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Correlation between oxalic acid production and copper tolerance in *Wolfiporia cocos*

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Abstract

The increased interest in copper-based wood preservatives has hastened the need for understanding why some fungi are able to attack copper-treated wood. Due in part to accumulation of oxalic acid by brown-rot fungi and visualization of copper oxalate crystals in wood decayed by known copper-tolerant decay fungi, oxalic acid has been implicated in copper tolerance by the formation of copper oxalate crystals. Nineteen isolates of the brown-rot fungus *Wolfiporia cocos* were evaluated for oxalic acid production and weight loss on wood treated with 1.2% copper citrate. Twelve of 19 isolates that caused moderate to high weight losses in copper citrate-treated wood produced low oxalic acid in liquid culture, whereas isolates with high oxalic acid production had low weight losses in treated wood. Seven *W. cocos* isolates demonstrated enhanced weight loss in Cu-treated wood. Wood weight loss was unaffected by the presence of copper citrate for two *W. cocos* isolates and weight loss was lower for 10 isolates compared to weight losses in untreated wood. Citrate did not significantly influence oxalic acid production in liquid culture. Previous hypotheses linking oxalic acid and copper tolerance were based upon observations of single isolates of *Postia* and *Tyromyces*. Although most isolates produced more oxalic acid in copper citrate-treated wood than in untreated wood. we found no statistical relationship between the amount of oxalic acid production in liquid culture or wood and copper tolerance in *W. cocos*. Production of oxalic acid does not seem to be the factor controlling copper tolerance in *W. cocos*. The diversity seen within *W. cocos* demonstrates that caution should be used when reporting results, so that generalizations are not based on the behavior of a single isolate. Published by Elsevier Science Ltd.

Keywords: Oxalic acid; Copper tolerance; Copper citrate; Preservatives; *Wolfiporia cocos*

1. Introduction

Interest in copper-based wood preservatives has increased in recent years in response to public concern about the environment. Copper exhibits good biocidal activity (Nicholas and Schultz, 1997), but a major requirement of any formulation of copper-based wood preservative is efficacy against copper-tolerant fungi. Fungi can be extremely tolerant of toxic metals (Gadd, 1993; Schmidt and Ziemer, 1976). Early reviews of preservative tolerance by Zabel (1954) and Cowling (1957) reported comparative tolerances of economically important decay fungi to current preservatives. Cowling reported unusual tolerance to one or more preservatives in 14 of 18 fungi tested. Up to 40 times the amount of preservative needed to control the most susceptible fungi was required to prevent decay by the most tolerant fungi. Brown-rot wood decay fungi in the genus *Poria* (Pers. ex Gray), and other genera related to *Poria*, such as *Serpula* [(Pers.) Gray], *Antrodia* (D.C.: Fr.) Ryv., and *Wolfiporia* (Schw syn. *Poria cocos*), are known to be copper tolerant (Davidson and Campbell, 1954; Collet, 1992; Leithoff et al., 1995; Schmidt and Moreth, 1996; Tsunoda et al., 1997). Duncan (1955, 1958) was the first to report resistance to copper naphthenate by *P. cocos*. She suggested that variations in preservative tolerance within a species may equal that between species. DeGroot and Woodward (1999) recently showed that the decay capacity of

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certain *Wolfiporia cocos* isolates in samples of copper-treated wood was greater than in untreated controls, but considerable variation among individual isolates was noted. Collet (1992) reported that isolates of *Antrodia vaillantii* differed significantly in their tolerance to copper. Variation in preservative tolerance among isolates of individual fungal species has not been adequately investigated (Collet, 1992).

Although metals such as copper and zinc are considered essential for fungal growth, most of them are toxic even at low concentrations. Baldrian and Gabriel (1997) examined the effect of inorganic salts of metals on growth of selected wood-rotting Basidiomycetes in agar medium. They reported that copper as $CuSO_4$ was not toxic even in millimolar concentrations. A study on tolerance of specific groups of decay fungi to copper showed a greater tolerance of copper by fungi in wood than in agar (DaCosta and Kerruish, 1964). Tsunoda et al. (1997) reported a general correlation between tolerance of copper by decay fungi in wood and in agar. Studies by Thornton and Tighe (1987) and Wazny and Thornton (1986) of strains of *Serpula lacrymans* (Schum. Ex. Fr.) S.F. Gray indicated that the ability of some fungi to decay wood is stimulated by low levels of copper in the wood. lllman and Highley (1996) demonstrated that two isolates of *Meruliporia incrassata* were tolerant to high retentions of $CuSO_4$ in soil block tests. Woodward and DeGroot (1999) also reported stimulated growth in seven of 20 *W. cocos* isolated on agar medium containing low levels of $CuSO_4$, but in general found that variations to copper tolerance were significantly different among isolates tested.

