al. 2009), aiding in the chelation and detoxification of metal ions (Schilling and Jellison 2005), solubilization and translocation by hyphal transport, and functionality as both an organic acid and chelator (Kartal et al. 2015).

Copper-tolerant fungi continue to be problematic necessitating the use of co-biocides in copper-containing preservative treatments in use today. This study evaluates if readily available oxalate added to untreated and copper-treated test blocks has an effect on fungal decay of copper-tolerant and copper-sensitive fungi. In this study, we exposed both untreated and copper-treated blocks soaked in sodium oxalate to two isolates of copper-tolerant *Fibroporia radiculosa* (FP-90848-T and L-9414-SP) and compared them to those exposed to the copper-sensitive fungus, *Gloeophyllum trabeum* (MAD 617). Specifically, we aimed to determine if soaking both untreated and copper-treated test blocks in sodium oxalate solution would increase decay by *G. trabeum* and *F. radiculosa*, which could indicate oxalate is an essential component of copper-tolerance. A better understanding of the importance of oxalate in brown-rot decay is necessary to develop improved protection of preservative-treated wood products from copper-tolerant organisms.

2. MATERIALS AND METHODS

2.1 Fungal Organisms

Two copper-tolerant isolates of *Fibroporia radiculosa* (Peck) Gilb & Ryvarden (L-9414-SP and FP-90848-T) (USDA-NRS-FMHC Forest Products Laboratory, Madison, WI) were used in this study. A copper-sensitive fungus, *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. isolate MAD 617 (USDA Forest Products Laboratory, Madison, WI) was also used in this study for comparison. Fungal cultures were previously grown on malt extract agar (MEA) (BD, Fisher Scientific) at 27°C and 70% relative humidity (RH).

2.2 Treatments

Southern yellow pine (SYP) test blocks (10mm³) were vacuum-treated with 1.2% ammoniacal copper citrate according to the AWP A E10-16 Standard (AWPA 2016). Untreated SYP blocks served as controls. Following treatments, all blocks were conditioned at 27°C and 30% RH for two weeks before secondary treatment with sodium oxalate.

2.2.1 Sodium Oxalate Treatment

Untreated and copper-treated test blocks were then soaked in 50mM sodium oxalate solution (following Schilling and Jellison 2005) for ten minutes or two hours. Table 1 outlines the configuration of the treatments used in this study.

Table 1: Treatment configurations

Sodium Oxalate (50mM)	Untreated (UN)	Copper-treated (CC)
No soak	UN	CC
10 minute soak	UNox10m	CCox10m
2 hour soak	UNox2h	CCox2h

Following the secondary sodium oxalate treatment, test blocks were reconditioned at 27°C and 30% RH for two weeks and steam-sterilized (122°C) for 20 minutes prior to being subjected to decay.

2.3 Decay Tests

Decay tests were set up according to the AWP A E10-16 Standard (AWPA 2016) at 27°C and 70% RH. Test fungi were allowed to colonize SP feeder wafers for two weeks prior to the introduction of the SYP test blocks. After addition of the test blocks, the decay tests were incubated at 27°C and 70% RH for up to eight weeks. Sampling occurred at both four and eight weeks (n=9). All test blocks were oven-dried overnight (60°C) and reconditioned (27°C and 30% RH) for two weeks prior to calculating percent weight loss (%).

3. RESULTS

The aim of this study was to determine if decay of copper-tolerant *F. radiculosa* and copper-sensitive *G. trabeum* increased when exposed to untreated and copper-treated test blocks soaked in sodium oxalate.

3.1 Effect of Sodium Oxalate on Decay of Untreated Wood

Decay values (% weight loss) at weeks four and eight of untreated SYP blocks (UN), untreated SYP blocks soaked in the sodium oxalate solution for ten minutes (UNox10m), and untreated SYP blocks soaked in sodium oxalate for two hours (UNox2h) are presented in the series of graphs below for the three fungal isolates used in this study (Figures 1-3). Decay values represent the average of nine biological replicates.

