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ABSTRACT

Serpula lacrymans (Wulfen : Fries) Schröter, the dry rot fungus, is considered the most economically important wood decay fungus in temperate regions of the world i.e. northern Europe, Japan and Australia. Previously copper based wood preservatives were the most commonly used preservatives for pressure treatment of wood for building constructions. Because of a suspicion about tolerance toward copper components, a soil block test was undertaken to clarify the effect of two copper based preservatives, copper citrate and ACQ-D, on the dry rot fungus, *Serpula lacrymans* compared to an alternative non-copper containing wood preservative. The extensive use of copper-based wood preservatives has hastened the need for understanding why some fungi are able to attack copper-treated wood. The copper tolerance of *S. lacrymans* and other brown-rot fungi is thought to be due in part to oxalic acid production and accumulation. Oxalic acid has been implicated in copper tolerance by the formation of copper oxalate crystals. Twelve isolates of the dry rot fungus, *S. lacrymans* and four other brown rot species were evaluated for weight loss on wood treated with 1.2% copper citrate, 0.5% ACQ-D and 0.5% N'N-naphthaloylhydroxylamine (NHA). Eleven out of 12 *S. lacrymans* were shown to be tolerant towards copper citrate. ACQ-D and NHA, on the other hand, were both effective against the dry rot isolates. These wood preservatives are less toxic toward the environment than traditional copper based preservatives.

Keywords: *Serpula lacrymans*, dry rot, copper tolerance, copper citrate, ACQ-D, NHA

1. INTRODUCTION

Copper tolerance can be defined as the ability of an organism to grow and thrive in the presence of copper ions. Within recent years the focus on copper-based wood preservatives has increased following the debate about the environmental effect of these components. Copper-based preservatives have been widely and successfully used for more than a century (Humar *et al.*, 2001), since copper exhibits good biocidal activity (Nicholas and Schultz, 1997). It is still the most frequently used formulation for wood preservation (Peek *et al.*, 1993) but a major requirement of any formulation of copper-based wood preservative is efficacy against copper-tolerant fungi. The copper-based preservatives have been used for treatment of wood for protection against wood decaying fungi especially the dry rot fungus, *Serpula lacrymans.*

S. lacrymans has become an increasing hazard in houses in the temperate regions of the world and it is of great economical importance to be able to hinder damage by this fungus.
S. lacrymans has previously been tested for copper tolerance with contradicting results. Schmidt and Moreth (1996) tested the influence of increasing copper concentrations on mycelial growth, when grown on malt agar. *S. lacrymans* was only able to grow at the lowest concentration of 1 mM Cu, and at a concentration of 5 mM Cu no growth was reported (Schmidt and Moreth, 1996). Others found low levels of copper in the wood were able to stimulate decay in of some strains of *S. lacrymans* and that the relative copper tolerance of strains varied with the formulation of the preservative (Woodward and De Groot, 1999). This variation in formulation was tested using both copper citrate and AQC-D (copper quaternary ammonia-type D). Again others have found *S. lacrymans* not to be copper tolerant. (Tsunoda *et al.*, 1997) found a single isolate of *S. lacrymans* not to be copper tolerant. It was possible for them to inhibit the growth of *S. lacrymans* with at copper concentration of 1250-2500 ppm. The decay ability of *S. lacrymans* was completely suppressed at a retention of 2.0 kg/m^3 copper(II) sulphate (Tsunoda *et al.*, 1997).
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 (1933) was the first to link the production of oxalic acid with copper tolerance and he demonstrated precipitation of copper oxalate when fungi were grown on an agar plate containing copper sulphate. Rabanus (1933) 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 (Rabanus, 1933;Schimazono and Takubo, 1952). Since then copper tolerance has been linked to oxalic acid production in a number of fungal species such as *Wolfiporia cocos* and *Poria placenta* (Sutter *et al.*, 1983;Clausen *et al.*, 2000). In contrary, Schmidt and Moreth (1996) found that despite considerable oxalic acid production by the brown rot *Antrodia sinusa,* this fungus did not show copper tolerance. Furthermore, 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). The same may be true for *S. lacrymans*. Green and Clausen (2003) found rapid induction of oxalic acid (exceeding 600 μmole/g) by 11 copper tolerant brown-rot fungi effectively decayed copper citrate-treated southern pine blocks (Green and Clausen, 2003).
Oxalic acid reacts, in preserved wood, with copper to form copper oxalate, which is subsequently precipitated (Woodward and De Groot, 1999; Humar *et al.*, 2001). This mechanism is considered to contribute to the detoxification of copper in copper-treated wood, which enables fungi to tolerate environments containing high concentrations of toxic metals. Since copper oxalate is insoluble, copper in this form has a greatly reduced inhibitory effect on fungal growth (Humar *et al.,* 2001).
The objective of this study was to evaluate a population of *S. lacrymans* for copper tolerance to ammoniacal copper citrate and copper quaternary ammonia-type D in comparison to an alternative wood preservative, N'N-naphthaloylhydroxylamine (NHA). This is the first report to examine the effect of NHA within the species of *S. lacrymans*.
Copper citrate has been used as a preservative model to study copper-tolerance fungi in wood because it lacks co-biocides that are found in most copper-based preservatives (Clausen *et al.*, 2000). For the purpose of studying copper tolerance, the presence of co-biocides, such as chromium and arsenic, could confound the results (De Groot and Woodward, 1998). ACQ-D