A relationship between copper tolerance and oxalic acid production has been implicated, due to copper oxalate crystal formation in decayed wood (Murphy and Levy, 1983). Rabanus (1931) and Shimazono and Takubo (1952) suggested that tolerance of brown-rot fungi is linked to oxalic acid production, which presumably precipitates copper into the insoluble form of the oxalate, rendering the copper metabolite inert. Both groups concluded that lowering of the pH by oxalic acid had more to do with copper tolerance than low solubility of copper oxalate. In support of this hypothesis, Young (1961) studied *W. cocos,* isolated from failed copper naphthenate-treated fence posts. He demonstrated a striking increase in tolerance to copper when the pH of agar medium supplemented with copper sulfate was lowered from pH 6 to pH 2.

Chou (1971) reported that copper oxalate crystals could not be detected in wood infested with *Fibroporia vaillantii* (syn. *A. vailantii*) (D.C.) Cke., reportedly one of the most copper tolerant *Poria* species. He concluded that fungal metabolism may differ in wood from that in artificial medium. Clausen et al. (1994) also noted fungal physiological differences in wood versus an artificial medium for *Postia placenta.* More recent studies by Tsunoda et al. (1997) and Sutter et al. (1983, 1984) have closely examined copper tolerance in wood decay fungi. Both studies, involving copper (II) sulfate and copper naphthenate, concluded that copper tolerance in *Poria* species is a function of the precipitation of copper oxalate. These studies examined decay capacity and microscopic copper oxalate crystal formation, but did not correlate oxalic acid production with an increase in decay capacity. Our objective was to evaluate a population of *W. cocos* (Schw syn. *P. cocos*) for copper tolerance to ammoniacal copper citrate in relationship to oxalic acid production. This is the first report to examine the relationship of oxalic acid production to copper tolerance among 19 isolates within a species.

2. Experimental

2.1. Fungul cultures

Nineteen isolates of *W. cocos,* provided by the Center for Forest Mycology Research, Forest Products Laboratory, Madison, WI., were maintained on 2% malt extract agar (Difco Laboratories, Detroit, MI). Isolate designation is given in Table 1.

Table 1
Decay capacity of brown rot fungal isolates on untreated and copper citrate-treated southern pine

Fungal isolate	Mean weight loss (%)	
	Treated	Untreated
W. cocos		
MD 106R	50.15 ± 13.09	55.99 ± 3.80
FP 97438 sp	42.88 ± 3.33	42.46 ± 3.74
FP 71284-A	42.15 ± 4.11	28.19 ± 5.18
MD 104R	42.14 ± 0.73	56.43 ± 5.94
FP 71691-R	41.19 ± 5.48	64.80 ± 2.17
FP 71730-T	41.07 ± 11.85	24.66 ± 3.44
FP 71693-T	38.39 ± 8.77	32.87 ± 1.08
LogWH45A	36.34 ± 28.01	45.85 ± 11.34
L(61)l-8-A	34.54 ± 4.63	25.77 ± 3.94
MD 104	33.40 ± 5.39	15.65 ± 2.19
FP 104264	32.84 ± 9.13	28.03 ± 4.16
MD 215	32.05 ± 17.23	61.12 ± 4.33
H + E 45	27.63 ± 12.97	50.89 ± 4.25
FP 71692-R	27.19 ± 8.98	19.85 ± 3.72
Priel B25	26.24 ± 1.62	28.63 ± 3.21
MD 275	23.48 ± 19.30	55.25 ± 1.59
FP 71054	23.32 ± 12.41	32.73 ± 5.82
FP 90850-R	15.08 ± 17.44	35.72 ± 3.08
FP 90850sp	13.34 ± 10.45	55.43 ± 11.80
P. placenta		
MAD 698	28.61 ± 12.91	52.18 ± 5.87
G. trabeum		
MAD 617	1.64 ± 0.35	42.46 ± 5.09

2.2. Decay test

Southern yellow pine sapwood blocks (19 × 19 × 19 mm) were conditioned to a 6% equilibrium moisture content and weighed. The blocks were then treated with 1.2% solution of ammoniacal copper citrate giving an average retention of approximately 8.5 kg/m^3. After treatment, blocks were returned to the conditioning room for 4 weeks and again weighed. The blocks were then subjected to soil-block test (ASTM, 1994) with *Gloeophyllum trabeum, P. placenta* and 19 isolates of *W. cocos,* following the guidelines of AWPA Standard E-10 (AWPA, 1997). Five replicates of treated and untreated blocks for each fungus were tested. All blocks were steam-sterilized for 30 min without pressure prior to setting them onto the fully-grown mycelial mat of the test fungus. Soil-bottles cultures were incubated at 26°C/70% RH for 12 weeks. Following incubation, blocks were removed from bottles, brushed free of mycelium, conditioned to 6% equilibrium moisture content and weighed. Percentage of weight losses was calculated from the treated weights before as well as after decay testing.

