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# Tolerance of brown-rot and dry-rot fungi to CCA and ACQ wood preservatives

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**Abstract:** Copper remains the primary biocide component used today to protect wood. Increased interest in the use of non-arsenic copper-based wood preservatives has also led to increased studies on copper-tolerant decay fungi. Oxalic acid production by brown-rot fungi is proposed as one mechanism of copper tolerance. This study evaluated oxalic acid production and copper losses from ACQ- and CCA-treated *Pinus sylvestris* L. and *Populus* × *euramericana* I 214 wood by brown-rot fungi: *Postia placenta*, *Gloeophyllum trabeum*, *Tyromyces palustris*, and 2 strains of *Serpula lacrymans*. There was no clear linear correlation among mass losses, oxalic acid production, or copper losses in most cases. However, *T. palustris* and one strain of *S. lacrymans* showed copper tolerance in treated wood. *P. placenta* caused considerable mass losses in ACQ-treated specimens only. *G. trabeum* produced very low oxalic acid and was inhibited by ACQ and CCA wood preservatives. We concluded that wood species and preservative formulation affected the oxalic acid production, mass losses, and copper tolerance of the tested fungi.

**Key words:** ACQ, CCA, brown-rot, copper loss, copper tolerance, oxalic acid

## Esmer ve kuru çürüklük mantarlarının CCA ve ACQ emprenye maddelerine karşı toleransı

**Özet:** Bakır, günümüzde ağaç malzemenin korunması amacıyla kullanılan en önemli biyositlerden biridir. Arsenik içermeyen bakır esaslı emprenye maddelerinin kullanımına yönelik ilginin artması, bakıra tolerans gösteren mantarlarla yapılan çalışmaların da artmasına neden olmuştur. Esmer çürüklük mantarlarının oksalik asit üretmesi bakır toleransını açıklayan bir mekanizma olarak ileri sürülmektedir. Bu çalışmada, esmer çürüklük mantarları, *Postia placenta*, *Gloeophyllum trabeum*, *Tyromyces palustris* ve *Serpula lacrymans*'ın iki farklı ırkı tarafından ACQ ve CCA emprenye maddeleriyle işlem görmüş sarıçam ve kavak odunlarında meydana gelen oksalik asit oluşumu ve bakır kayıpları incelenmiştir. Genel olarak mantarların etkileri sonucu meydana gelen ağırlık kaybı, oksalik asit oluşumu ve bakır kayıpları arasında doğrusal bir ilişki bulunamamıştır. Ancak, *T. palustris* ve *S. lacrymans* mantarının bir ırkı emprenyeli örneklerde bakıra tolerans göstermiştir. *P. placenta* ACQ ile emprenye edilmiş örneklerde önemli ağırlık kayıpları meydana getirmiştir. *G. trabeum* tarafından çok az miktarda oksalik asit üretilmiş, ACQ ve CCA emprenye maddeleri bu mantarın gelişimini engellemiştir. Sonuç olarak, ağaç türü ve emprenye maddesi formülasyonunun mantarların oksalik asit üretimlerini, oluşturdukları ağırlık kayıplarını ve bakır toleransını etkilediği saptanmıştır.

**Anahtar sözcükler:** ACQ, CCA, esmer çürüklük, bakır kaybı, bakır toleransı, oksalik asit

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## Introduction

Efficacy against copper tolerant fungi is an important issue for any formulation of copper-based wood preservatives. Present at higher levels in its free ionic form ($Cu^{2+}$), copper is both an essential micronutrient and a toxic heavy metal for most living cells. Copper's toxicity is mainly due to its interactions with nucleic acids, to the alteration of enzyme active sites, and to the oxidation of membrane components, processes that can be related to the ability of copper to generate toxic hydroxyl free-radicals. Copper tolerance in fungi has been ascribed to diverse mechanisms involving the trapping of the metal by cell-wall components, altered uptake of copper, extracellular chelating or precipitation by secreted metabolites, and intracellular complexing by metallothioneins and phytochelatins, although only the metallothionein chelation mechanism has been approached with molecular detail (Cervantes and Gutierrez-Corona 1994). Copper tolerance is the ability of an organism to grow and thrive in the presence of copper ions. Various brown-rot fungi such as *Serpula, Tyromyces, Poria (Postia), Antrodia,* and *Wolfiporia* spp. are known to be copper tolerant (Zabel 1954; Da Costa and Kerruish 1964; Chou et al. 1973; Thornton and Tighe 1987; Collett 1992; Williams and Fox 1994; Schmidt and Moreth 1996; Tsunoda et al. 1997; De Groot and Woodward 1999; Clausen et al. 2000; Clausen and Green III 2003; Green III and Clausen 2003; Köse 2006; Arango et al. 2009). The mechanism for tolerance in wood-degrading fungi is attributed to their ability to immobilize copper by precipitating copper oxalate. A relation between oxalic acid production and copper tolerance has been implied due to copper oxalate crystal formation in decayed wood (Murphy and Levy 1983; De Groot and Woodward 1999; Clausen and Green III 2003; Hastrup et al. 2005). Oxalic acid ($C_2H_2O_4$) is produced in measurable amounts by most brown-rot fungi and is known to be a metabolic byproduct of the decay process (Bech-Andersen 1987; Collett 1991; Espejo and Agosin 1991; Dutton et al. 1993; Hastrup et al. 2006; Sierra-Alvarez 2007; Arango et al. 2009). De Groot and Woodward (1999) reported that the hyphae are surrounded by an extracellular mucilaginous sheath containing dissolved oxalic acid, which in turn extracts or reacts with cations, such as calciumor copper, that are precipitated and deposited around the hyphae (Sutter et al. 1983). A reduction in the toxicity of copper with increased acidity was observed in several fungi (Gadd and White 1985), and the lowering of the pH by oxalic acid had more to do with copper tolerance than with the low solubility of copper oxalate (Hastrup et al. 2005).