Results for copper-sensitive *G. trabeum* MAD 617 decay of untreated blocks soaked in sodium oxalate are shown in Figure 1. By eight weeks, decay by *G. trabeum* had reached 42% on untreated controls. Interestingly, decay by *G. trabeum* of untreated SYP blocks soaked in sodium oxalate for two hours was slightly higher (46%) compared to the ten minute soak (40%) after eight weeks.

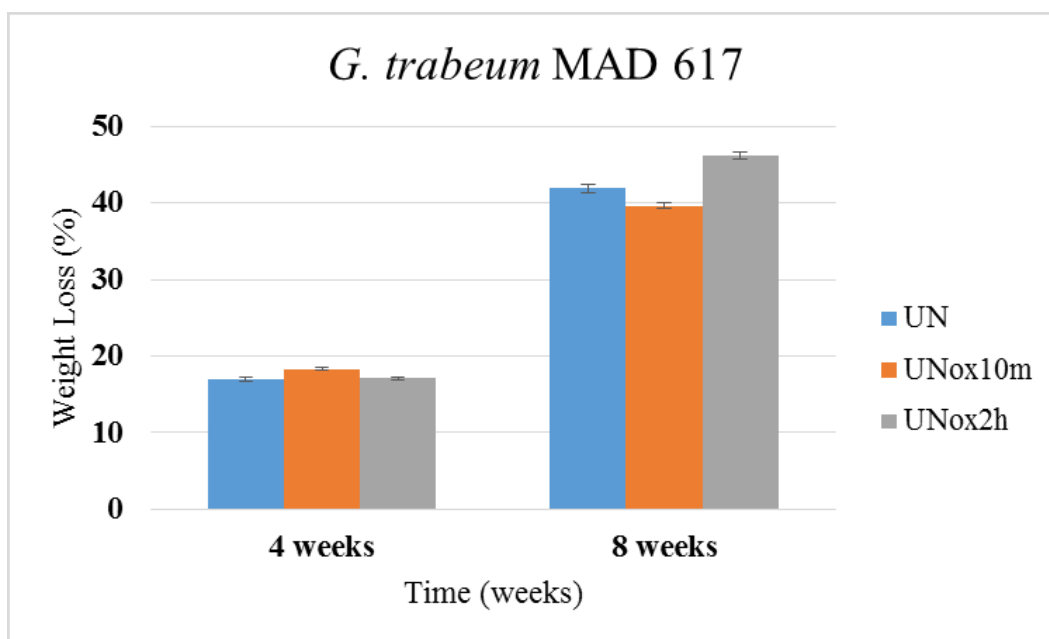


Figure 1: Weight loss (%) of copper-sensitive *G. trabeum* MAD 617 exposed to SYP blocks: UN (■), UNox10m (■), and UNox2h (■) for four and eight weeks.

Decay results of untreated blocks soaked in sodium oxalate and exposed to copper-tolerant *F. radiculosa* FP-90848-T are presented in Figure 2. By eight weeks, decay by FP-90848-T had only reached 13% for the untreated controls. Decay rates for the ten minute sodium oxalate soak was 13% while the decay of the two hour sodium oxalate soak was 12% after eight weeks.

