Evaluation of Decay, Oxalic Acid Production and Strength Loss in Wood by the Dry Rot Fungus, *Serpula lacrymans*

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Abstract

***Serpula lacrymans* (Wulfen:Fr.) Schroeter, the dry rot fungus, is generally accepted as one of the most economically important wood degrading fungi in some temperate regions of the world. This study evaluated decay capacity of one isolate of *S. lacrymans* at four different wood species by the two different decay tests by using wood blocks and stakes. Besides mass losses in the specimens, strength losses and oxalic acid (OA) production in wood by the fungus during decay process were also measured. Higher mass losses were observed in the wood blocks in soil block tests when compared to the stakes in a modified soil bed tests. Losses in modulus of rupture (MOR) in bending were more distinctive in the stakes than mass losses. In the specimens subjected to decay tests, there was a good correlation between both mass losses and OA production and MOR losses and OA production. Further studies are in progress for treated wood specimens to understand copper tolerance ability of *S. lacrymans* to copper-based wood preservatives.**

Keywords: *Serpula lacrymans*, oxalic acid, mass loss, MOR loss, decay

1. Introduction

Decay caused by brown-rot fungi is the most prevalent and destructive type of wood deterioration because it can cause rapid structural failure. The dry rot fungus, *Serpula lacrymans* (Wulfen:Fr.) Schroeter is one of the most destructive and important decay fungi in buildings in Northern and Central Europe and it may cause decay and

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structural damage in both timber and masonry (Hastrup et al., 2006). Depolymerization of cellulose in wood by brown-rot fungi has been explained by different pathways; by the oxidative radical reactions, such as the Fenton reaction, initiated by the production of extracellular hydrogen peroxide (Koenigs, 1974; Highley, 1987; Ritschkoff and Viikari, 1991) by one-electron oxidation (Enoki et al., 1990, 1991) and by production of oxalic acid (OA) (Schmidt et al., 1981; Bech-Anderson, 1987; Green et al., 1991). Brown-rot fungi contain at least two different OA producing enzymes, glyoxylate oxidase (dehydrogenase) and oxalate hydrolase, and the production of OA is connected to the tricarboxylic acid (TCA) cycle (Shimada et al., 1991). OA plays an important role in both brown and white rot decay. It is also assumed to be a metabolic byproduct of incomplete glucose oxidation either via malate in the TCA cycle or glyoxylate in the glox cycle (Gadd, 1999; Munir et al., 2001). It is secreted by the majority of brown-rot fungi, including *S. lacrymans*, but only in limited amounts by white-rot fungi because of the presence of the OA-degrading enzyme oxalate decarboxylase (Akamatsu et al., 1992).

There might be no visible damage to the wood although brown rot decay fungi initiate colonization and start to release enzymes and organic acids such as OA. During early decay, color or texture of wood slightly changes, but decay may not be yet obvious (Clausen and Kartal, 2003; Clausen et al., 2001; Zabel and Morrell, 1992; Köse 2006). However, due to chemical changes during initial colonization, considerable losses in strength before measurable mass losses can occur (Clausen and Kartal 2003; Kim et al., 1996; Imamura 1993, Schmidt et al., 1978; Wilcox 1978).

It is of great importance to be able to prevent damage by *S. lacrymans* since this fungus is able to cause considerable failure in wooden structures. Since a relationship between copper tolerance and OA production has been implicated (Hastrup et al., 2005; Murphy and Levy, 1983), it is beneficial to know the resistance of *S. lacrymans* to wood treated with copper-containing wood preservatives. However, before tolerance tests, it is important to know the ability of the dry-wood fungus, *S. lacrymans* to produce OA during its decay process considering mass and strength losses in untreated wood. The aim of this study was to evaluate OA production and decay capacity in untreated wood blocks and stakes from various softwood and hardwood species exposed to the dry rot fungus, *S. lacrymans*. In the study, wood specimens were subjected to the fungus in a soil block test and a modified soil-bed test and strength and mass losses in the specimens were determined after a 3, 6 and 12 weeks incubation period together with pH and OA production.

2. Materials and Methods

2.1. Fungal culture

One isolate of *Serpula lacrymans* (Wulfen:Fr.) Schroeter (ATTC 36335) provided by the Center for Forest Mycology Research, Forest Products Laboratory, Madison, WI, USA was maintained on 2% malt extract agar.