Oxalic acid is a key fungal metabolite in the initial stages of brown rot (Green III et al. 1991) and the precipitation and neutralization of copper by brown-rot fungi (Clausen et al. 2000; Munir et al. 2001). The actual role of oxalic acid in tolerance seems to vary with treatment and fungal species. Wood species may also play a role in oxalic acid production by fungi, and, in turn, in copper tolerance.

A considerable number of studies on the mechanism of decay have stated that oxalic acid is a major factor leading to tolerance of various copper-based wood preservatives. Copper-tolerant organisms are of great interest to researchers. The copper tolerance of various isolates can have a significant practical impact on the long-term performance of treated wood products or the bioprocessing of spent wood materials treated with copper-based preservatives (Woodward and De Groot 1999). Increasing perceptions of toxicity, leaching, and other potential environmental effects have stimulated the development of alternative systems that, while still containing copper, incorporate materials other than chromium or arsenic (Lebow et al. 2000). There are few studies on the comparison of relative tolerance and oxalic acid production of isolates of decay fungi to ACQ preservatives. This study aimed to determine whether oxalic acid contributes to the tolerance to CCA and ACQ wood preservatives of various brown-rot and dry-rot fungi, as well as the effects of preservative type and wood species on oxalic acid production.

## Materials and Methods

### Fungal Cultures

Isolates of *Serpula lacrymans* (Wulfen:Fr.) Schroeter (ATTC 36335), *Postia placenta* (Fr.) Larsen & Lombard (MAD 698-R), and *Gloeophyllum trabeum* (Pers.:Fr.) Murrill (MAD 617-R) were provided by the Center for Forest Mycology Research,

Forest Products Laboratory, Madison, WI, USA, and isolates of *Serpula lacrymans* (Wulfen:Fr.) Schroeter (SEL 8501) and *Tyromyces palustris* (Berk. et Curt.) Murr. (TYP 6137) were provided by the Research Institute for Sustainable Humanosphere (RISH), Laboratory of Innovative Humano-habitability, Kyoto University, Japan. Isolates were maintained on 2% malt extract agar (Merck KGaA, 64271 Darmstadt, Germany).

**Preservative Treatments and Decay Test**

Wood specimens (19 × 19 × 19 mm) were prepared from sapwood portions of *Pinus sylvestris* L. and *Populus* × *euramericana* I 214 logs. Specimens were free of knots and visible concentration of resins, and showed no visible evidence of infection by mold, stain, or wood-destroying fungi. All specimens were conditioned at 20 °C and 65% relative humidity (RH) and weighed. Specimens were then vacuum-treated with 0.3% and 1.2% solutions of CCA-C (47.5% $CrO_3$, 18.5% CuO, 34.0% $As_2O_5$) and ACQ (copper 13.4% as ammoniacal salt, and 5.7% N-Alkylbenzyldimethylammonium chloride) wood preservatives (Table 1). After treatment, specimens were then reconditioned at 20 °C and 65% RH for 4 weeks and again weighed. All specimens were steam sterilized for 30 min without pressure before being placed onto wood block feeders covered with mycelium of the test fungus. The specimens were subjected to *G. trabeum, P. placenta, T. palustris,* and 2 isolates of *S. lacrymans* in a soil block test (ASTM 1998). Test bottles were incubated at 27 °C and 70% relative humidity (RH) for 3, 6, and 12 weeks. The percentage of mass loss in the specimens was then calculated from the weights of the specimens before and after the decay tests. Six replicates of treated and untreated specimens for each wood species and duration were used in the tests.