2.3. MIC determination

The minimal inhibitory concentration (MIC) of copper citrate was determined by a dose response to 0.50, 0.01, 0.005 and 0.001 % copper citrate (2:1 CuO:citrate $(C_6H_4)_7$, Osmose, Buffalo, NY) in 2% malt extract agar (Difco Laboratories). Average radial growth of each isolate was measured (two measurements per plate at right angles to each other) after 7 days incubation at 27°C. A single concentration of citric acid (0.50%) in malt extract agar was tested; no signs of inhibition at this concentration were observed.

2.4 Mycelial weight

The mycelial weight of each fungal mat was determined by filtering mycelium from each culture filtrate described in Section 2.5 by aspiration through Whatman No. 1 filter paper. Samples were oven dried (60°C) for 24 h and weighed.

2.5. Oxalic acid assay

Oxalic acid was determined by microassay (Sigma, St. Louis, MO). Two weeks incubation at 27°C was determined to represent the peak production of oxalic acid for *W. cocos* isolates in duplicate liquid cultures consisting of 100 ml modified Bailey's Minimal medium (MM) (Highley, 1973) with 0.5% polygalacturonic acid as a carbohydrate source (Green et al., 1991). Cultures were grown in MM and MM amended with the MIC of either 0.5% citric acid or 0.001% copper citrate as determined in Section 2.3. The culture filtrate was assayed for oxalic acid. Oxalic acid determinations were also made on *W. cocos*-infected southern pine wafers (42 × 29 × 3 mm), both untreated and treated with 1.2% copper citrate as described in Section 2.2, incubated at 27°C, 70% RH for 2 weeks, and extracted with DI water for 2 h.

2.6. Microscopic observations

Treated and untreated wood blocks, decayed by six selected *W. cocos* isolates, were examined using a JEOL 840 scanning electron microscope (Peabody. MA) with a Tracor Northern energy dispersive X-ray analysis (EDAX) attachment for copper oxalate crystals.

3. Results and discussion

3.1. Decay test

The decay capacity of *W. cocos* in untreated wood was compared to that in copper citrate-treated wood (Table 1). Mean percent weight loss (n = 5) in treated wood is ranked from highest to lowest and ranges from 50.15 to 13.34%. Two isolates decayed treated and untreated wood to the same degree (FP 97438sp and Priel B25), two isolates were significantly inhibited by copper citrate (58 and 76% less weight loss compared to untreated controls for FP 90850R and FP 90850sp, respectively), and eight isolates were moderately inhibited by copper citrate (29948% less weight loss in treated wood than in untreated controls). Copper citrate-treatment of the wood stimulated the decay capacity in the remaining seven isolates. A 5–18% increase in weight loss for treated wood was seen compared to untreated wood for these seven isolates (Table 4). Decay capacity is shown for comparison for *P. placenta,* a known copper tolerant decay fungus and *G. trabeum,* a copper sensitive decay fungus, which is a nonaccumulator of oxalic acid (Table 1)

3.2. MIC

The MIC of copper citrate and citric acid were determined in malt extract agar as a preliminary step to studying the effects of copper and citric acid on fungal tolerance in situ. Results (not shown) determined that citric acid at 0.5% had no inhibitory effect on fungal growth. *W. cocos* isolates were sensitive to 0.001–0.05% copper citrate. The lowest concentration (0.001% copper citrate and 0.5% citric acid) to give uninhibited growth on agar was selected for oxalic acid production studies in liquid culture. It should be

noted that ammoniacal copper citrate agar has a pH of 9.7, while that for citric acid agar is 2.5.

3.3. Mycelial weight

Weights of mycelial mats collected from liquid cultures are given in Table 2. Mycelial weights were similar for nonamended cultures and those supplemented with citric acid. Nine isolates showed a 35–67% increase in mycelial weight in cultures supplemented with copper citrate compared to nonamended medium. This finding combined with the citric acid results suggest that it is the copper and not the citrate influencing growth for these seven isolates. The isolates with increased mycelial growth did not correlate with isolates demonstrating a stimulated decay capacity in copper citrate-treated wood (Fig. 1).

3.4. Oxalic acid production

In order to make comparisons in situ, oxalic acid production needed to be determined in liquid culture with and without ammoniacal copper citrate. Likewise, in order to determine whether citric acid influences the oxalic acid production, citric acid amended medium was tested for oxalic acid production. These results and those in unamended medium are shown in Table 3; they indicate that the effect of citric acid on oxalic acid production was not statistically significant (Spearman correlation coefficient = -0.02385). Citric acid enhanced the oxalic acid production of some isolates compared to controls, while the presence of citric acid seemed to inhibit oxalic acid production for others. Results did not correlate between treatments or isolates in liquid medium. In this study, we were unable to distinguish between free oxalic acid in the liquid culture and copper oxalate although no crystal formation was visible in liquid cultures.

