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Premature failure of utility poles in Switzerland and Germany related to wood decay basidiomycetes

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Abstract: In contact with soil, copper (Cu) formulations as preservatives are expected to inhibit wood decay by fungi and other soil-borne microorganisms. However, Cu-resistant brown-rot (BR) fungi lead to premature failures of utility poles at some sites. In this study, the service lives of 111 utility poles of Norway spruce (*Picea abies* (L.) H. Karst) (73 from Switzerland and 38 from Germany) impregnated with Cu-based wood preservatives were investigated. Three segments of each utility pole were analyzed. The severity of decay was dependent on the preservative formulation. BR fungi and in particular *Antrodia* species were predominantly isolated from utility poles that were not treated with a co-biocide, e.g. boron (B). Cu-sensitivity of several isolated BR fungi was confirmed in studies on Cu-amended medium and in Cu-treated wood. Isolates of *Fibroporia vaillantii* and *Serpula himantioides* showed a higher Cu-tolerance than the highly Cu-tolerant Empa isolate *Rhodonia placenta* (Empa 45) or *Antrodia serialis*.

Keywords: *Antrodia serialis*, Cu-based wood preservatives, *Fibroporia vaillantii*, field tests, *Rhodonia placenta*, *Serpula himantioides*, wood poles (WPs)

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Introduction

Wood has numerous ecological, biological, and economic advantages and is an important natural resource. Increasing demand for wood necessitates material saving, prolonged service life, and improved technologies of recycling. Wood products in soil contact (utility class 4) such as terraces, pergolas, stakes in vineyards, or utility poles, must be protected against microorganisms and insects (BS EN 460:1994). Cu-based preservatives are effective in this regard and have a low mammalian toxicity. Their fungicity is based on the sulfhydryl groups, which produce the coagulation of cytoplasmic proteins (Hughes and Poole 1989).

Approximately 800 M utility wood poles (WPs) are currently in service in Europe. In Switzerland and Germany, 0.56 and 4.8 M WPs are in use, respectively, and 6.000–8.000 and 200.000–300.000 new WPs are installed in these countries annually. However, 1% (service life <10 years) of these replacements are due to premature failures. Thus, the total economic damage because of premature failure amounts to approx. 36 M € per annum (Swisscom AG, BASF Wolman GmbH, pers. communication). In soil contact, brown-rot (BR) fungi are responsible for wood decay, and the mode of BR action in decay and the decay resistance in modified woods without biocides was reviewed by Ringman et al. (2014). BR biodeterioration is frequently tested in soil block tests also in the context of commercial Cu-type preservatives (Schultz and Nicholas 2012); see also soil block results of Little et al. (2010) and Janzen and Nicholas (2016). Accelerated field stake tests for rapid assessment of wood preservative systems were proposed by Schultz and Nicholas (2010). Freitag et al. (2011) tested up to 15 years long protection of borate-treated Douglas fir WPs in field tests and Häger et al. (2001) reported the results of 31-year-long exposure of *Pinus sylvestris* L. stakes treated with ammoniacal Cu-based preservatives. Nevertheless, results of long-time decay behavior of WPs are seldom reported in the literature.

Since 1968, the majority of WPs in Germany have traditionally been impregnated with Cu-Cr (CC) by means of vacuum pressure processes. The recommended retention of CC for utility class 4 is 9 kg m^{-3} in Germany and 12 kg m^{-3} in Switzerland (Quitt 2014; Hach and Schwarze 2016). Nevertheless, these retentions do not completely protect wood against wood decay fungi. Several BR fungi have the ability to grow and survive at Cu^{2+} concentrations up to 1.6 mM (Hughes 2004) or 100 mg kg^{-1} (Gadd 2007). Diverse mechanisms involved in the Cu-tolerance process have been described such as ability to absorb Cu into cell wall, extracellular chelation by metabolites, and intracellular complexion with metallothioneins (Young 1961; Cervantes and Gutierrez-Corona 1994; Civardi et al. 2015b; Kartal et al. 2015). For example, *Wolfiporia cocos*, *Gloeophyllym trabeum*, and *Rhodonia placenta* (Empa 45) can degrade wood treated with Cu-based preservatives even at retention rates of 34.4 kg m^{-3} (De Groot and Woodwards 1999, Sierra-Alvarez 2007).