**Oxalic Acid Production**

Wood specimens were ground into sawdust. Sawdust samples were extracted in 3.0 mL of 0.1 M phosphate buffer, pH 7.0, for 2 h with shaking. For each extracted sample, oxalic acid was determined by microassay with a diagnostic kit (Trinity Biotech Plc, Bray, Co. Wicklow, Ireland). The amount of oxalic acid was expressed as micromoles of oxalic acid per gram of final dry weight of wood. The enzymatic reactions involved in the assay procedure are as follows:

$$HOOC\text{-}COOH \xrightarrow[\text{Oxalate oxidase}]{O_2} 2CO_2 + H_2O_2$$

$$H_2O_2 + MBTH + DMAB \xrightarrow{\text{Peroxidase}} \text{Indamine dye} + H_2O$$

Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye, which has an absorbance maximum of 590 nm. The intensity of the color produced is directly proportional to the concentration of oxalate in the sample.

**Elemental Analysis**

Treated wood specimens were ground, digested, and analyzed for copper (Cu) content by inductively coupled plasma emission spectrometry (Thermo Elemental X7 Series ICP-MS). Analyses were done at the Advanced Analysis Laboratory of İstanbul University. Copper concentrations in the wood following decay were also compared with those in the wood prior to decay.

Table 1. Wood preservatives and retentions.

| Wood species | Active ingredient (%, w $w^{-1}$) | CCA (kg $m^{-3}$) | ACQ (kg $m^{-3}$) |
|---|---|---|---|
| *Populus* × *euramericana* I 214 | 0.3 | 2.3 | 2.3 |
| | 1.2 | 9.2 | 9.0 |
| *Pinus sylvestris* L. | 0.3 | 1.9 | 1.8 |
| | 1.2 | 7.7 | 7.1 |

## Results

### Decay Resistance

The decay capacities of *G. trabeum, P. placenta, T. palustris,* and 2 isolates of *S. lacrymans* in untreated *P. sylvestris* and *P.* × *euramericana* wood are given in Tables 2 and 3, respectively, in comparison with those in ACQ- and CCA-treated wood. The lowest mass loss in the *P.* × *euramericana* control wood was obtained with *S. lacrymans* (SEL 8501) isolate in 12-week exposure (17%); however, *G. trabeum, P. placenta, T. palustris*, and *S. lacrymans* (ATTC 36335) isolates caused substantial amounts of mass loss. In ACQ-treated wood specimens, average mass losses decreased significantly for *G. trabeum* and *P. placenta*. *T. palustris* and *S. lacrymans* (ATTC 36335) were not inhibited by ACQ at either concentration level, and mass losses in the wood specimens treated at 0.3% concentration were as high as those in the control specimens. *G. trabeum* and *P. placenta* were significantly inhibited by ACQ and mass losses decreased considerably when compared to untreated wood. Interestingly, *S. lacrymans* (SEL 8501) caused more mass loss in wood specimens treated at 0.3%

Table 2. Mass losses in *P.* × *euramericana* specimens exposed to decay tests.

| Preservative | Active ingredient (%) | *G. trabeum* | | | *S. lacrymans* SEL 8501 | | | *S. lacrymans* ATCC 36335 | | | *P. placenta* | | | *T. palustris* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks |
| Control | 0.0 | 35.5 (11.45) | 63.3 (9.01) | 73.9 (5.50) | 18.2 (4.54) | 21.6 (6.16) | 16.9 (8.96) | 40.2 (3.97) | 54.7 (5.73) | 63.5 (2.90) | 19.5 (11.68) | 44.5 (3.26) | 61.1 (4.58) | 33.2 (6.67) | 46.2 (15.77) | 56.7 (7.21) |
| ACQ | 0.3 | 0.8 (0.97) | 2.6 (2.84) | 14.1 (12.41) | 24.1 (8.31) | 21.6 (4.65) | 27.9 (7.19) | 22.6 (8.48) | 47.4 (8.39) | 59.8 (4.69) | 1.3 (1.12) | 12.7 (11.92) | 23.8 (12.99) | 14.8 (7.85) | 40.9 (6.38) | 55.1 (6.41) |
| | 1.2% | -3.7 (0.31) | -5.6 (0.86) | -5.2 (0.80) | -0.5 (1.59) | 16.1 (6.24) | 16.3 (6.28) | -4.3 (2.03) | 13.7 (8.21) | 39.9 (13.20) | -4.2 (0.48) | -4.4 (1.11) | -6.8 (1.37) | -1.0 (1.65) | 9.2 (9.77) | 46.8 (8.82) |
| CCA | 0.3 | 0.5 (0.04) | -0.6 (0.35) | -0.5 (0.77) | 4.7 (1.31) | 11.4 (3.26) | 19.4 (12.45) | 1.8 (1.08) | 4.2 (2.73) | 2.1 (3.64) | -0.3 (0.23) | -1.2 (0.42) | -2.1 (0.99) | 3.6 (2.49) | 9.3 (4.90) | 17.6 (3.03) |
| | 1.2% | 2.0 (0.42) | 0.7 (0.32) | -0.6 (0.67) | 3.8 (1.00) | 4.5 (1.52) | 5.1 (1.76) | 1.2 (0.49) | 0.8 (0.26) | -1.1 (0.92) | 1.0 (0.34) | -0.5 (0.18) | -2.5 (2.35) | 3.0 (1.25) | 5.0 (1.46) | 4.4 (3.08) |