Fig. 1 shows a three-dimensional comparison of weight loss in copper-treated wood, oxalic acid production in liquid medium containing copper citrate, and mycelial weight of isolates grown in liquid culture containing copper citrate. Isolates with moderate to high decay capacity are clustered in the lower quadrant for oxalic acid production and mycelial weight. This shows that the highest weight loss correlates with low oxalic acid production and low mycelial weight. This trend is also evident in Fig. 2A, which shows a two-dimensional graph of oxalic acid production in liquid culture versus decay capacity; the slope of the linear regression line is zero.

Oxalic acid production and a rapid lowering of pH by decay fungi is important in the initial stages of brown-rot (Green et al., 1991). Schmidt et al. (1981) implicated oxalic acid in nonenzymatic wood decay by brown-rot fungi. However, in a study on a nondegradative isolate of *P. placenta* (ME-20) Micales and Highley (1988) demonstrated that oxalic acid production was not related to the inability of this isolate to decay wood. In later work, Micales (1995) showed that ME-20 produced oxalate decarboxylase which rapidly broke down the OA. It was also shown in a

Table 2
Mycelial weight in vitro of 19 *W. cocos* isolates grown in liquid culture

W. cocos isolate	Mycelial weight[a] (mg)		
	A	*B*	*C*
FP 71054	81[b]	56	113
FP 90850-SP	51	43	86
FP 104264	37	31	70
FP 71693-T	50	68	66
MD 106-R	36	34	66
MD 275	31	24	51
FP 90850-R	56	32	46
FP 71284-A	16	15	42
FP 71691-R	9	16	23
H + E45	19	10	19
L (61) 1-8-A	9	7	18
Priel B25	6	11	18
FP 71730-T	16	11	18
FP 97438-SP	11	17	17
MD 215	10	11	13
MD 104-R	8	13	9
FP 71692-R	12	12	8
WH 45-A	5	12	4
MD 104	7	13	2

[a] A = Bailey's + 0.5% polygalacturonic acid. B = Bailey's + 0.5% polygalacturonic acid + 0.5% citric acid. C = Bailey's + 0.5% polygalacturonic acid + 0.001% copper citrate.
[b] Mean of duplicate samples.

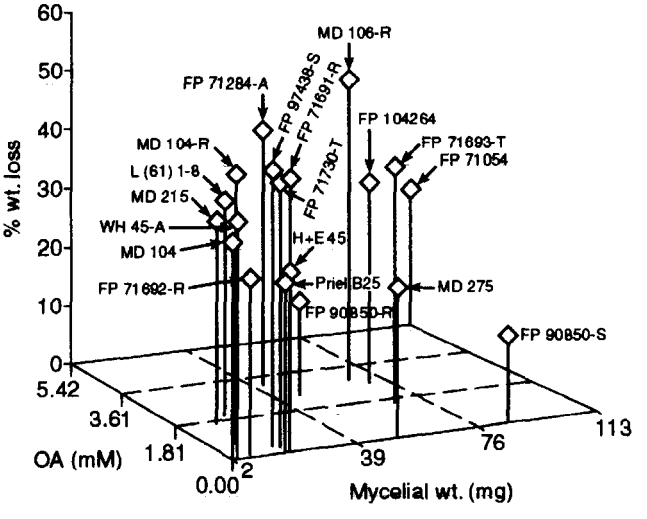

Fig. 1. Three-dimensional view of the decay capacity of *W. cocos* isolates on copper citrate-treated wood versus oxalic acid production in liquid medium containing copper citrate versus mycelial growth in the presence of copper citrate.

Table 3
Oxalic acid production in nineteen *W. cocos* isolates grown in liquid culture and on wood

W. cocos isolate	Oxalic acid (mM)				
	Bailey's medium			Southern pine	
	Untreated	Citric acid	Cu citrate	Untreated	Cu citrate
FP 90850-R	2.46	0.03	2.44	14.71	53.72
MD 215	0.44	0.04	1.64	70.36	0
FP 71691-R	0.38	0.03	0.44	8.99	48.79
FP 90850-sp	2.09	0.34	0.09	11.92	54.25
FP 71693-T	1.90	0.48	1.52	6.06	83.94
FP 104264	1.30	0	2.66	47.46	86.49
MD 275	1.05	0.02	0	19.50	83.67
FP 97438-sp	0.32	0.53	0.34	65.17	72.22
FP 71284-A	0.45	0	3.23	10.85	56.38
MD 104-R	0.22	1.57	0.65	22.03	82.34
FP 71054	2.11	0.73	5.42	3.93	53.19
MD 106-R	0.72	0	2.90	14.98	49.33
L (61) 1-8-A	0.09	0.06	1.91	34.28	65.43
Priel B25	0.06	0.04	0.07	36.28	95.65
H + E45	0.10	0.09	0.04	22.03	68.36
MD 104	0.02	0	0.02	22.97	61.84
FP 71692-R	0.04	0.25	0.08	34.41	135.73
Log WH 45-A	0	0.28	0.07	1.53	60.00
FP 71730-T	0	0.04	0.25	13.25	70.36

