IRG/WP 00-50145

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

SECTION 5 ENVIRONMENTAL ASPECTS

Bioprocessing Preservative-Treated Waste Wood

By

Barbara L. Illman, Vina W. Yang and Les Ferge

USDA Forest Service
Forest Products Laboratory
Madison, WI 53705

Prepared for the 31st Annual Meeting
Kona, Hawaii USA
May 14 – 19, 2000

IRG Secretariat
SE-10044 Stockholm
Sweden

Bioprocessing Preservative-Treated Waste Wood

Barbara L. Illman, Vina W. Yang and Les Ferge
USDA Forest Service, Forest Products Laboratory, Madison, WI, USA

Abstract

Disposal of preservative-treated waste wood is a growing problem worldwide. Bioprocessing the treated wood offers one approach to waste management under certain conditions. One goal is to use wood decay fungi to reduce the volume of waste with an easily managed system in a cost-effective manner. Wood decay fungi were obtained from culture collections in the Mycology Center and Biodeterioration research unit at the USDA-FS Forest Products Laboratory (FPL), Madison, Wisconsin, and from FPL field sites. The 95 isolates had originally been taken from at least 66 sites from around the United States. Isolates were screened in a bioassay (known as the 'choice test') for tolerance to CCA, ACQ, creosote and pentachlorophenol. A tolerant rating was based on fungal growth toward or on treated wood, with 17 tolerant to CCA, 21 to ACQ, 12 to creosote and 5 to pentachlorophenol. Decay capacity of the tolerant isolates was determined as percent weight loss by the ASTM D-1413-76 soil bottle method. We identified 8 isolates for experiments on preservative remediation. Isolates of *Meruliporia incrassata* and *Antrodia radiculosa* gave the highest percent degradation of ACQ and CCA-treated wood. Several *A. radiculosa* isolates and a *Neolentinus lepideus* isolate grew on creosote-treated wood, but had only a 4-5% weight loss. In this paper we discuss the potential use of decay fungi to degrade or remediate preservative-treated wood.

Introduction

The disposal of preservative-treated waste wood is a growing problem worldwide (Cooper, 1993; Falk, 1997; Leithoff, 1995). Potential waste management systems are currently being studied for composting or bio- remediation of the spent wood (Clausen and Smith, 1998; DeGroot and Woodward, 1998); Kamden et al, 1998; Stephen et al, 1996). Most spent wood is treated with waterborne preservatives due to the large quantity in service. Over 98 percent of the wood treated in the United States in 1993 was treated with waterborne preservatives (Micklewright, 1994) such as chromated copper arsenate (CCA), ammoniacal copper arsenate (ACA) and ammoniacal copper zinc arsenate (ACZA). Utility poles and railroad ties treated with creosote are being taken out of service and accumulating as problem waste products.

Bioprocessing of waste wood has potential as a management system under some conditions. Several isolates of wood decay fungi have long been reported to be tolerant to and even degrade treated wood, especially wood treated with copper-based preservatives (Cowling, 1957; DeCosta and Kerruish, 1964; Duncan, 1957; Thornton and Tighe, 1987; Young, 1961; Zabel, 1954).

A long-term goal of this research is to develop a method to reduce the volume of waste wood with an easily managed, cost-effective system using preservative-tolerant wood decay fungi. In this paper we present the biological assay for screening fungal isolates exhibiting preservative-tolerance and the degradation tests to determine capacity of the isolates to degrade preservative-treated wood.

Materials and Methods

Fungal Cultures

Fungal isolates were obtained from five facilities supported by the USDA Forest Service, Forest Products Laboratory: the Biodeterioration RWU4502, the Center for Forest Mycology Research, four research field sites located in Gulfport, Mississippi and Middleton and Madison, Wisconsin. These brown- and white-rot fungi were selected because they were originally taken from failed preservative-treated wood in field tests, were reported to be preservative tolerant or were taken from discarded utility poles and railroad ties.

Fungi were transferred from stock cultures to 2% malt extract agar (MEA) plates and allowed to grow in the dark for 7-10 days at 80°F and 70% relative humidity (RH) prior to the bioassay for preservative-tolerance or ASTM soil bottle assay for degradation efficiency. The 13 isolates that have been screened for CCA-tolerance and degradation of CCA-treated wood (Illman and Highley, 1996) have been added to this study.