Values in parentheses are standard deviations

Table 3. Mass losses in *P. sylvestris* specimens exposed to decay tests.

| Preservative | Active ingredient (%) | *G. trabeum* | | | *S. lacrymans* SEL 8501 | | | *S. lacrymans* ATCC 36335 | | | *P. placenta* | | | *T. palustris* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks | 3 weeks | 6 weeks | 12 weeks |
| Control | 0.0 | 25.9 (5.38) | 35.7 (10.57) | 57.0 (7.37) | 27.2 (2.61) | 44.8 (2.86) | 57.1 (4.67) | 12.6 (3.16) | 13.3 (2.55) | 10.7 (4.45) | 25.6 (6.29) | 36.3 (5.60) | 55.3 (6.44) | 21.8 (5.53) | 32.0 (4.91) | 42.4 (6.36) |
| ACQ | 0.3 | 0.4 (0.97) | 1.2 (2.08) | 11.6 (9.85) | 2.0 (2.50) | 5.3 (3.24) | 5.5 (3.12) | 0.8 (1.30) | 0.8 (7.65) | -4.5 (3.13) | 14.2 (7.13) | 24.8 (7.54) | 48.8 (11,68) | 15.1 (3.41) | 28.6 (7.55) | 54.4 (5.26) |
| | 1.2% | 0.0 (0.21) | -0.6 (0.21) | -1.0 (0.83) | 2.2 (0.79) | 4.8 (1.26) | 39.1 (2.61) | 0.6 (1.19) | 0.0 (1.99) | -4.8 (1.49) | 2.5 (2.72) | 18.4 (8.29) | 9.9 (12.79) | 2.8 (1.47) | 9.3 (6.82) | 14.8 (7.30) |
| CCA | 0.3 | 0.2 (0.21) | -0.5 (0.37) | -1.4 (0.68) | 1.9 (2.02) | 3.1 (3.98) | 19.7 (9.19) | 1.6 (0.83) | 5.1 (3.12) | 15.4 (4.04) | 0.1 (0.12) | -1.0 (0.33) | -2.4 (0.42) | 1.3 (1.19) | 4.4 (3.50) | 6.1 (3.30) |
| | 1.2% | 0.5 (0.10) | -0.4 (0.16) | -1.3 (0.67) | 1.8 (0.97) | -1.4 (1.42) | 1.3 (1.19) | 0.9 (0.13) | 0.1 (0.84) | -3.5 (1.08) | 0.6 (0.36) | -0.8 (0.42) | -2.5 (1.45) | 1.1 (0.36) | -2.2 (0.75) | 2.7 (2.43) |

Values in parentheses are standard deviations

concentration and nearly the same amount of mass loss at 1.2% concentration in comparison with untreated control wood. In the CCA treatments, *S. lacrymans* (ATTC 36335), *P. placenta*, *G. trabeum*, and *T. palustris* were significantly inhibited at both concentration levels of CCA wood preservative; however, the *S. lacrymans* (SEL 8501) isolate resulted in higher mass loss when compared to the untreated control at 0.3% concentration. These results suggested that the 2 isolates of *S. lacrymans* and *T. palustris* were copper-tolerant fungi for ACQ treatments. However, wood specimens treated with CCA were resistant against all the fungi tested, except for the 0.3% concentration level of *S. lacrymans* (SEL 8501). Results of this study indicated that the copper tolerance of the fungi tested for *P.* × *euramericana* wood treated with copper-based preservatives was largely dependent on the preservative system that was used.

In the *P. sylvestris* wood specimens, all fungi except for *S. lacrymans* (ATTC 36335) resulted in significant mass losses. *P. placenta* and *T. palustris* were not inhibited by ACQ at 0.3% concentration level; however, mass losses by the other tested fungi considerably decreased during ACQ treatments. Wood specimens treated with CCA at both concentration levels were resistant against *G. trabeum, P. placenta*, and *T. palustris*; however, *S. lacrymans* (SEL 8501) and *S. lacrymans* (ATTC 36335) isolates were able to cause 20% and 15% mass losses in the respective wood specimens after 12-week exposure at 0.3% concentration level. These 2 isolates were significantly inhibited at the 1.2% concentration level in CCA treatments. In general, the fungi tested did not show any copper tolerance in the CCA treatments; however, at a lower concentration level of ACQ wood preservative, *P. placenta* and *T. palustris* were able to cause substantial amounts of mass loss. In contrast to *P. sylvestris* wood treated with ACQ, *S. lacrymans* (ATTC 36335) showed copper tolerance in *P.* × *euramericana* wood, suggesting that wood species might also be important for copper tolerance of fungi.