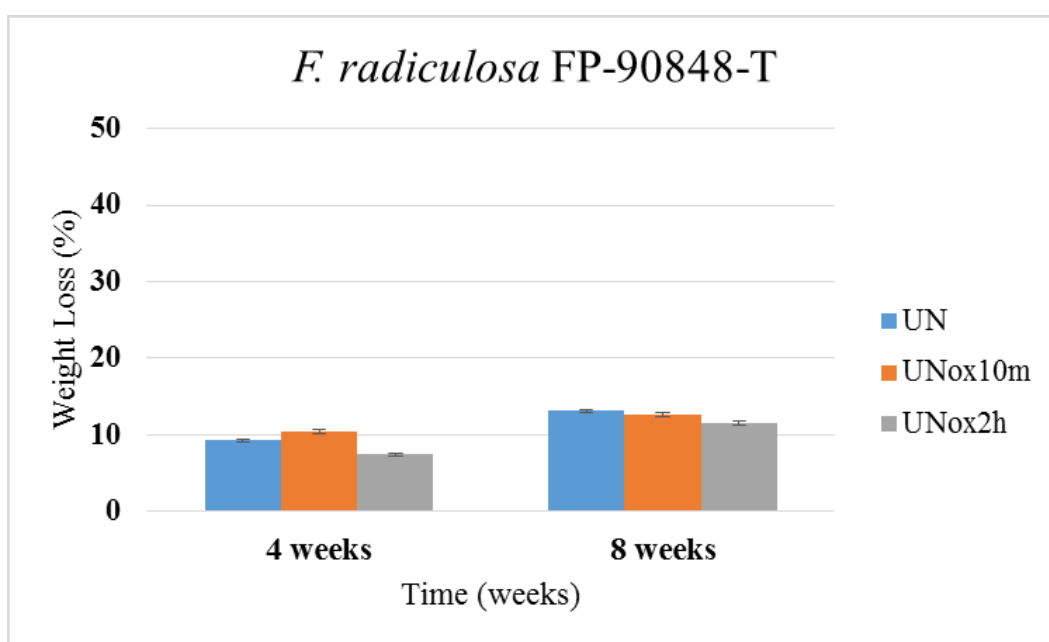


Figure 2: Weight loss (%) of copper-tolerant *F. radiculosa* FP-90848-T exposed to UN (■), UNox10m (■), and UNox2h (■) for four and eight weeks.

Decay results of untreated blocks soaked in sodium oxalate for copper-tolerant *F. radiculosa* L-9414-SP are presented in Figure 3. Decay by L-9414-SP had reached 36% on untreated controls by eight weeks. Decay of untreated blocks soaked in sodium oxalate for ten minutes was higher (43%) than when soaked in sodium oxalate for two hours (40%) after eight weeks. Interestingly, decay of both sodium oxalate soaks were higher than the untreated controls after eight weeks.

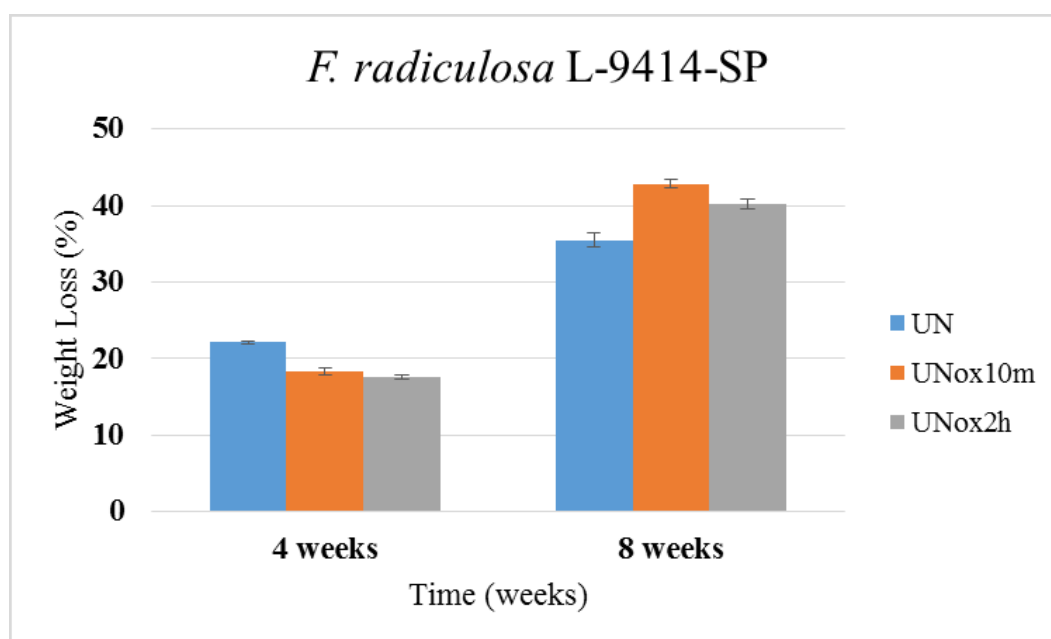


Figure 3: Weight loss (%) of copper-tolerant *F. radiculosa* L-9414-SP exposed to UN (■), UNox10m (■), and UNox2h (■) for four and eight weeks.