Preservative-Treated Wood

Southern yellow pine lumber treated with CCA-C to 0.04 pounds per cubic foot (pcf) or ACQ-D treated to 0.40 pcf was obtained from a commercial lumber company in Madison, Wisconsin. The lumber had been treated by Quality Wood Treating Co., Prairie du Chein, WI, and Northern Crossarm Co. Inc., Chippewa Falls, WI, respectively. Creosote-treated Southern yellow pine test blocks were cut from utility poles supplied by Madison Gas and Electric Power Company, Madison, WI, and from railroad pilings supplied by Wisconsin Southern Railroad. Pentachlorophenol-treated Southern yellow pine wood was treated at the Forest Products Laboratory. Wood blocks were vacuum impregnated with technical grade pentachlorophenol (96% active ingredients) from Monsanto Co., St. Louis, MO, to retain 0.40 pcf pentachlorophenol by weight.

Assay for Preservative Tolerance

The bioassay used to determine preservative-tolerance was described by Leithoff et al (1995) as a 'choice test' screening method to identify CCA-tolerant. A disk of mycelia taken from the growing edge of fungal culture was placed in the center of water agar petri dish containing a woodblock treated with preservative and a control non-treated block at opposite sides of the dish. The fungi were incubated for 7-14 days and growth recorded. A total of 84 isolates were subjected to the 'choice test'.

Degradation Test

The capacity of preservative-tolerant fungi to degrade treated wood was determined as per cent weight loss by the standard soil bottle method (ASTM D-1413-76). Five replicate wood blocks (2.5 by 2.5 by 0.9 cm) treated with CCA, ACQ, creosote or pentachlorophenol were preconditioned in a humidity room, exposed to the appropriate fungus, incubated for 12 days at 80°F and 70% RH. Blocks were removed from bottles, dried, weighed and weight loss determined as per cent of original weight. Fast growing isolates from the 'choice test' and the Biodeterioration RWU collection isolates (Illman and Highley, 1996) were selected for the ASTM degradation test.

Results and Discussion

Wood inhabiting fungi were screened as potential candidates for bioprocessing waste wood treated with CCA, ACQ, creosote or pentachlorophenol. Fungi were originally collected from at least 66 sites in the United States as shown in Figure 1.

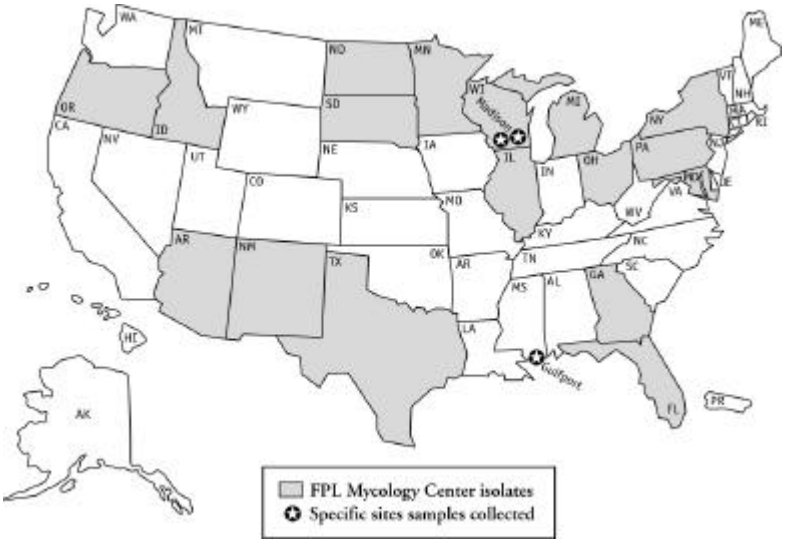

Figure 1. Geographical Origin of Fungal Isolates

A total of 95 fungal isolates were obtained from around the United States. They include 21 from the FPL Biodeterioration RWU4502, 62 from the FPL Center for Forest Mycology Research, 12 from the research field sites. On the map in Figure 1, the field sites are designated with a star and the Mycology Center collection sites are designated gray. Fungi that were newly isolated from field sites, railroad ties, pressure-treated wood and utility poles were given an isolate number. All but 7 of the fungi were basidiomycetes and were

identified to species. The names of the basidiomycetes are given in Tables 1 and 2 below. The species of the non-basidiomycetes remain unknown. Cultures of all isolates are maintained in the FPL Biodeterioration RWU.