remediation study by Clausen and Smith (1998) that exposure of CCA-treated wood to oxalic acid is effective in removing 81% copper. In this study, oxalic acid analysis of wood wafers exposed to *W. cocos* showed a marked increase in oxalic acid production in copper citrate-treated wafers compared to untreated control wafers despite little visible mycelial growth (Table 3). This was true for all isolates except MD 215 which produced no detectable oxalic acid on copper citrate-treated wood.

Overall, oxalic acid production was enhanced on wood compared to liquid medium (Table 3). Despite the increase in oxalic acid seen in treated wood versus untreated wood, those isolates with the highest oxalic acid production, e.g. FP 71692R, Priel B25 and FP 104264, showed moderate decay capacity in copper-treated wood (26.2-32.8%). MD 215 also showed moderate decay capacity in copper-treated wood (32.1%), and although it had the highest oxalic acid production on untreated wood, its decay capacity for copper citrate-treated wood was reduced 52% compared to untreated wood.

Statistical analyses of the weight loss of copper citrate-treated wood and oxalic acid production in liquid culture medium containing copper citrate revealed that no correlation exists for these two parameters, either linearly or by rank (Pearson correlation coefficient = 0.3417, Spearman correlation coefficient = 0.24309). Statistical analyses of weight loss of copper-treated wood and oxalic acid production in copper citrate-treated wood indicated an inverse correlation for these two parameters. The Spearman correlation coefficient indicates that as weight loss for copper citrate-treated-wood increases, the oxalic acid production on copper citrate-treated wood decreases (-0.05965). A similar result was obtained with the Pearson linear correlation coefficient (-0.0730 1) for this population of *W. cocos.*

Table 4 ranks seven isolates by the increase in weight loss in copper citrate-treated wood over the

Table 4
Seven isolates[a] ranked by percentage increase in weight loss on copper citrate-treated wood versus untreated wood and corresponding oxalic acid values on treated wood

W. cocos isolate	Increase (%)	Oxalic acid (mM)
MD 104	IX	61.84
FP 71730-T	16	70.36
FP 71284-A	14	56.38
L (61) 1-8-A	9	65.43
FP 71692-R	7	135.73
FP 71693-T	6	83.94
FP 104264	5	86.49

[a] Only these seven isolates demonstrated enhanced weight loss in Cu-treated wood versus untreated wood.

weight loss in untreated wood. The presence of copper citrate stimulated the decay capacity of these seven isolates. Oxalic acid production, although higher in copper citrate-treated wood than in untreated wood (Fig. 2B and C), did not directly correlate with weight loss.