**Oxalic Acid Production and Copper Loss**

Tables 4 and 5 show the mean maximum values of oxalic acid produced for each fungus, the mass loss, and the copper losses from CCA- and ACQ-treated wood specimens. These values represent the maximum values achieved for each fungus over the 12-week evaluation period. In general, maximum mass loss was achieved at 12 weeks (Table 4 and Table 5); in most cases, maximum oxalic acid production was achieved at 12 weeks in the evaluation period (Figures 1 and 2).

## Discussion

*G. trabeum* produced lower oxalic acid during the incubation period than the other fungal strains did, and oxalic acid accumulation in CCA- and ACQ-treated wood specimens was slightly higher than in untreated control specimens. Failure to exhibit copper tolerance appears linked to lack of oxalic acid accumulation in *G. trabeum* MAD 617 (Green III et al. 1992), even though this species is particularly tolerant to phenolic acid and arsenic compounds (De Groot and Woodward 1999; ASTM 1998). Green III and Clausen (2003), Green III and Clausen (2005), and Arango et al. (2009) have previously shown that *G. trabeum* produces low amounts of oxalic acid during incubation periods, suggesting that *G. trabeum* is not copper tolerant and that copper easily inhibits this species. *Postia placenta* is known as a copper-tolerant fungus and is referenced in standard decay tests as a copper-tolerant fungus. Arango et al. (2009) stated that *P. placenta* has been studied extensively and is also known to be tolerant to zinc compounds (AWPA 2003). In our study, *P. placenta* caused 24% and 49% mass losses in poplar and pine wood specimens treated with ACQ at lower concentration levels. However, in CCA-treated wood specimens at both low and high concentration levels, no mass loss occurred even though *P. placenta* is a copper-tolerant fungus species, probably due to the presence of arsenic and chromium in the CCA formulation. Copper losses caused by *P. placenta* in poplar wood specimens varied from 15% to 48%, while the losses in pine wood specimens varied from 0% to 39%. A number of studies have shown *T. palustris* to be copper-tolerant and a prolific producer of oxalic acid (Green III and Clausen 2003; Green III and Clausen 2005; Köse 2006; Arango et al. 2009). In our study, *T. palustris* caused 55%, 47%, 18%, and 4% mass losses in ACQ-treated (0.3% and 1.2% concentration levels) and CCA-treated (0.3% and 1.2%

Table 4. Mass loss, oxalic acid production, and Cu loss in *P.* × *euramericana* specimens exposed to the fungi (12 weeks).

| Preservative | Active ingredient (%) | Fungi | Mass loss (%) | Oxalic acid (μmol $g^{-1}$) | Cu (mg $kg^{-1}$) | Cu loss (%) |
|---|---|---|---|---|---|---|
| Untreated control | | *G. trabeum* Mad-617-R | 73.87 | 8.49 | - | - |
| | | *S. lacrymans* SEL 8501 | 16.91 | 1355.31 | - | - |
| | | *S. lacrymans* ATCC 36335 | 63.48 | 597.88 | - | - |
| | | *P. placenta* Mad-698-R | 61.07 | 978.22 | - | - |
| | | *T. palustris* TYP 6137 | 56.69 | 332.82 | - | - |
| ACQ | 0.3 | *G. trabeum* Mad-617-R | 14.06 | 19.9 | 862.8 | 17.7 |
| | | *S. lacrymans* SEL 8501 | 27.94 | 1343.3 | 376.3 | 64.1 |
| | | *S. lacrymans* ATCC 36335 | 59.79 | 982.7 | 1002.1 | 4.4 |
| | | *P. placenta* Mad-698-R | 23.79 | 176.6 | 545.8 | 47.9 |
| | | *T. palustris* TYP 6137 | 55.13 | 142.0 | 1045.2 | 0.3 |
| | 1.2 | *G. trabeum* Mad-617-R | -5.18 | 44.9 | 2871.0 | 5.0 |
| | | *S. lacrymans* SEL 8501 | 16.27 | 1257.7 | 2805.1 | 7.1 |
| | | *S. lacrymans* ATCC 36335 | 39.89 | 463.6 | 2443.6 | 19.1 |
| | | *P. placenta* Mad-698-R | -6.76 | 61.3 | 1893.9 | 37.3 |
| | | *T. palustris* TYP 6137 | 46.78 | 50.9 | 1937.7 | 35.9 |
| CCA | 0.3 | *G. trabeum* Mad-617-R | -0.45 | 18.1 | 1238.5 | 20.3 |
| | | *S. lacrymans* SEL 8501 | 19.41 | 30.1 | 1448.3 | 6.8 |
| | | *S. lacrymans* ATCC 36335 | 2.13 | 35.7 | 1544.7 | 0.6 |
| | | *P. placenta* Mad-698-R | -2.07 | 62.4 | 1316.7 | 15.3 |
| | | *T. palustris* TYP 6137 | 17.56 | 40.8 | 1448.6 | 6.8 |
| | 1.2 | *G. trabeum* Mad-617-R | -0.64 | 29.2 | 4849.1 | 31.9 |
| | | *S. lacrymans* SEL 8501 | 5.13 | 55.6 | 5184.2 | 27.2 |
| | | *S. lacrymans* ATCC 36335 | -1.06 | 35.5 | 4381.2 | 38.5 |
| | | *P. placenta* Mad-698-R | -2.52 | 115.5 | 4263.8 | 40.1 |
| | | *T. palustris* TYP 6137 | 4.38 | 292.3 | 6467.9 | 9.2 |