3.2 Effect of Sodium Oxalate on Decay of Copper-treated Wood

In the subsequent graphs, decay (%) of copper-treated SP blocks (CC), copper-treated SP blocks soaked in sodium oxalate for ten minutes (CCox10m), and copper-treated SP blocks soaked in sodium oxalate for two hours (CCox2h) are presented over time for the three test fungi. Again, decay rates presented are the average of nine biological replicates. Decay results of copper-treated blocks soaked in sodium oxalate for copper-sensitive *G. trabeum* MAD 617 clearly shows copper-sensitivity on all treatments (Figure 4). Decay rates by *G. trabeum* only reached 1% by eight weeks on copper-treated blocks. In addition, *G. trabeum* was unable to decay the copper-treated blocks soaked in sodium oxalate after eight weeks.

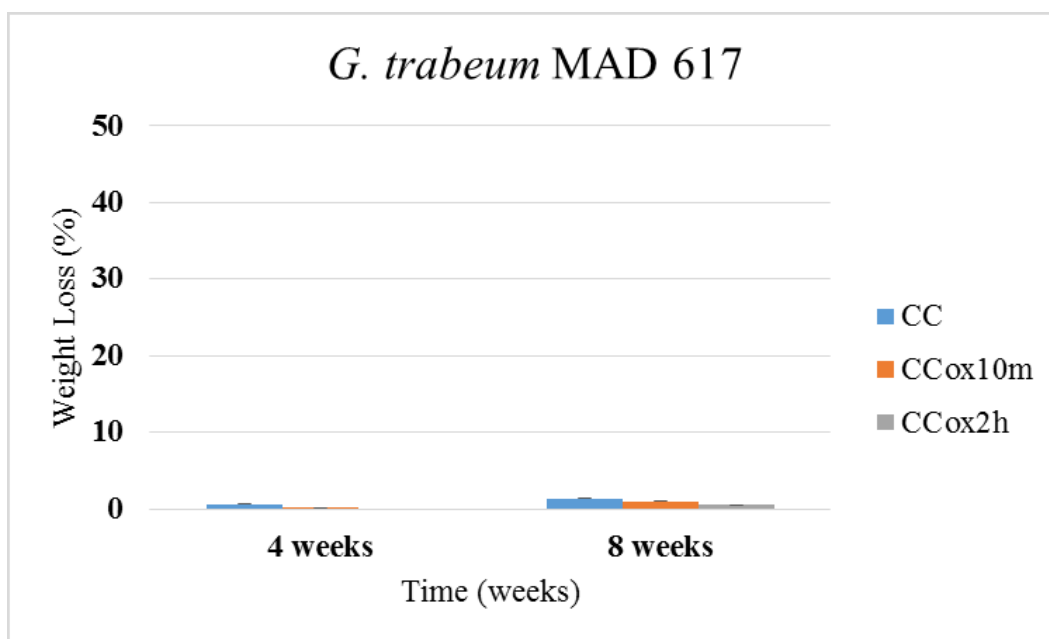


Figure 4: Weight loss (%) of copper-sensitive *G. trabeum* MAD 617 exposed to SYP blocks: CC (■), CCox10m (■), and CCox2h (■) for four and eight weeks.

Decay results of copper-treated blocks soaked in sodium oxalate for copper-tolerant *F. radiculosa* FP-90848-T are shown in Figure 5. By eight weeks, decay by FP-90848-T had reached 21% on the copper-treated controls. FP-90848-T decay of the copper-treated blocks soaked in sodium oxalate was slightly lower (19%) when compared to the copper-treated controls.