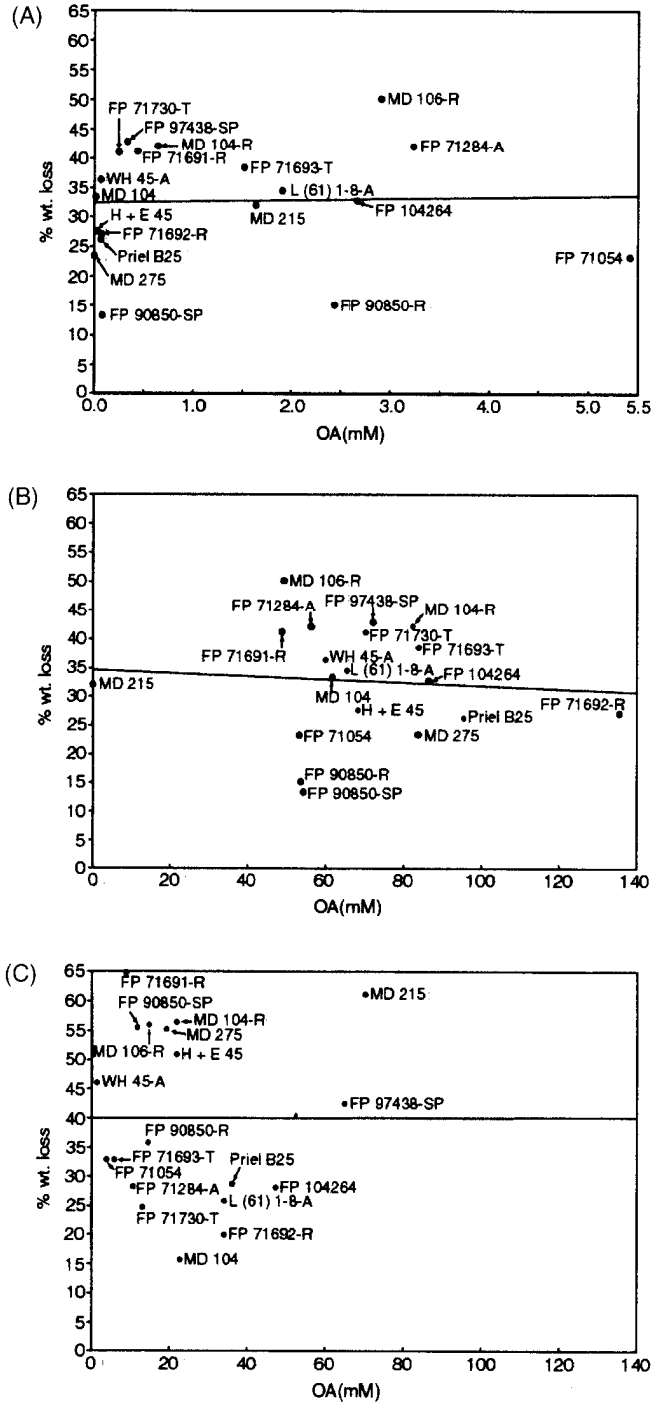

Fig. 2. The percent wood weight loss in southern pine following exposure to 19 isolates of *W. cocos* versus oxalic acid production (mM), (A) weight loss in copper citrate-treated pine blocks versus OA in liquid medium containing copper citrate, (B) weight loss in Cu citrate-treated pine blocks versus OA produced by the fungi in Cu citrate-treated pine blocks, (C) weight loss in untreated pine blocks versus OA produced by the fungi in untreated pine blocks.

3.5. SEM observations

Copper oxalate crystals were seen in six *W. Cocos* selected to represent selected to represent both high and low decay capacities in copper citrate-treated wood (23.3–50.1% wood weight loss). Fig. 3 shows a copper oxalate crystal overlaid with the EDAX spectra indicating the elemental composition of the crystal typically seen in copper citrate-treated wood decayed by *W. cocos.*

4. Conclusions

Several conclusions can be drawn from this study of 19 isolates of *W. cocos*. There was a great deal of physiological intraspecies diversity among the *W. cocos* isolates. This is believed to be the first report to look at a large number of isolates of copper-tolerant decay fungi in a single genus. Most studies on wood decay fungi include representative organisms from genera of both brown- and white-rot fungi, and often generalizations are drawn for all brown and white-rot organisms based on results from the few representatives in a study. We conclude from this study that not only should research on decay fungi include more representatives from each genus, but caution should be used when conclusions are based on a single isolate and do not represent the entire intraspecies population.

All *W. cocos* isolates in this study were copper tolerant. While copper citrate showed some degree of decay inhibition for 10 isolates, only two isolates were significantly inhibited compared to decay capacity in untreated wood. Seven isolates thrived on copper citrate, i.e. the presence of copper citrate in the treated wood apparently stimulated the decay capacity for these seven isolates. Of course, this study tests only one treatment retention of 1.2% copper citrate under optimal decay conditions, and does not characterize potential performance of this preservative under all field conditions.

Finally, all *W. cocos* isolates in this study produced oxalic acid, but no statistical correlation was seen between oxalic acid production and decay capacity. On copper citrate-treated wood, isolates with the highest decay capacity generally had low to moderate oxalic acid production in both liquid culture and wood. Oxalic acid production by *W. cocos* in liquid culture plus copper citrate (up to 5 mM) was notably less than OA production in copper citrate-treated wood (up to 136 mM). Likewise, oxalic acid production on copper citrate-treated wood wafers was four to forty times higher than that in untreated wood despite little visible