Table 5. Mass loss, oxalic acid production, and Cu loss in *P. sylvestris* specimens exposed to the fungi (12 weeks).

| Preservative | Active ingredient (%) | Fungi | Mass loss (%) | Oxalic acid (μmol $g^{-1}$) | Cu (mg $kg^{-1}$) | Cu loss (%) |
|---|---|---|---|---|---|---|
| Untreated control | | *G. trabeum* Mad-617-R | 56.99 | 10.93 | - | - |
| | | *S. lacrymans* SEL 8501 | 57.13 | 945.16 | - | - |
| | | *S. lacrymans* ATCC 36335 | 10.72 | 860.28 | - | - |
| | | *P. placenta* Mad-698-R | 55.31 | 843.6 | - | - |
| | | *T. palustris* TYP 6137 | 42.42 | 65.77 | - | - |
| ACQ | 0.3 | *G. trabeum* Mad-617-R | 11.58 | - | - | - |
| | | *S. lacrymans* SEL 8501 | 5.46 | 825.0 | 353.2 | 29.3 |
| | | *S. lacrymans* ATCC 36335 | -4.49 | 1444.4 | 410.0 | 17.9 |
| | | *P. placenta* Mad-698-R | 48.83 | 197.2 | 433.7 | 13.1 |
| | | *T. palustris* TYP 6137 | 54.38 | 264.3 | 344.0 | 31.1 |
| | 1.2 | *G. trabeum* Mad-617-R | -0.98 | 33.8 | 1580.4 | -0.1 |
| | | *S. lacrymans* SEL 8501 | 39.12 | 833.2 | 762.0 | 51.8 |
| | | *S. lacrymans* ATCC 36335 | -4.85 | 30.6 | 1102.8 | 30.2 |
| | | *P. placenta* Mad-698-R | 9.92 | 115.2 | 962.1 | 39.1 |
| | | *T. palustris* TYP 6137 | 14.75 | 41.9 | 1337.8 | 15.3 |
| CCA | 0.3 | *G. trabeum* Mad-617-R | -1.44 | 11.1 | 650.9 | 5.1 |
| | | *S. lacrymans* SEL 8501 | 19.74 | 338.8 | 508.0 | 26.0 |
| | | *S. lacrymans* ATCC 36335 | 15.36 | 101.5 | 633.3 | 7.7 |
| | | *P. placenta* Mad-698-R | -2.36 | 41.0 | 515.9 | 24.8 |
| | | *T. palustris* TYP 6137 | 6.13 | 127.3 | 415.8 | 39.4 |
| | 1.2 | *G. trabeum* Mad-617-R | -1.33 | 22.4 | 2920.0 | 5.4 |
| | | *S. lacrymans* SEL 8501 | 1.29 | 44.9 | 3088.3 | 0.0 |
| | | *S. lacrymans* ATCC 36335 | -3.55 | 28.6 | 3060.0 | 0.9 |
| | | *P. placenta* Mad-698-R | -2.45 | 38.4 | 3077.0 | 0.3 |
| | | *T. palustris* TYP 6137 | 2.71 | 33.8 | 2967.7 | 3.8 |