The number of premature failures of utility poles shows increasing tendencies (Bollmus et al. 2012) but there are only very few field studies examining the role of destroying fungi in these cases in Europe. In contrast, in North America, chromated-copper-arsenate (CCA) is still common for protection of utility poles (Bolin and Smith 2011) and thus problems in this regard are negligible (Freeman and McIntyre 2008).

The present study is dedicated to this problem, and 111 utility poles from Switzerland and Germany will be investigated in terms of the preservative formulation used and the service lives of the poles. Moreover, the type of fungal decay and the decay basidiomycetes should be identified. The copper sensitivity of the isolated strains will also be examined. As indicated above, long-term field decay results are seldom reported, and thus the expectation is that the result of the present paper will contribute to the practical evaluation of wood-protecting systems with service lives between 1 and 68 year.

Materials and methods

Distribution of the poles: A total number of 111 utility WPs of Norway spruce (*Picea abies*) were collected from various sites in Germany and Switzerland. The collection sites were selected from regions with pole stability problems. The segments were excised from each pole at ground level, at 30 cm above ground, and 30 cm below ground level (Figure 1a). The segments were immediately wrapped in kraft paper after marking for shipping to limit contamination during transport to the laboratory. The exact place where the WPs were collected can be requested from the research laboratory of the corresponding author.

Wood preservatives and decay types: Wood preservatives in treated WPs were identified according to Theden and Kottlors (1965) and AWPA (1999). Most WPs in Switzerland and all WPs in Germany were incised, i.e. penetration of preservatives was homogenously distributed in the sapwood (Figure 1b). Cu-based wood preservatives were qualitatively analyzed with a reagent containing 2% potassium ferrocyanide (Sigma-Aldrich, Buchs, Switzerland). The reagent induces a brownish color in Cu-treated wood due to the reaction of Cu to copper hexacyanoferrate. The presence of boron (B) in the WP segments was observed after applying two different reagents: 1. A solution of 20 ml of HCl (Sigma-Aldrich) and 80 ml of ethanol (Sigma-Aldrich) with 5 g of salicylic acid (Sigma-Aldrich) was applied to the wood surface. 2. After 5 min, a 2nd solution of 10 g of curcumin (Sigma-Aldrich) dissolved in 100 ml of ethanol (Sigma-Aldrich) was sprayed onto the wood surface. After 40 min, a reddish color was observed as a sign of positive reaction. Cr was determined by applying a solution of 1 g of diphenylcarbazide (Sigma-Aldrich) with 5 ml of acetic acid (Sigma-Aldrich) and 15 ml of ethanol 95% (Sigma-Aldrich). This reagent induces a purple color in the presence of Cr (III). The wood decay type was determined by its typical appearance (Figure 2), e.g. cubical rot typical of BR and/or wood erosion from the surface, which

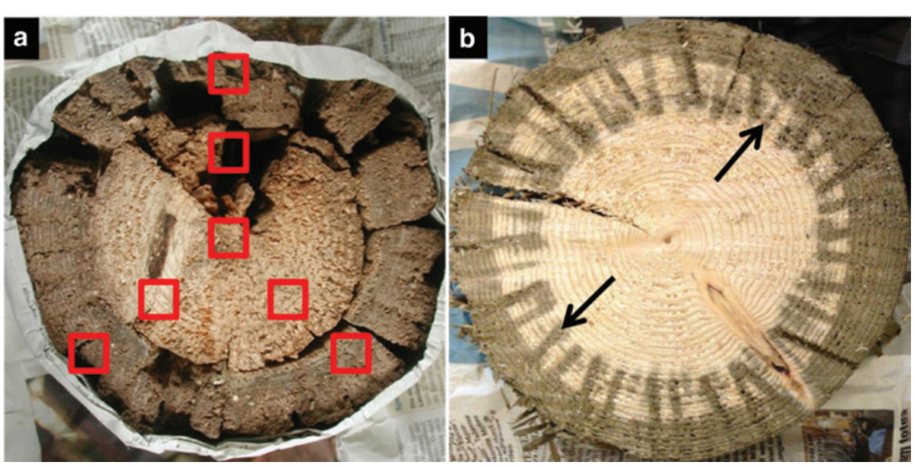

Figure 1: (a) Wood regions from which wood-destroying basidiomycetes were randomly isolated. (b) Cross section with numerous incisions (arrows).