Preservative tolerance of the fungi was determined by the ‘choice test’, with the exception of some fungi previously reported to be preservative tolerant. A tolerant rating was based on fungal growth toward or on treated wood.

The fungi identified by the choice test included 17 tolerant to CCA, 21 to ACQ, 12 to creosote and 5 to pentachlorophenol. We identified 8 isolates for future experiments on preservative remediation. From the fast growing isolates in the 'choice test' and the Biodeterioration collection, 15 brown-rot and 5 white-rot isolates were tested for degradation efficacy of creosote and pentachlorophenol treated southern yellow pine and 37 were tested for degradation of ACQ and CCA wood.

Table 1. Screening fungi for tolerance to preservatives by 'choice test' procedure

Fungus	CCA	ACQ	creosote	PCP
Antrodia radiculosa (FP-103272)	+	-	-	-
Antrodia radiculosa (MJL-630)	+	-	-	-
Antrodia radiculosa (FP-90848)	-	+	-	-
Antrodia radiculosa (FP-103272-sp)	+	-	-	-
Antrodia xantha (ME-268)	+	-	-	-
Antrodia xantha (FP-100046)	-	+	-	-
Ceriporia spissa (FP-133233-sp)	-	+	-	-
Ceriporia spissa (12A4ck)	-	-	+	-
Chain chlamydospora (ME-681)	-	+	-	-
Chain chlamydospora (Aho-TM-38)	-	+	-	-
Chain chlamydospora (Piirto 23B)	-	+	-	-
Ceriporia spissa (RLG-6838-sp)	-	+	-	-
Crustoderma dryinum (FP-133453-sp)	-	+	-	-
Crustoderma dryinum (Kropp62C1)	-	-	+	-
Diplomitoporus lindbladii (FP-105349-sp)	-	+	-	-
Diplomitoporus lindbladii (FP-134600)	+	+	-	-
Gloeophyllum subferrugineum (FRI-417/R)	+	-	-	-
Gloeophyllum subferrugineum (FRI-88(a)C)	-	+	-	-
Gloeophyllum subferrugineum (FPRI-508)	-	-	+	-
Gloeophyllum trabeum (Boat-228)	+	-	-	-
Gloeophyllum trabeum (Mad-617-R)	-	-	+	-
Gymnopilus sp (HHB-14860T)	-	+	-	-
Irpex lacteus (HHB-7328)	-	-	-	+
Melanoporia niger (MD-278)	-	-	+	-
Meruliporia incrassata (Mad-563)	+	-	+	+
Meruliporia incrassata (TFFH -294)	+	-	-	-
Neolentinus lepideus (RLG-7891-sp)	-	-	+	-
Neolentinus lepideus (HHB-13625)	+	-	-	-
Neolentinus lepideus (Mad-534)	-	-	+	-
Peniphora pseudopini (HHB-4876-sp)	-	+	-	-
Peniphora pseudopini (HHB-11655)	-	+	-	-
Peniphora pseudopini (TCS-13)	-	-	+	-
Phanerochaete chrysosporium (P6G)	+	-	-	-
Phanerochaete chrysosporium (F43G)	+	+	-	-
Phanerochaete chrysosporium (F65F)	+	+	-	-
Phanerochaete chrysosporium (O55B)	+	+	-	-
Phanerochaete chrysosporium (F66C)	+	+	-	-
Phanerochaete omnivorum (KKN-112-sp)	+	+	-	-
Phanerochaete omnivorum (HHB-5969)	+	+	-	-
Phanerochaete sordida (FP-101975)	-	+	-	-
Polyporus sp (FP-101605-sp)	-	-	+	-
Phanerochaete sordida (Kropp 36T2)	-	-	+	-
Phanerochaete chrysosporium (BKM-F1767)	-	-	-	+
Phanerochaete sordida (Kropp-36T2)	-	-	-	+
Phlebia concentrica (L-10540-sp)	-	+	-	-
Polyporus sp. (FP-134933)	+	-	-	-
Polyporus sp (FP-101605)	-	-	-	+
Pycnoporus sanguineus (RLG-10851)	-	+	-	-
Schizophyllum commune (TJV-93-5)	-	+	-	-
Sistotrema brinkmannii (HHB-10096-sp)	-	+	-	-
Trichaptum byssogenum (FP-105308-R)	+	-	-	-