is included in this study because it is one of several wood preservatives that has been developed in recent years as an alternative to CCA. NHA is a water-soluble calcium capture agent (Green *et al.*, 1997;Green *et al.*, 2002) and is a relatively benign compound with lower toxicity in aquatic environments than copper (around 130 times less) (Crawford and Green, 2004), therefore it could become a very important alternative to copper based preservatives if it proves efficient.

2. EXPERIMENTAL METHODS

2.1 Fungal cultures

12 isolates of *Serpula lacrymans*, 2 isolates of *Serpula himantioides,* and one isolate of each of; *Tyromyces palustris, Gloeophyllum trabeum, Postia placenta* provided by Technological Institute, Denmark; Håvard Kauserud, Oslo University, Norway and Forest Products Laboratory, Madison, WI, USA, were maintained on 2% malt extract agar (Difco Laboratories, Detroit, MI, USA) (Table 1).

Table 1. Strains used in soil block test

Fungal species	Fungal isolates	Origin	Growth temperature
S. lacrymans	Bb 29	Belgium: Baisy-Thy	20° C
	Sl 199	Japan: Asahikawa	20° C
	Sl 200	Poland: Warsaw	20° C
	Sl 202	France: Xylochimie	20° C
	Sl 207	England: Rothesay	20° C
	Sl 209	Germany: Velbert	20° C
	Sl 210	Germany: Krefeldt	20° C
	Sl 216	Norway: Fannrem	20° C
	Sl 217	Norway: Oslo	20° C
	Sl 219	Finland: Helsinki	20° C
	Sl 221	Finland: Helsinki	20° C
	Bam Ebers 315	Germany: Eberswalde	20° C
	ATCC 11485	USA: N. Carolina, Asheville	20° C
S. himantioides	ATCC 36335	USA: Mississippi, Gulfport	20° C
	Sh 100	Germany: Wilsede	27° C
G. trabeum	Mad 617	USA: Wisconsin, Madison	27° C
P. placenta	Mad 698	USA: Maryland	27° C
T. palustris	TYP 6137	Japan: Uji	27° C

2.2 Decay test

Southern yellow pine (SYP) sapwood blocks (10 x 10 x 10 mm) and SYP sapwood feeder blocks (3 x 28 x 35 mm) were conditioned to 6% equilibrium moisture content and weighed. The blocks were then pressure-treated with 0.5% ACQ-D (copper quaternary ammonia-type D), 1.2% ammoniacal copper citrate (CuCit), or 1% NHA (N'N-naphthaloylhydroxylamine), respectively (Table 2). Preweighed blocks were submerged in respective treating solutions and subjected to a vacuum of -165.5 kPa gage pressure twice for 20 min each. Blocks were weighed, dried in fume cupboard overnight, returned to the conditioning room for one week, and reweighed. The blocks were subjected to *T. palustris, G. trabeum, P. placenta,* 2 isolates of *S. himantioides,* and 12 isolates of *S. lacrymans* (Table 1), in a soil-block test following the guidelines of AWPA Standard E10-01 (AWPA, 2003).