2.2. Decay test

Wood blocks (19 x 19 x 19 mm) were prepared from sapwood portions of two softwood (*Pinus sylvestris* L., *Abies bornmülleriana* Mattf.) and two hardwood (*Fagus orientalis* Lipsky, *Populus* x *euramericana* I 214) logs. The blocks were free of knots, and visible concentration of resins, and showed no visible evidence of infection by mold, stain on wood destroying fungi. All blocks were conditioned at room temperature prior to steam sterilization for 30 minutes. Blocks were then subjected to *S. lacrymans* in soil block test (ASTM 1998). Test bottles were incubated at 27°C and 70% relative humidity (RH) for 3, 6 and 12 weeks. Mass loss in the blocks caused by fungus was then calculated from the weights before and after decay tests. Six replicate blocks for each wood species and duration were used in the tests.

Wood stakes from the sapwood portions of the four wood species (250 mm (L) by 25 mm (T) by 10 mm (R) were exposed to *S. lacrymans* in a modified cake pan test. One liter of a 1:1 soil and vermiculite mixture was placed in an aluminum cake pan. The surface of the mixture was covered with rows of southern pine feeders (42 mm by 29 mm by 3 mm). The moisture content of the soil/vermiculite mixture was adjusted to 50% of the water-holding capacity, and the test apparatus was autoclaved at 103 kPa and 121°C for 45 min. When the pans were cooled, the feeders were inoculated with the fungus by pipetting 100 ml/pan of a macerated 3-week-old liquid culture of *S. lacrymans* evenly over the feeders. The test pans were sealed in a plastic bag to prevent drying and incubated at 27°C and 70% RH for 3 weeks until the feeders were completely covered by fungal growth. Steam-sterilized test specimens were then placed on the top of the feeders and the pans were incubated at 27°C and 70% RH for 3, 6 and 12 weeks. Mass loss in the stakes caused by fungus was then calculated from the weights before and after decay tests. Six replicate stakes for each wood species and duration were used in the tests.

2.3. Determination of modulus of rupture (MOR)

Stakes were conditioned at 20°C and 65% RH prior to modulus of rupture (MOR) in bending determinations. MOR was conducted according to ASTM D4761 (2005). Wood stakes were tested on a Losenhausen Universal Testing System equipped with a load cell with a capacity of 10,000 N.

2.4. Determination of pH value and oxalic acid production

The stakes approximately 25 mm-long by full cross-section were cut from the decay zone near the mechanical failure of specimen. The pH of wood was determined by an extraction method. Wood blocks and stakes samples were ground into sawdust. Sawdust of 2.0 g was then immediately added to 50 ml of boiling de-ionized water and stirred for 5 min. in an Erlenmeyer flask with reflux. The mixture was standing in the closed flask for 30 minutes and was then rapidly cooled to room temperature. The

extract was then filtered and pH of the solution was measured with a glass electrode. The experiment was performed in three replicates.

Soluble OA was measured in the same sawdust samples for pH determinations. Sawdust samples were extracted in 3.0 ml 0.1 M phosphate buffer, pH 7.0, for 2 h with shaking. For each extracted sample, OA was determined by microassay with a diagnostic kit (Trinity Biotech Plc Ida Business Park Bray, Co., Wicklow, Ireland). Amount of OA was expressed as micromoles OA 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 at 590 nm. The intensity of the color produced is directly proportional to the concentration of oxalate in the sample.

3. Results and Discussion

Mass losses in wood blocks exposed to *S. lacrymans* for 3, 6 and 12 weeks are given in Figure 1. Average mass losses in the wood blocks ranged from 40% to 60% for 12 week incubation period. Highest mass loss was seen in *P. euramericana* specimens whilst the study revealed mass loss hierarchies of *P. euramericana* > *P. sylvestris* > *A. bornmülleriana* > *F. orientalis*. Hastrup et al. (2006) tested southern yellow pine wood blocks (10 x 10 x 10 mm) against various strains of *S. lacrymans* and the strain ATTC 36335 in soil block tests for 10 weeks. In their study, mass losses ranged from 28% and 53%.