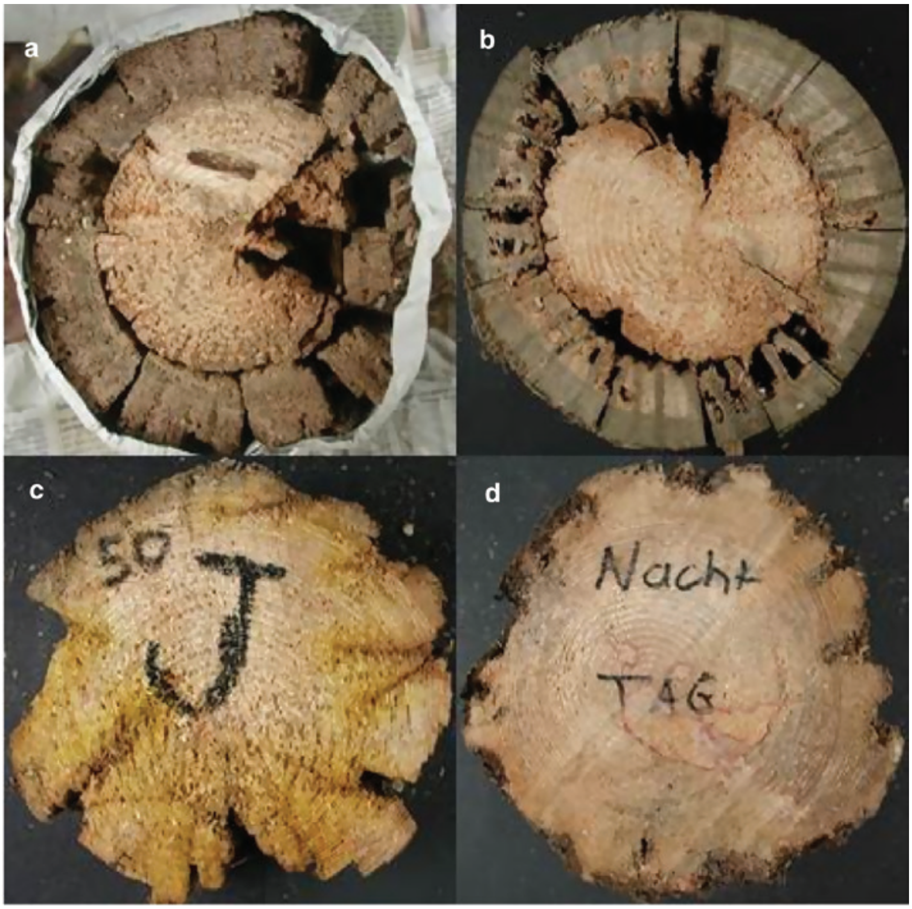

Figure 2: Damage of the selected poles by brown-rot (BR) (a,b) and soft-rot (SR) (c,d) fungi.

is typical of soft-rot (SR) (Schwarze 2007; Arantes et al. 2012; Arantes and Goodell 2014; Daniel 2014).

Wood decay basidiomycetes: For the isolation of wood decay basidiomycetes, seven increment cores were extracted aseptically from each WP segment and three smaller wood samples (3×3 mm) from each core were placed into Petri dishes (Figure 1a) containing 20 ml of 2% Malt Extract Agar (MEA, OXOID, Pratteln, Switzerland) with 2 ml of thiabendazole (Sigma-Aldrich) dissolved in lactic acid (Merck, Darmstadt, Germany), and then incubated at 22±1°C and 70% relative humidity (RH) for 10 days. Thiabendazole-malt extract agar allows the growth of wood decay fungi but suppresses the growth of other fungi (Sieber 1995; Baum et al. 2003; Hopkins et al. 2005, Schwarze et al. 2012). Isolates were subcultured on Petri dishes containing 2% MEA and incubated at 22±1°C and 70% RH in the dark. Isolates were identified microscopically (Stalpers 1978; Lombard and Chamuris 1990; Schmidt and Moreth 2003; Huckfeldt and Schmidt 2006). Additionally, the rDNA was extracted according to the protocol described in the kit “Extract N-Amp Plant PCR Kit” (Sigma Aldrich). In cases where the above kit did not extract the target rDNA, a second procedure described in “Fungi/Yeast Genomic DNA isolation Kit” (NorGen Biotek corp., Thorold, Canada) was used. The internal transcribed spacer (ITS1-5.8S-ITS4) of the extracted rDNA was amplified and sequenced for each strain. The primers for identification of the isolated fungi were designed according to the standard primers from White et al. (1990) (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′) and synthesized at Microsynth (Balgach, Switzerland). Amplification of the ITS region was achieved according to the polymerase chain reaction (PCR) protocol (White et al. 1990) with the following cycling parameters: Initial denaturation for 3 min at 94°C, 25 cycles of annealing for 1 min at 94°C, extension for 1 min at 48°C, denaturation for 2 min at 72°C, and final extension for 10 min at 72°C. PCR products were sequenced and the obtained sequences were matched based on the basic local alignment search tool of National Centre for Biotechnology Information (NCBI).