Table 2. Wood preservatives and retentions

Preservative treatment	Copper composition	Active ingredient (w/w%)	ICP calculated retention (mg/g)	Calculated retention (kg/m^3)
Ammoniacal copper quat D	66.7% CuO	0.5 Cu	1.4 Cu	2.9
Copper citrate (CuCit)	62.3% CuO	1.2 Cu	4.6 Cu	4.3
N'N-napthaloylhydroxylamine	0% CuO	0.5%	N/D	3.2

All blocks were steam-sterilized for 30 min without pressure prior to setting them onto the wood block feeders covered with mycelium of the test fungus. Soil bottles were incubated at 20°C or 27°C, respectively (Table 1) and 70% RH for 10 weeks. Five replicates oftreated and untreated blocks for each fungal isolate were harvested after incubation for 1, 2, 4, 6, 8 or 10 weeks. Blocks were removed from bottles, brushed free of mycelium, weighed, dried at 60°C for 24 hours, weighed, conditioned to 6% equilibrium moisture content, and weighed. Percentage of weight loss was calculated from the treated weights before as well as after decay testing.

3. RESULTS AND DISCUSSION

The decay capacity of *S. lacrymans* in untreated wood was compared to that in copper citrate-treated wood (Table 3). Mean percent weight loss (n = 5) in treated wood is ranked from highest to lowest and ranges from 46.68 to 9.81%.

Three isolates decayed copper citrate treated and untreated wood to the same degree (S1 221, S1 200 and S1 199), one isolate (Bam Ebers 315) was significantly inhibited by copper citrate (78.2% less weight loss compared to untreated controls), and seven isolates were moderately inhibited by copper citrate (12.5 to 38% less weight loss in treated wood than in untreated controls). In Bb 29 CuCit-treatment of the wood seemed to increase the decay capacity. A 21.50% average increase in weight loss for CuCit-treated wood was seen compared to untreated wood (Table 3). A 95% confidence interval analysis found this increase in weight loss not to be statically significant.

Decay capacity is for comparison shown for *S. himantioides,* also known as 'the wild dry rot fungus', *T. palustris* and *P. placenta,* which are known copper tolerant decay fungi and *G. trabeum,* a copper sensitive decay fungus, which is a non-accumulator of oxalic acid (Table 1). The *S. himantioides* isolate Sh 100 showed no difference between CuCit-treated and untreated wood for decay capacity, whereas ATCC 36335 was moderately inhibited by copper citrate. *T. palustris* and *P. placenta* were also moderately inhibited by copper citrate in this test. *G. trabeum* was significantly inhibited.

The ACQ-D and NHA-treated treated wood showed in general markedly better inhibition of the *S. lacrymans* isolates. For the isolates grown on AQC-treated wood, the decrease in decay ranged from 84 to 99%. For the other isolates there was the same tendency, that ACQ-D showed a significant effect in inhibiting wood decay. All weight losses in the treated wood ranged from 59 to –100% less than in the untreated wood.

NHA-treated wood showed a 45 to 90% decrease in decay by S1 216 and S1 219, respectively. The other isolates showed the same pattern towards NHA-treated wood, except the T. *palustris* (now *Fomitopsis palustris*) isolate. The *T. palustris* isolate TYP 6137 show almost no inhibition in decay (8.1% less decay in blocks treated with NHA).

Table 3: Decay capacity of 17 brown rot fungal isolates after 10 weeks growth on untreated and CuCit-, ACQ-D-, and NHA-treated southern pine.