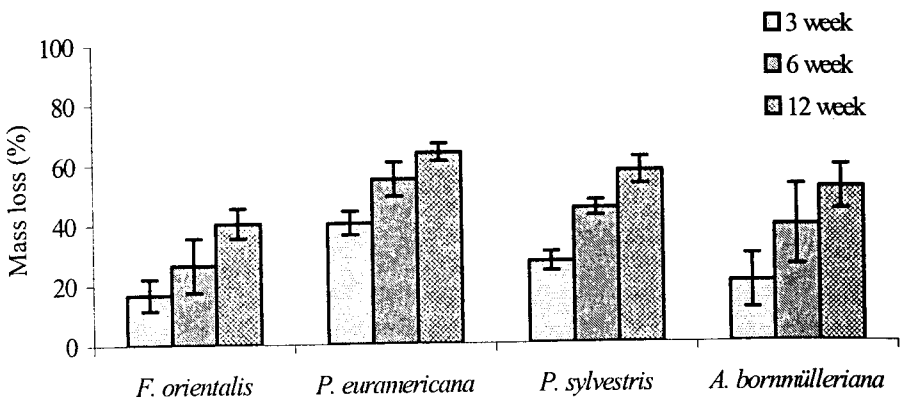

Figure 1. Mass losses occurred in the wood blocks.
Şekil 1. Odun bloklarında meydana gelen ağırlık kayıpları.

Relationship between mass losses and OA production in wood blocks exposed to *S. lacrymans* for 3, 6 and 12 weeks are given in Figure 2. The highest amount of OA was measured in *P. sylvestris* wood blocks; however, when compared to 6-week results, amount of OA decreased after 12-week-exposure period. Even though higher mass losses occurred in *P. euramericana* wood blocks than the other wood species, less OA production was seen in those blocks. The OA concentration and the percentage mass loss increased in blocks from week 3 to week 6. In *F. orientalis* and *P. euramericana* wood blocks, amount of OA increased from week 6 to week 12; however, no increases were seen in *P. sylvestris* and *A. bornmülleriana* blocks. *S. lacrymans* continued producing mass losses in *P. sylvestris* and *A. bornmülleriana* blocks even though those blocks showed no more OA production by the fungus.

Relationship between pH and oxalic acid accumulation in the wood blocks is given in Figure 3. There was a clear relation between pH and OA production in the blocks prepared from *F. orientalis*. In those blocks, as amount of OA increased, pH of wood decreased as expected. Some fluctuations were observed in *P. euramericana* and *A. bornmülleriana* blocks, even though OA production by the fungus continued. In *P. sylvestris* wood blocks, after 12 week exposure period, pH decreased when compared to week 6; however, OA production also decreased in the respective wood blocks for the same exposure periods.

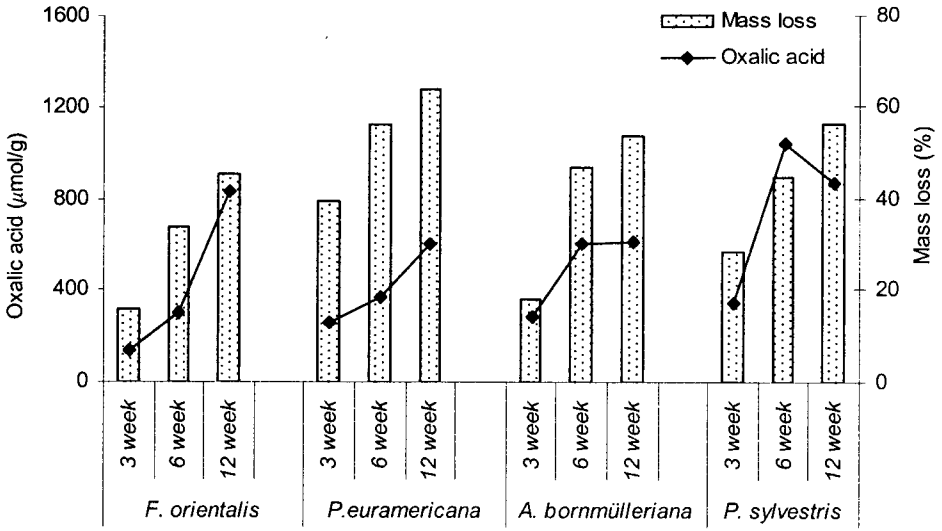

Figure 2. Relationship between mass loss and oxalic acid accumulation in the wood blocks.
Şekil 2. Odun bloklarında oksalik asit üretimi ile ağırlık kaybı arasındaki ilişki.