Table 2. Fungal Degradation of Preservative-Treated Wood*

Fungus	Untreated	ACQ	CCA
Meruliporia incrassata (TFFH-294)	62.2 ± 2.9	9.7 ± 5.7	36.8± 2.7
Antrodia radiculosa (MJL-630)	32.6 ± 4.8	6.7 ± 6.8	26.6± 2.9
Meruliporia incrassata (Mad-563)	62.5 ± 2.5	3.5 ± 0.1	23.7± 7.0
Antrodia radiculosa (FP-90848)	39.5 ± 4.1	29.9 ±14.3	20.1± 7.7
Antrodia radiculosa (FP-103272-sp)	24.6 ± 6.0	0.7 ± 0.1	6.5 ± 4.7
Antrodia radiculosa (FP-105309-R)	27.2 ± 3.0	4.4 ± 4.0	2.3 ± 0.8
Antrodia radiculosa (L-11659-sp)	23.1 ± 2.7	0.8 ± 0.3	1.3 ± 1.3
Phanerochaete omnivorum (KKN-112)	6.0 ± 1.1	1.3 ± 0.4	1.1 ± 0.3
Gloeophyllum subferrugineum (FRI-88(a)c)	24.8 ± 0.6	1.6 ± 0.2	0.6 ± 0.4
Gloeophyllum trabeum (Boat-228)	33.7 ± 1.7	1.4 ± 0.3	0.6 ± 1.9
Antrodia xantha (Boat-173)	33.8 ± 6.5	2.0 ± 0.8	0.4 ± 0.1
Antrodia xantha (ME-268)	48.6 ± 6.6	2.1 ± 0.5	0.4 ± 0.1
Pycnoporus sanguineus (RLG-10851)	16.5 ± 2.1	1.0 ± 0.1	0.4 ± 0.1
Phanerochaete omnivorum (HHB-5969-sp)	5.6 ± 4.3	1.7 ± 0.2	0.2 ± 0.4
Phanerochaete chrysosporium (F43G)	1.3 ± 1.1	1.2 ± 0.2	0.2 ± 0.1
Antrodia xantha (ACC-111)	29.6 ± 3.1	1.3 ± 0.6	0.1 ± 0.1
Phanerochaete chrysosporium (055B)	0.5 ± 0.2	1.3 ± 0.1	0.1 ± 0.1
Phanerochaete chrysosporium (F66C)	0.5 ± 0.2	1.1 ± 0.4	0.0 ± 0.2
Antrodia xantha (ACC-109)	23.8 ± 1.8	0.9 ± 0.3	-0.1 ± 0.1
Sistrotrema brinkmannii (HHB-10096-sp)	0.2 ± 0.1	1.1 ± 0.4	-0.1 ± 0.1
Diplomitoporus lindbladii (FP-134600)	19.7 ± 1.3	2.7 ± 0.9	-0.2 ± 0.3
Phanerochaete sordida (FP-101975)	18.7 ± 1.0	1.6 ± 0.6	-0.2 ± 0.1
Sistrotrema sp. (ME-681)	0.3 ± 0.6	1.3 ± 0.4	-0.2 ± 0.1
Antrodia xantha (FP-100046-R)	20.7 ± 2.2	2.2 ± 0.4	-0.3 ± 0.0
Antrodia xantha (ME-550)	31.0 ± 2.0	1.0 ± 0.4	-0.3 ± 0.0
Phlebia concentrica (L-10540-sp)	9.4 ± 1.0	1.2 ± 0.2	-0.3 ± 0.0
Schizophyllum commune (TJV-93-5)	0.2 ± 0.3	1.1 ± 0.4	-0.3 ± 0.0
Phanerochaete chrysosporium (F65F)	0.2 ± 0.1	1.3 ± 0.3	-0.3 ± 0.4
Ceriporia spissa (FP-133233)	0.1 ± 0.3	0.9 ± 0.2	-0.4 ± 0.1
Diplomitoporus lindbladii (FP-105349)	4.5 ± 5.2	1.0 ± 0.4	-0.4 ± 0.4
Phanerochaete chrysosporium (P6G)	0.1 ± 0.3	1.4 ± 0.2	-0.4 ± 0.5
Peniophora pseudopini (HHB-11655-sp)	0.8 ± 0.9	1.0 ± 0.4	-0.6 ± 0.4
Polyporus sp. (FP-134933)	3.7 ± 2.1	0.7 ± 0.3	-0.6 ± 0.4
Gloeophyllum subferrugineum (FRI-417/R)	8.9 ± 1.5	1.4 ± 0.1	-0.7 ± 0.3
Neolentinus lepideus (HHB-13625-sp)	38.8 ± 5.3	1.4 ± 0.3	-0.7 ± 0.4
Gymnopylus sp. (HHB-14860-T)	4.8 ± 0.9	1.4 ± 0.8	-0.7 ± 0.6

* Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures, ASTM D-1413-76

Table 3. Fungal Degradation of Preservative-Treated Wood*

Fungus	Untreated	Creosote	PCP
Antrodia radiculosa (MJL-630)	27.0 ± 2.9	1.7 ± 0.2	1.5 ± 0.1
Antrodia radiculosa (FP-90848)	51.0 ± 2.1	2.1 ± 0.2	2.6 ± 0.5
Antrodia radiculosa (FP-103272-sp)	20.8 ± 6.1	5.5 ± 2.0	4.7 ± 2.3
Antrodia radiculosa (FP-105309-R)	20.1 ± 4.2	2.9 ± 0.8	2.4 ± 0.6
Antrodia radiculosa (L-11659-sp)	15.2 ± 3.7	3.2 ± 1.8	5.3 ± 1.8
Antrodia radiculosa (HHB-11414-sp)	38.4 ± 4.0	1.5 ± 0.2	1.2 ± 0.3
Gloeophyllum subferrugineum (FPRI-508)	-0.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Gloeophyllum trabeum (Mad-617-R)	34.7 ± 0.7	2.1 ± 0.4	1.3 ± 0.0
Gloeophyllum trabeum (Boat-228)	33.1 ± 1.5	1.7 ± 0.6	1.6 ± 0.3
Irpex lacteus (HHB-7328-sp)	21.5 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
Melanoporia niger (MD-278)	55.3 ± 5.2	1.4 ± 0.1	1.3 ± 0.0
Meruliporia incrassata (TFFH-294)	46.1 + 1.7	1.8 ± 0.2	1.9 ± 0.3
Meruliporia incrassata (Mad-563)	59.7 ± 3.0	1.5 ± 0.0	4.1 ± 2.5
Neolentinus lepideus (RLG-7891-s)	27.3 ± 5.9	1.8 ± 0.2	1.6 ± 0.1
Neolentinus lepideus (Mad-534)	22.3 ± 1.9	4.1 ± 0.7	1.5 ± 0.1
Neolentinus lepideus (HHB-13625-sp)	33.6 ± 3.9	1.5 ± 0.1	1.2 ± 0.2
Phanerochaete chrysosporium (ME-461)	-0.8 ± 0.2	1.2 ± 0.1	1.2 ± 0.1
Phanerochaete chrysosporium (BKMF-1767)	0.3 ± 0.9	0.9 ± 0.1	1.1 ± 0.1
Phanerochaete sordida (Kropp 36T2)	15.4 ± 1.0	1.3 ± 0.0	1.3 ± 0.2
Polyporus sp. (FP-101605-s)	17.4 ± 1.2	1.3 ± 0.1	1.1 ± 0.1

*Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures, ASTM D-1413-76

The fungi identified by the choice test included 17 tolerant to CCA, 21 to ACQ, 12 to creosote and 5 to pentachlorophenol. We identified 8 isolates for future experiments on preservative remediation. From the fast growing isolates in the 'choice test' and the FPL Biodeterioration RWU collection, 15 brown-rot and 5 white-rot isolates were tested for efficacy to degrade creosote and pentachlorophenol treated southern yellow pine, 37 were tested for degradation of ACQ and CCA wood.

Isolates of *Meruliporia incrassata* and *Antrodia radiculosa* gave the highest percent degradation of ACQ and CCA-treated wood. Several *A. radiculosa* isolates and a *Neolentinus lepideus* isolate grew on creosote-treated wood, but had only a 4-5% weight loss. Although some fungi were identified as preservative tolerant by the 'choice test', they did not degrade treated wood. We have selected at least 8 of these isolates for future studies on bioremediation. White- and brown-rot fungi exhibited differences in tolerance and decay capacities. The white-rot fungi did not degrade untreated wood, but appeared to have an effect on creosote and pentachlorophenol treated wood. White-rots were more tolerant to organic while brown-rots were more tolerant to waterborne preservatives. Results from these tests will be used for degradation of waste wood as an alternative to landfills, referred to as composting by some authors, or for bioremediation of preservatives or clean-up sites.