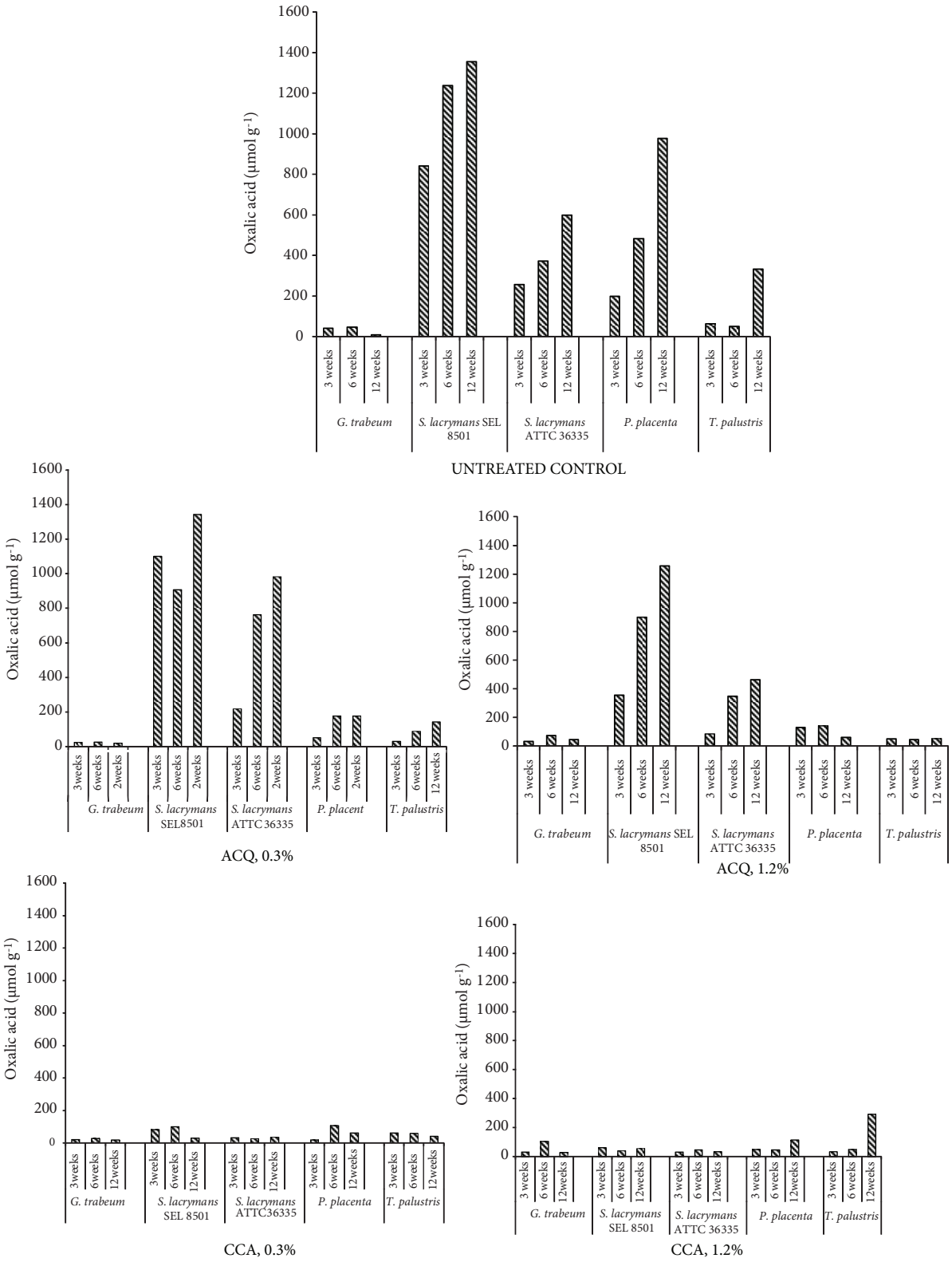


Figure 1. Oxalic acid production in *P.* × *euramericana* specimens treated with 2 copper-based wood preservatives and exposed to the fungi.