Growth of BR fungi on $CuSO_4$-amended MEA and decay evaluation: The Cu-sensitivity of each isolated basidiomycete species was evaluated with $CuSO_4$ (Sigma-Aldrich). *R. placenta* (Empa 45) served as a positive control due to its known high Cu-tolerance (Sierra-Alvarez 2007; Civardi et al. 2015a). Petri dishes containing 20 ml of 2% MEA and a range of $CuSO_4$ concentrations (0.000, 0.025, 0.050, 0.075, 0.1, 0.5, and 1%) were prepared according to Schmidt and Moreth (1996). All Petri dishes were inoculated centrally and incubated at 22(±1)°C and 70% RH for 10 days. Each fungal isolate was tested on five replicates per Cu concentration. The growth rate (mm day^{-1}) was determined by colony diameter measurements carried out along two perpendicular axes after 24 h (Schubert et al. 2008; Schwarze et al. 2012). Additionally, sapwood specimens of Scots pine 0.5×0.5×0.2 cm^3 were vacuum-pressure impregnated with $CuSO_4$ solutions (0.00, 0.025, 0.050, 0.075, 0.1, 0.5, or 1%) according to

EN 113 (1996). Before incubation, wood samples were sterilized with ethylene oxide (Sigma-Aldrich) and then placed onto 20 ml of 2% MEA. Each fungal isolate was inoculated as described above. After 8 weeks, the wood specimens were removed, carefully cleaned with a brush, dried at room temperature for 2 weeks, oven dried (103°C), and the mass loss (ML) was recorded.

Statistical analysis: Growth data were log-transformed and %-data, such as the wood weight loss (WL), were arcsine-transformed prior to analysis of variance (ANOVA) and back-transformed to numerical values for presentation (expressed as mean ± standard error). Means were separated using Dunett's test at significance levels of $P < 0.05$. The statistical package SPSS® (Version 22, SPSS Inc., Chicago, IL, USA) was adopted for this purpose.

Results and discussion

Determination of wood preservatives and decay types in utility poles

Important differences in the utility WPs from both countries are evident (Table 1). In Germany, the mean service life of WPs (9.6 year) was far lower than in Switzerland (32 year) (Table 1). A great diversity of wood preservative formulations was identified in WP segments from Switzerland (Table 1). Only 33% of the analyzed segments from the WPs in Switzerland contained CC or Cu-Cr-B (CCB). The rest of the analyzed segments (77%) contained a range of other preservative formulations. Around 90% of the WPs impregnated with wood preservative formulations other than CC or CCB were installed before 1990. CC or CCB were identified in all WPs from Germany and all of them were installed after 1990. Expectedly, the different formulations were applied in the last 50 y. During this period, about 1500 wood preservative formulations were available on the German market (Mai and Militz 2007).

Table 1: Analysis of removed utility wood poles (WPs).

Parameter	Germany	Switzerland
Number of poles (*n*)	38	73
Range of service life (y)	1–22	7–68
Mean service life (y)	9.6	32
Identified preserv.: CC (%)	87	16
Identified preserv.: CCB (%)	13	17
Other preservatives[a] (%)	0	67
Main decay type (%)	BR	SR
	98.5	63
BR fungi isolations (%)	45	10

[a]Others: Pole segments without CC or CCB, e.g. CCF (fluorine) or CCA (arsenic). BR, Brown-rot; SR, soft-rot.

In the 1990s, the EU started to regulate active ingredients against wood decay organisms in order to guarantee a low impact on human beings and the environment. Hence, the use of some compositions was restricted (BS 335-1 1992; European directive 98/8/EG 1998).