Fungal isolate	Mean weight loss [%]			
	Treated			Untreated
	Copper citrate (CuCit)	ACQ-D	NHA	Control
S. lacrymans				
Sl 221	46.7% ± 5.3%	1.6% ± 1.9%	7.8% ± 6.5%	46.2% ± 5.2%
Bb 29	38.3% ± 5.8%	2.5% ± 1.9%	6.1% ± 3.0%	31.5% ± 13.7%
ATCC 11485	33.1% ± 10.8%	2.5% ± 1.4%	8.1% ± 1.4%	38.1% ± 7.1%
Sl 219	32.9% ± 18.7%	0.4% ± 2.8%	5.2% ± 0.6%	53.1% ± 6.9%
Sl 216	32.3% ± 14.0%	0.4% ± 0.4%	24.7% ± 12.4%	44.6% ± 12.5%
Sl 202	31.6% ± 3.2%	4.8% ± 6.7%	12.3% ± 11.2%	44.0% ± 10.6%
Sl 200	29.3% ± 11.5%	1.2% ± 0.4%	3.2% ± 0.7%	31.4% ± 9.2%
Sl 217	29.0% ± 9.9%	0.8% ± 0.6%	20.0% ± 6.2%	42.2% ± 5.5%
Sl 207	29.0% ± 7.9%	5.3% ± 9.6%	5.8% ± 4.5%	33.1% ± 8.6%
Sl 199	27.7% ± 6.5%	1.2% ± 0.4%	4.3% ± 4.8%	27.5% ± 4.2%
Sl 209	27.4% ± 11.6%	0.5% ± 0.9%	8.0% ± 6.2%	38.7% ± 5.8%
Bam Ebers 315	9.8% ± 6.5%	2.9% ± 1.0%	5.2% ± 1.7%	45.0% ± 9.0%
S. himantioides				
Sh 100	45.9% ± 10.5%	18.7% ± 4.7%	18.8% ± 14.0%	47.7% ± 3.8%
ATCC 36335	28.4% ± 15.3%	0.8% ± 0.4%	21.3% ± 12.6%	38.7% ± 4.5%
P. placenta				
Mad 698	57.0% ± 11.5%	1.8% ± 0.4%	3.7% ± 2.5%	68.5% ± 1.9%
T. palustris				
TYP 6137	39.6% ± 9.4%	19.7% ± 12.3%	44.3% ± 4.8%	48.2% ± 6.4%
G. trabeum				
Mad 617	4.8% ± 7.2%	0.1% ± 0.1%	8.8% ± 3.7%	59.4% ± 8.0%

CONCLUSION

The conclusion from this study was that *S. lacrymans* and *S. himantioides* display a considerable copper tolerance towards the wood preservative, copper citrate. Of the 12 *S. lacrymans* isolates tested, only one (Bam Ebers 315) was significantly inhibited to copper, whereas ten isolates showed moderate to no inhibition by copper. Wood blocks treated with copper citrate showed an average of 31% weight loss when challenged with isolates of *S. lacrymans*. In comparison, the untreated blocks had an average decay of 40% after 10 weeks of incubation. Thus, the treatments with copper citrate caused an average decrease in decay by 21%. This can not be considered an efficient wood protection agent against dry rot decay. The two alternative wood protection solutions tested in this study (ACQ-D and NHA) were much more effective in preventing wood decay from the test fungi than copper citrate. The ACQ-D-treated wood blocks showed an average of only 2% weight loss after 10 weeks of incubation with *S. lacrymans* isolates. The wood blocks treated with NHA solution showed an average of 9% weight loss after 10 weeks of incubation with *S. lacrymans* isolates. In these blocks, the variance was higher between the isolates, ranging from 3 to 25% weight loss in the wood blocks. The *S. himantioides* isolate (Sh 100) and *T. palustris,* seemed to be fairly aggressive no matter what treatment the wood blocks had been exposed to.

The results from this study show that a greater caution should be taken when choosing wood protection agents now the traditional solutions are no longer acceptable. The soil block test shows that most isolates of *S. lacrymans* are not inhibited by copper. The alternative solutions, which are less environmentally damaging, give much better resistance to dry rot decay in wood.