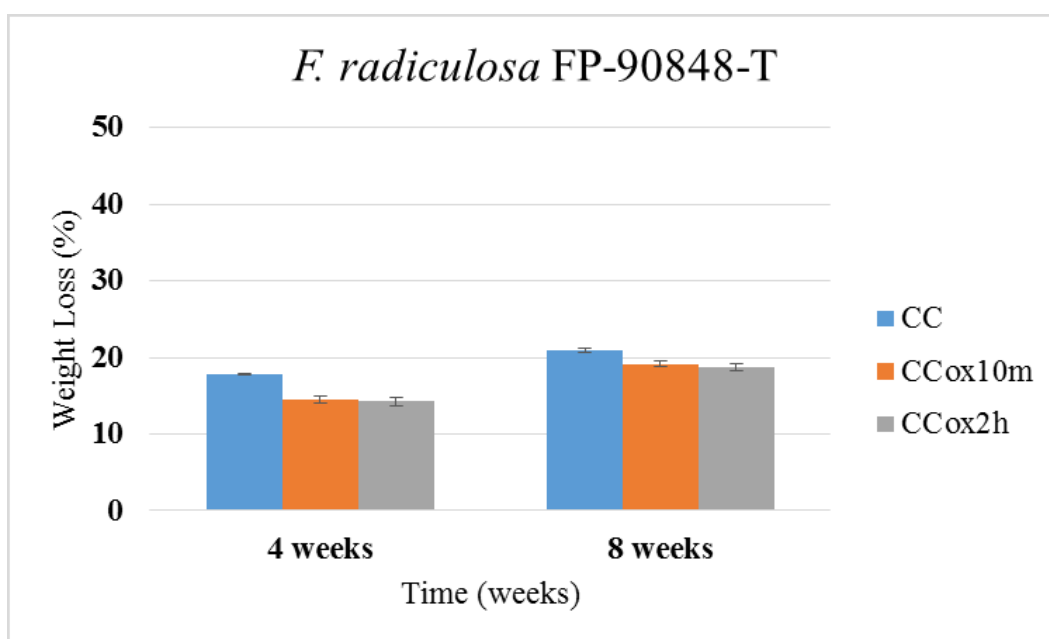


Figure 5: Weight loss (%) of copper-tolerant *F. radiculosa* FP-90848-T exposed to CC (■), CCox10m (■), and CCox2h (■) for four and eight weeks.

Results of copper-tolerant *F. radiculosa* L-9414-SP decay of copper-treated blocks soaked in sodium oxalate are presented in Figure 6. L-9414-SP decay of the copper-treated controls had reached 43% by eight weeks. Decay of copper-treated blocks soaked in sodium oxalate was 38% by eight weeks. In addition, decay of the copper-treated blocks soaked in sodium oxalate was lower than the copper-treated controls.