References

ASTM. 1998. Standard Test Method for Wood Preservatives by Laboratory Soil-Block Cultures. Designation D 1413-76. Annual Book of ASTM Standards, Vol. 04.10 Wood. American Society for Testing and Materials, West Conshohocken, PA.

Bryde, R.J.W. 1961. Non-aromatic organics. In: Fungicides II, T.C. Torgeson, ed., Academic Press, N.Y. and London.

Clausen, C.A. and R.L. Smith 1998. Removal of CCA from treated wood by oxalic acid extraction, steam explosion, and bacterial fermentation. J Indust. Microbiol. & Bact. 20:251-257.

Cooper, P.A. 1993. Leaching of CCA: Is it a problem? Disposal of treated wood removed from service: The issues. Proceedings from the Environmental Considerations in the Manufacture, Use and Disposal of Preservative Treated Wood. Carolinas'Chesapeake Section of the Forest Products Society. May 13. Richmond, VA. pp.45-54.

Cowling, E.B. 1957. The relative preservative tolerances of 18 wood-destroying fungi. Forest Products Journal 7:355-359.

DaCosta, E.W.B. 1959. Abnormal resistance of *Poria vaillantii* (D.C.exFr.) Cke. Strain to copper chrome-arsenate wood preservatives. Nature 1843: 910-911.

DaCosta, E.W.B. and Kerruish, R.M. 1964. The tolerance of *Poria* species to copper-based wood preservatives. Forest Products Journal 14:106-112.

Falk, Bob. 1997. Opportunities for the Woodwaste Resource. Forest Products Journal 47(6): 17-22.

DeGroot, r.C., and B. Woodward. 1999. Wolfiporia cocos-A potential agent for composting or bioprocessing Dougls-fir wood treated with copper-based preservatives. Material u. Organismen pp 195-215.

Duncan, C.G. 1958a. Evaluating wood preservatives by soil block tests: 10. Effect of species of wood on preservative threshold values. Proceedings American Wood Preserver's Association 54:172-177.

Duncan, C.G. 1958b. Studies of the methodology of soil block testing USDA Forest Service Report 2114. 126pp.

Horsfall, J.G. 1956. Principles of fungicidal action. Chronica Botonica. Co., Waltham, Mass.

Illman, B.L. and Highley, T.L. 1996. Fungal degradation of wood treated with metal-based preservatives: 1. Fungal tolerance. The Inter. Res. Group on Wood Preserv. Document No: IRG/WP96-10163.

Kamdem, D.P., Wanli Ma, J. Zhang, and J. Zyskowski. 1998. Recovery of copper chromium and arsenic from old CCA treated commodities. The Inter. Res. Group on Wood Preserv. Document No: IRG/WP98-50118.

Leithoff, H., Stephan, I., Lenz, M.T., Peek, R-D. 1995. Growth of the copper tolerant brown-rot fungus *Antrodia vaillantii* on different substrates. The Inter. Res.Group on Wood Preserv. Document No: IRG/WP95-10121.

Micklewright, J.T. 1994. Wood preservation statistics, 1993. American Wood Preservers' Association. 9 pp.

Shimazono, H. and Takubo, K. 1952. The biochemistry of wood-destroying fungi (1st report) the Bavendam's reaction and the accumulation of oxalic acid by wood-destroying fungi. Bull. of the Japan Government Forest Experiment Station 53:117-125.

Stephan, I., H. Leithoff, and R.D. Peek. 1996. Microbial conversion of wood treated with salt preservatives. Material u. Organismen 30:179-200.

Thornton, J.D. and Tighe, M.A. 1987. Comparative testing of strains of the dry rot fungus *Serpula lacrymans* (Schum. ex Fr.) S.F. Gray. III. The action of copper naphthenate in wood. Holzforschung 41:195-197.

Young, G.Y. 1961. Copper tolerance of some wood rotting fungi. USDA Forest Products Laboratory Report 2223. 7pp.

Zabel, R.A. 1954. Variations in preservative tolerance of wood-destroying fungi. Journal of the Forest Products Research Society 4:166-169.