In Germany, the susceptibility of WPs for BR colonization was higher than in Switzerland (Table 1). Around 98% of the WPs showed symptoms of BR decay, which were all treated with CC or CCB. In contrast, in Switzerland, mainly SR decay was detected in 63% of the segments and only three of them were impregnated with CC or CCB. Moreover, 92% of the WPs decayed by SR were installed before the 1990s and strong decay was not detectable. Accordingly, the WP susceptibility to BR fungi is strongly related to the application of recent wood preservative formulations without an effective co-biocide and only with a weak fixation of Cu in the wood (Humar et al. 2007). The finding in the present paper confirms the early observations of Leithoff et al. (1995), who demonstrated in a choice test the preference of *Antrodia* spp. for Cu-treated wood in combination with different amended media. As shown earlier, Cu-based salts are effective for protecting wood in soil contact against SR fungi, but are less effective against BR fungi (Woodward and De Groot 1999; Green and Clausen 2003; Freeman and McIntyre 2008).

Although the heaviest damage by BR fungi was observed at ground level, in some cases the damage had also spread above and below ground level. Twenty and 58% of the WPs from Switzerland and Germany, respectively, had decay below ground level. The decay observed above ground level was similar in both countries (9 and 13% in Switzerland and Germany, respectively). Various factors may explain the activity of BR fungi at ground level. For instance, the moisture content in the first layers of the soil maintains the wood above 30%, which is optimal for BR decay (Palfreyman and Bruce 1994; Kües et al. 2007). Additionally, elevated preservative leaching has been related to the soil composition due to the affinity of the organic soil constituents to bound preservative compounds and the solubilization of metals (Cu and Cr) at low pH in soils due to the high content of organic acids (Freeman and McIntyre 2008).

Isolation and identification of wood decay basidiomycetes

In the present study, the total isolation ratio of BR fungi from WPs (45 and 10% in Germany and Switzerland, respectively) was comparable to that in previous studies (seven up to 60%) (Eslyn 1970; Zabel et al. 1985; Bollmus et al. 2012). Although almost 7000 isolations were made, it was impossible to isolate all BR fungi present in the wood due to contamination by other fungi from the environment that were able to rapidly colonize the substrate, e.g. *Trichoderma* spp., *Penicillium* spp., and *Mucor* spp. For this reason, the presence of other BR fungi in the WPs cannot be completely excluded, as described by Pfeffer et al. (2012).

Fungi were differentiated based on macro- and micromorphological features, such as clamp morphology, hyphal diameter, presence of hyphal strands, or color and odor of the mycelium (Nobles 1965; Stalpers 1978; Lombard and Chamuris 1990; Schmidt and Moreth 2003; Huckfeldt and Schmidt 2006). Such keys are useful for fungi identification, but nevertheless, confusions, doubts, or misidentification may occur at species level (Schimdt and Kebernik 1989; Palfreyman and Bruce 1994). Therefore, in the present study, all isolates were additionally identified by molecular biological methods.

The main wood decay basidiomycete was identified as *Antrodia serialis*, which was present on 32 and 75% of WPs from Germany and Switzerland, respectively (Table 2). *Fibroporia vaillantii* was isolated from 23% of the WPs, *Serpula himantioides* was the third most-abundant isolated fungus (18%), and *Sistotrema brinkmannii* was isolated twice from poles in Germany. The other species were isolated only once from the WP segments in both countries (Table 2). All identified wood decay basidiomycetes have been reported previously in association with decay of wood products (Duncan and Lombard 1965; Wilcox and Dietz 1997).

Growth of BR fungi on $CuSO_4$-amended MEA. Decay evaluation of $CuSO_4$-impregnated specimens

The suppressive effect of Cu-amended medium was higher for *A. serialis* than formerly reported by Civardi et al. (2015a) (Figure 3a). Statistical analysis revealed that the

Table 2: Brown-rot (BR) fungi isolated from utility poles.

BR fungi	Abundancy (%) in	
	Germany	Switerland
Antrodia serialis	31.8	75
Serpula himantioides	18.2	–
Sistotrema brinkmannii	9.2	–
Fibroporia vaillantii	22.8	–
Gloeophyllum sepiarium	4.5	–
Tapinella panuoides	4.5	–
Postia dissecta	4.5	–
Neolentinus lepideus	4.5	12.5
Unknown fungi	–	12.5