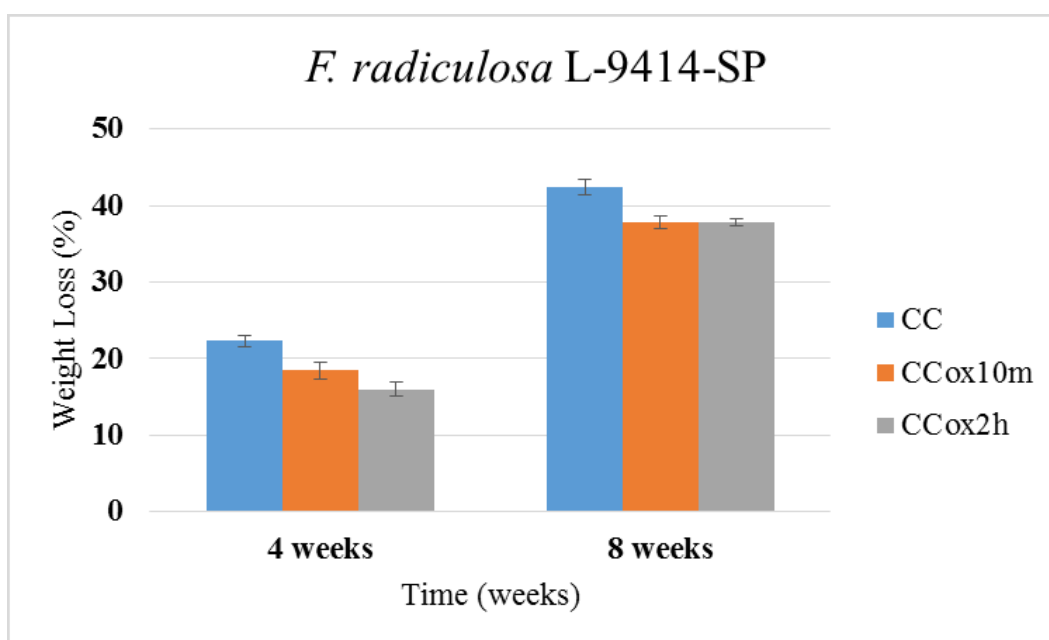


Figure 6: Weight loss (%) of copper-tolerant *F. radiculosa* L-9414-SP exposed to CC (■), CCox10m (■), and CCox2h (■) for four and eight weeks.

4. DISCUSSION

Untreated blocks soaked in sodium oxalate showed no major effect on decay of the three test fungi used in this study. *G. trabeum* decayed untreated blocks soaked in sodium oxalate for two hours slightly more (46%) than the ten minute soaked (40%) and the untreated controls (42%). *F. radiculosa* FP-90848-T was only able to decay all blocks slightly (12-13% in all treatments), which indicates this fungus was less vigorous in this study than what we typically observe. This could be a result of the age of the fungus. *F. radiculosa* L-9414-SP decayed untreated blocks soaked in sodium oxalate for ten minutes slightly higher (43%) than when soaked for two hours (40%) both of which were higher than and the untreated controls (36%).

Similar to the untreated sodium oxalate blocks, copper-treated sodium oxalate blocks showed no notable increase in decay of the three test fungi used in this study. *G. trabeum* was unable to decay copper-treated controls or copper-treated blocks soaked in sodium oxalate. *F. radiculosa* FP-90848-T decayed copper-treated controls slightly more (21%) than copper-treated blocks soaked in sodium oxalate (19%). However, FP-90848-T was stimulated by the copper treatment with and without sodium oxalate, which caused greater decay than in untreated blocks with and without sodium oxalate at both time intervals. *F. radiculosa* L-9414-SP decayed copper-treated controls slightly higher (43%) than when soaked in sodium oxalate (38%).

Schilling and Jellison (2005) found that the dry rot fungus, *Meruliporia (Poria) incrassata*, did not increase decay of spruce blocks amended with 50mM sodium oxalate. These results are comparable to the decay of untreated blocks soaked in sodium oxalate when exposed to *G. trabeum* and both *F. radiculosa* isolates. In addition, Schilling and Jellison (2005) showed that the brown-rot fungus, *Fomitopsis pinicola*, had a decrease in decay of spruce blocks amended with 50mM sodium oxalate. These results are similar to the decay of the copper-treated blocks soaked in sodium oxalate when exposed to the two *F. radiculosa* isolates.

5. CONCLUSION

From the results of this study, we determined that the addition of 50mM sodium oxalate to both untreated and copper-treated test blocks did not enhance the decay of *G. trabeum* MAD 617, *F. radiculosa* FP-90848-T, or *F. radiculosa* L-9414-SP. This study showed that the mechanism of copper-tolerance does not solely rely on oxalate (i.e. additional components are essential to the success of copper-tolerant fungi). In addition, this study only measured decay (%) when the three test fungi were subjected to untreated and copper-treated blocks soaked in sodium oxalate. Future studies should measure soluble and insoluble oxalate concentrations in the test blocks, pH values, and vary the sodium oxalate concentration of the soak treatments.

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