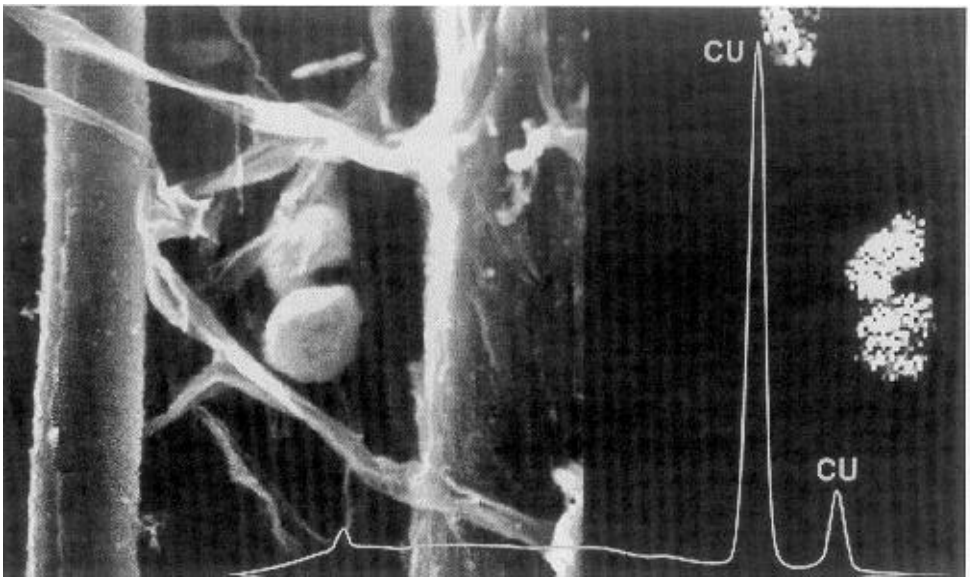

Fig. 3. A copper oxalate crystal overlaid with the EDAX spectra indicating the elemental composition of the crystal typically seen in copper citrate-treated wood decayed by *W. cocos*.

growth. The theory that lowering pH has more to do with copper tolerance than simple production of oxalic acid was not supported by the results of this study (Rabanus, 1931; Shimazano and Takubo, 1952; Young, 1961). Fifteen *W. cocos* isolates grew without inhibition at pH 9.7 on malt agar supplemented with 0.005% copper citrate. Nine isolates had increased in mycelial weight in liquid culture medium containing copper citrate 35–67%. Copper citrate stimulated decay capacity for seven isolates, but other isolates demonstrated either no increased growth, no oxalic acid production, or no increase in decay capacity on untreated wood. Clearly, high pH did not inhibit some *W. cocos* isolates and low pH was not the overriding factor for high decay capacity in other isolates. Copper oxalate crystals were seen in six selected *W. cocos* isolates representing both high and low decay capacities. If oxalic acid production, and copper oxalate crystal formation were solely responsible for copper tolerance, one might expect two outcomes from this study: (I) immobilization and detoxification of copper to decay fungi, and (2) precipitation of oxalate and thus, reduced decay capacity of the fungus. Our results do not support either of these hypotheses, but they do not exclude oxalic acid as the key factor in copper tolerance. Since it has been shown that oxalic acid is effective for removing significant amounts of copper from treated wood, we believe oxalic acid does play a role in the successful colonization of treated wood by copper-tolerant fungi. Alternative hypotheses to explain the copper tolerance of *W. cocos* include: (1) a second factor which works independent of or in concert with oxalic acid, or (2) that the production of oxalic acid and precipitation of copper simply are not linear.

In biological systems, we believe the interaction of diverse factors, i.e. growth rate, pH, production of OA, decay capacity, all contribute to copper tolerance, but may not be linear. In future experiments, oxalic acid will be measured during the course of decay by brown-rot fungi to determine if the time course of OA production correlates with copper tolerance.

References

ASTM. 1996. D 1413-76 (1994). Standard test method for wood preservatives by laboratory soil-block cultures. In: ASTM. Annual Book of Standards. vol. 4.10 Wood. American Society for Testing and Materials. West Conshohocken. PA. pp. 207-213.

AWPA. 1997. E10-97. Standard method of testing wood preservatives by laboratory soil-block cultures. In: Book of Standards. American Wood Preservers' Association, Granbury, TX. pp. 305–315.

Baldrian, P., Gabriel, J., 1997. Effect of heavy metals on the growth of selected wood-rotting Basidiomycetes. Folia Microbiology 42 (5), 521–523.

Chou, C.K., 1971. Degradation of wood by fungi as affected by preservatives. Ph.D. Thesis. Leeds, UK.

Clausen, C.A., Green III, F., Highley, T.L., 1994. Extracellular polysaccharide-degrading enzymes of *Postia placenta* isolated from wood or artificial media. In: Llewellyn, G.C., Dashek, W.V., O'Rear, C.E. (Eds.), Biodeterioration Research 4: Mycotoxins, Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration: Proceedings of 4th Meeting of the Pan American Biodeterioration Society; 1991 August 20–25. Plenum Press, New York, pp. 231–242.

Clausen, C.A., Smith, R.L., 1998. Removal of CCA from treated wood by oxalic acid extraction, steam explosion, and bacterial fermentation. Journal of Industrial Microbiology 20, 251–257.

Collet, O., 1992. Comparative tolerance of the brown-rot fungus *Antrodia vaillantii* (DC.: Fr.) Ryv. isolates to copper. Holzforschung 46, 293–298.