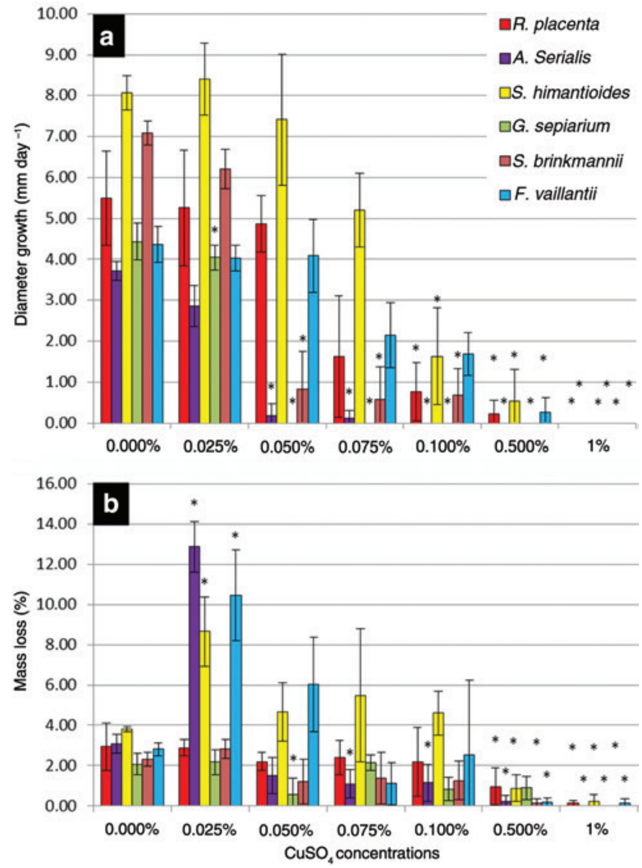

Figure 3: (a) Diameter growth of brown-rot (BR) isolates in MEA amended with different concentrations of $CuSO_4$. (b) Mass loss (ML) by BR isolates in Scots pine wood blocks treated with different concentrations of $CuSO_4$. *Indicates statistical differences in comparison with controls; *=$P<0.05$ (Dunnett's t-test two-way).

effect of $CuSO_4$ varied among fungal species, a finding which is in good agreement with that of Schmidt and Moreth (1996). *S. himantioides* and *F. vaillantii* showed the highest tolerance to Cu-amended medium (Figure 3a). A significant negative effect for Cu-amended medium ($P<0.05$) was recorded in comparison with the controls for all wood decay basidiomycetes at concentrations higher than 0.075% $CuSO_4$ (Figure 3a). Interestingly, the highly Cu-tolerant strain *R. placenta* (Empa 45) was less tolerant than one isolate of *S. himantioides*.

MLs caused by basidiomycetes on Cu-impregnated wood are presented in Figure 3b. Low concentrations of Cu in impregnated wood (0.025% $CuSO_4$) resulted in a significant increase of ML by *A. serialis*, *F. vaillantii*, and *S. himantioides* ($P<0.05$). Collet (1992) and Leithoff et al. 1995 demonstrated a similar stimulus for different isolates of *F. vaillantii*, when adding low Cu concentrations to the substrate. In comparison with controls, $CuSO_4$ concentrations higher than 0.1% had an adverse effect on ML

(Figure 3b), which was already reported by Schmidt and Moreth (1996), who found a high Cu-sensitivity for the basidiomycetes (*A. serialis, R. placenta, F. vaillantii, S. himantioides*). The highly Cu-tolerant fungus *R. placenta* Empa 45 and *S. himantioides* were tolerant to all $CuSO_4$ concentrations, whereas *S. brinkmannii* and *Gloeophyllum sepiarium* were highly susceptible to all $CuSO_4$ concentrations higher than 0.05% (Figure 3b).

Conclusions

Wood decay basidiomycetes associated with premature failure of utility WPs in Switzerland and Germany belong mainly to *Antrodia* spp. A direct correlation between fungal species and geographic regions was not evident but there is strong evidence that the type of wood preservative formulation has the highest selective pressure on BR fungal colonization and wood decomposition. In the absence of a co-biocide, WPs treated with CC formulations are highly susceptible to premature failures. The isolated BR fungi show significant differences concerning the Cu-resistance. *A. serialis* was the main wood decay basidiomycete isolated from WPs in both countries but the bioassays revealed that *A. serialis* is not highly tolerant to Cu concentrations. The isolates of *S. himantioides* and *F. vaillantii* demonstrated a greater Cu-tolerance threshold and thus they might play an important role in removal of Cu from treated wood. Further studies are currently in progress, in which the Cu-sensitivity of the isolated wood decay fungi on wood treated with a range of commercial Cu-based formulations and retentions are evaluated. Data about the locations where the WPs were sampled can be given by the corresponding author upon request.

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