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The Dry Rot Fungus, *Serpula lacrymans*: Copper Tolerance and Wood Decay

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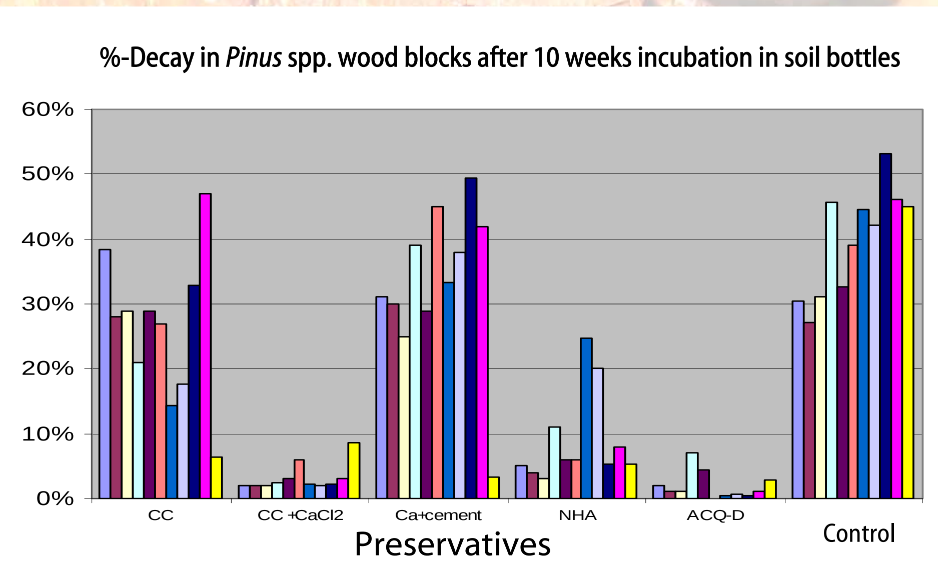
Visible decay: Wood blocks after 10 weeks of incubation. Blocks (from left) 1-5, 11-15, and especially 26-30 show obvious signs of decay. The chemicals used for preservation (from left): 1-5: CC, 6-10: CC + $CaCl_2$, 11-15: CC + cement, 16-20: NHA, 21-25: ACQ-D, 26-30 control.



Conclusion: The dry rot fungus, *Serpula lacrymans* is one of the most damaging and economically wood decay fungi in houses in temperate regions. Soil block test showed that the traditional copper containing wood preservatives have little effect on twelve strains of *S. lacrymans.* 11 of the 12 strains showed to be copper tolerant. Alternative, less toxic products showed promising results. These were NHA and ACQ-D.

Cu effect	Strain	CC	Control
Least	SI 221	46.7%	46.2%
Highest	Bam E. 315	9.8%	45.0%

Strain difference: The results showed a noteworthy difference in the amount of decay between the strains. The average decay for the copper citrate (CC) treated wood block being 31%, and 40% in average for the untreated wood blocks. Combined with $CaCl_2$, CC performed much better protection, this could be due to effect of Cl_2.

Result: The 10 week soil block test showed the tolerance of 11 *S. lacrymans* isolates towards different wood preservatives. The wood blocks were pressure treated with different wood preservatives before incubation. The preservatives were: CC (Copper citrate), NHA (N'N-naphthaloyl-hydroxylamine) , AQC-D (copper quaternary ammonia-type D). The higher the %-decay, the less effect the preservative had against the fungal isolate. The green dot is the mean of the 11 isolates with each 5 replicates.

Methods: *S. lacrymans* isolates were tested for copper tolerance. Wood block feeders were placed in glass bottles half filled with soil, incubated with fungal isolate. Pressure treated wood block were placed on top of them. One wood block was removed from each bottle after 1,2,4,6,8 or 10 weeks and weighed.

Decay: *Serpula lacrymans* causes brown rot.

Serpula lacrymans, the Dry Rot fungus, has only been collected a few places in nature but in houses it is a serious pest and therefore feared by house owners. Efficient wood preservatives, which follows the restrictions for low environmental toxicity, are of great economical importance.

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