Cowling. E.B., 1957. The relative preservative tolerances of 18 wood-destroying fungi. Forest Products Journal 7 (10), 355–359.

DaCosta, E.W.B., Kerruish, R.M., 1964. Tolerance of *Poria* species to copper-based wood preservatives. Forest Products Journal 3, 106–112.

Davidson. R.W., Campbell, W.S., 1954. *Poria cocos*, a widely distributed wood-rotting fungus. Mycologia 46, 234–237.

DeGroot, R.C., Woodward, B.M., 1999. Using copper-tolerant fungi to biodegrade wood treated with copper-based preservatives. International Biodeterioration and Biodegradation 44, 17–27.

Duncan, C.G., 1955. Forest Products Laboratory, Madison, WI, (unpublished manuscript).

Duncan, C.G., 1958. Studies of the methodology of soil-block testing. USDA Forest Products Laboratory, Madison, WI. Report 2114. 126 p.

Gadd, G.M., 1993. Tansley review No. 47: interactions of fungi with toxic metals. New Phytologist 124, 25–60.

Green. F., Larsen, M.J., Winandy, J.E., Highley, T.L., 1991. Role of oxalic acid in incipient brown-rot decay. Material und Organismen 26 (3), 191–213.

Highley. T.L., 1973. Influence of carbon source on cellulase activity of white-rot and brown-rot fungi. Wood and Fiber Science 5, 50 58.

Illman, B.L., Highley, T.L., 1996. Fungal degradation of wood treated with metal-based preservatives: 1. Fungal tolerance. International Research Group on Wood Preservation. IRG/WP 96-10163.

Leithoff, H., Stephan, H.L., Lenz, M.T., Peek, R.-D., 1995. Growth of the copper tolerant brown rot fungus *Antrodia vaillantii* on different substrates. International Research Group on Wood Preservation. Doc. No. IRG/WP/ 95-10121, 11 pp.

Micales, J.A., 1995. Oxalate decarboxylase in the brown-rot fungus *Postia placenta.* Material und Organismen 29 (3), 177–186.

Micales, J.A., Highley, T.L., 1988. Some physiological characteristics of a nondegradative strain of Postia (= *Poria*) *placenta.* International Research Group on Wood Preservation. IRG/WP/ 1341.

Murphy, R.J., Levy, J.F., 1983. Production of copper oxalate by some copper tolerant fungi. Transactions of the British Mycological Society 81, 165–168.

Nicholas, D.D., Schultz, T.P., 1997. Comparative performance of several ammoniacal copper preservative systems. International Research Group on Wood Preservation. IRG/WP 97-30151. 7 p.

Rabanus, A., 1931. Die Toximetrische Prunfung von Holzkonservierungsmitteln. In: Angew. Bot. 13, 352–371 (Partial translation in English) American Wood Preservers' Association Proceedings 1933, pp. 34–43.

Schmidt, O., Moreth, U., 1996. Biological characterization of *Poria* indoor brown-rot fungi. Holzforschung 50, 105–110.

Schmidt, C.J., Whitten, B.K., Nicholas, D.D., 1981. A proposed role for oxalic acid in non-enzymatic wood decay by brown-rot fungi. Proceedings of the American Wood Preservers' Association. 77, 157–164.

Schmidt, O., Ziemer, B., 1976. On the inhibitor tolerance of fungi Material und Organismen 11 (3), 215–230.

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

Sutter, H.P., Jones. E.B.G., Walchli, O., 1983. The mechanism of copper tolerance in *Poria placenta* (Fr.) Cke. and *Poria vaillantii* (Pers) Fr. Material und Organismen 18 (4), 241–262.

Sutter, H.P., Jones, E.B.G., Walchli, O., 1984. Occurrence of crystalline hyphal sheath in *Poria placenta* (Fr.) Cke. Journal of the Institute of Wood Science 19–23.

Thornton, J.D., Tighe, M.A., 1987. Comparative laboratory 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.

Taunoda, K., Nagashima, K., Takahashi, M., 1997, High tolerance of wood-destroying fungi to copper-based fungicides. Material und Organismen 31 (1), 31–44.

Wazny, J., Thornton. J.D., 1986. Comparative laboratory testing of strains of the dry rot fungus *Serpula lacrymans* (Schum. Ex Fr.) S.F. Gray. Holzforschung 40. 383–388.

Woodward, B., DeGroot, R.C., 1999. Tolerance of *Wolfiporia cocos* isolates to copper in agar media Forest Products Journal 49 (4), 87–94.

Young, G.A., 1961. Copper tolerance of some wood-rotting fungi. Report 2223. Madison. WI: U.S. Department of Agriculture. Forest Service. Forest Products Laboratory. 10 p.

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