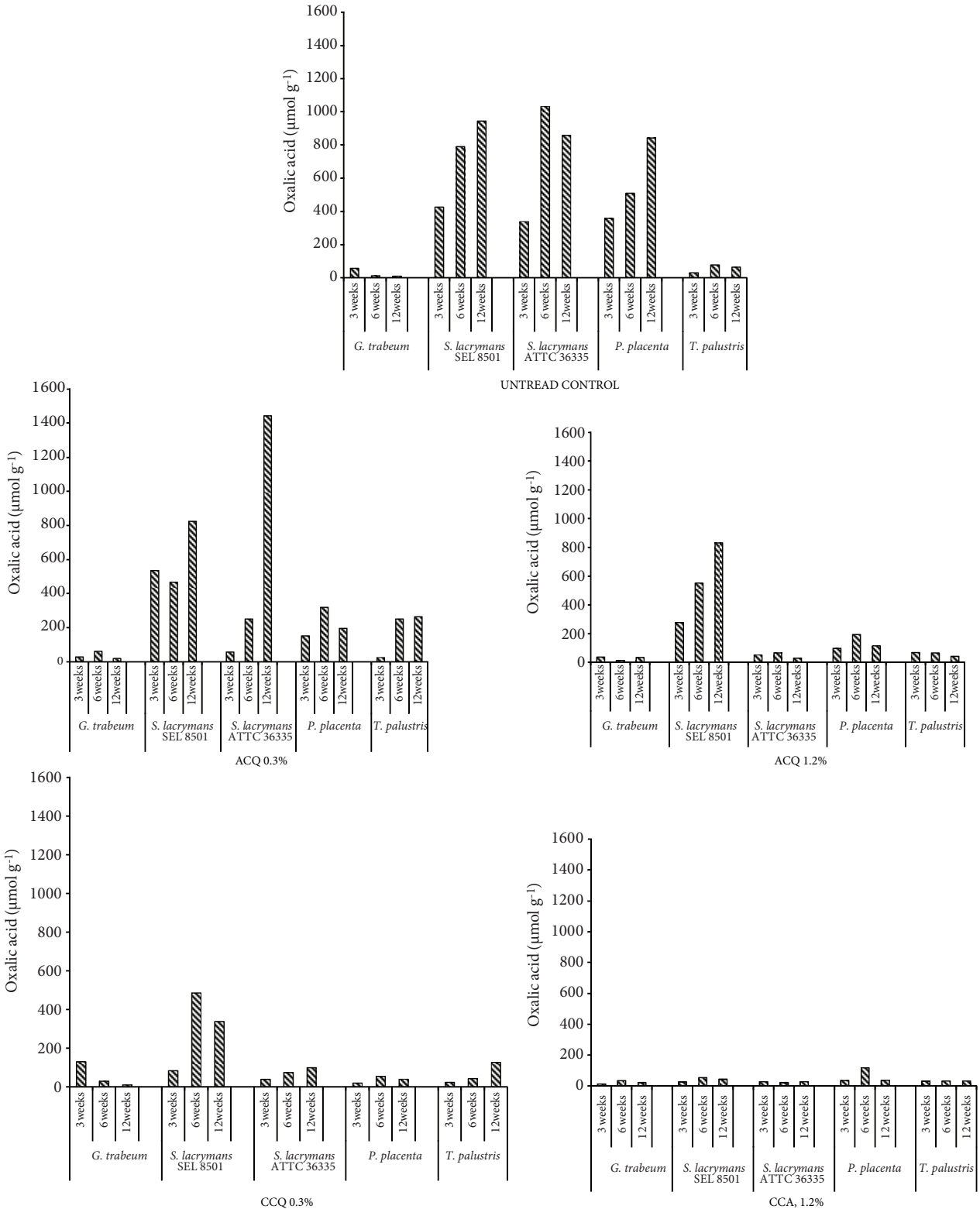


Figure 2. Oxalic acid production in *P. sylvestris* specimens treated with 2 copper-based wood preservatives and exposed to the fungi.

concentration levels) poplar specimens, respectively. In pine wood specimens, the fungus species were able to cause 54% and 15% mass losses in ACQ-treated specimens at 0.3% and 1.2% concentration levels; however, mass losses in CCA-treated wood specimens were much lower. *S. lacrymans*, the European dry-rot fungus, has also been shown to be copper-tolerant (Hastrup et al. 2005; Arango et al. 2009). Green III and Clausen (2003) found that oxalic acid production by 2 different strains of *S. lacrymans* was considerably lower than that of a number of brown-rot fungi tested in their study, and the strains tested exhibited sensitivity to copper citrate wood preservative. A study by Thornton (1991), however, showed that 8 strains of *S. lacrymans* were resistant to CCA inhibition in agar medium. In our study, the 2 strains of *S. lacrymans* produced higher amounts of oxalic acid when compared to other fungi tested for ACQ treatments. In CCA-treated wood specimens, oxalic acid production was lower for both pine and poplar wood because of the toxicity of the co-biocides in CCA formulation. In general, the *S. lacrymans* SEL 8501 strain resulted in more oxalic acid accumulation in treated wood specimens in comparison with the ATCC 36335 strain. Interestingly, the SEL 8501 strain caused high mass losses in pine wood (57%) and low mass losses in poplar wood (17%); however, the ATTC 36335 strain caused high mass losses in poplar wood (63%) and low mass losses in pine wood (11%). Copper losses from ACQ- and CCA-treated wood specimens were generally in accordance with oxalic acid production.

In our study, chromium and arsenic, as co-biocides in CCA formulation, affected the copper tolerance of the fungi tested when compared to ACQ treatments. Oxalic acid is a key factor in the degradation of treated wood by copper-tolerant fungi, since oxalic acid is known to form complexes with copper from treated wood. *S. lacrymans* SEL 8501 and *T. palustris* successfully colonized ACQ- and CCA-treated wood and caused high mass losses in treated wood, suggesting that those species were copper tolerant. *P. placenta* showed tolerance to copper in only ACQ-treated wood; in the presence of chromium and arsenic, CCA-C wood preservative inhibited this strain. Wood species, preservative type and formulation, growth rate and decay capacity, and oxalic acid production should be considered as important factors affecting the copper tolerance of any fungal strain.

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