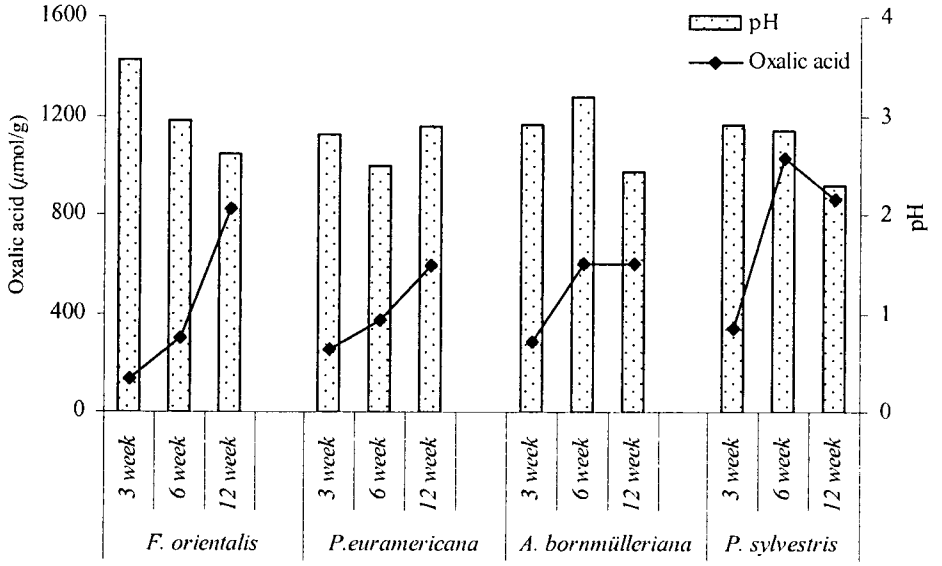

Figure 3. Relationship between pH and oxalic acid accumulation in the wood blocks.
Şekil 3. Odun bloklarında oksalik asit üretimi ile pH arasındaki ilişki.

Hastrup et al. (2006) studied correlations between the amount of soluble OA and the degree of wood decay in untreated southern yellow pine wood blocks. They found that OA levels declined in heavily degraded wood where average weight reduction of about 45–50% occurred. They stated that the amount of soluble OA were almost undetectable in wood blocks that were highly to completely degraded (Espejo and Agosin 1991; Itakura et al. 1994). In a study by Green III and Clausen (2003), two different *S. lacrymans* strains were tested to evaluate decay capacities and OA production in wood blocks. One strain caused mass loss of about 53% with OA production of about 169 mM; however, the other strain resulted in mass loss of 15% with OA production of 27 mM in southern yellow pine blocks. They state that OA production and a rapid lowering of pH by decay fungi are important in the initial stages of brown rot (Bech-Andersen, 1987; Green et al., 1991; Shimada et al., 1994). Micales and Highley (1988) state that OA production is not always directly related to the ability of fungi to decay wood.

Figure 4 illustrates MOR loss in the stakes exposed to *S. lacrymans* for 3, 6 and 12 weeks. Higher MOR losses were occurred in *P. euramericana*, *A. bormülleriana,* and *F. orientalis* stakes when compared to *P. sylvestris*. After 12-week exposure, MOR losses in the wood stakes in *F. orientalis*, *P. euramericana*, and *A. bornmülleriana* reached nearly 90%; however, P. sylvestris wood stakes had MOR losses of about 60%. In *P. euramericana*, *A. bormülleriana,* and *F. orientalis* stakes, MOR losses after 6 weeks were as high as those after 12 weeks. MOR losses in *F. orientalis*, *A. bornmülleriana*, and *P. sylvestris* wood stakes after 3-week-decay tests were around 40% while *P. euramericana* wood stakes showed MOR losses of nearly 70% in the same exposure period.

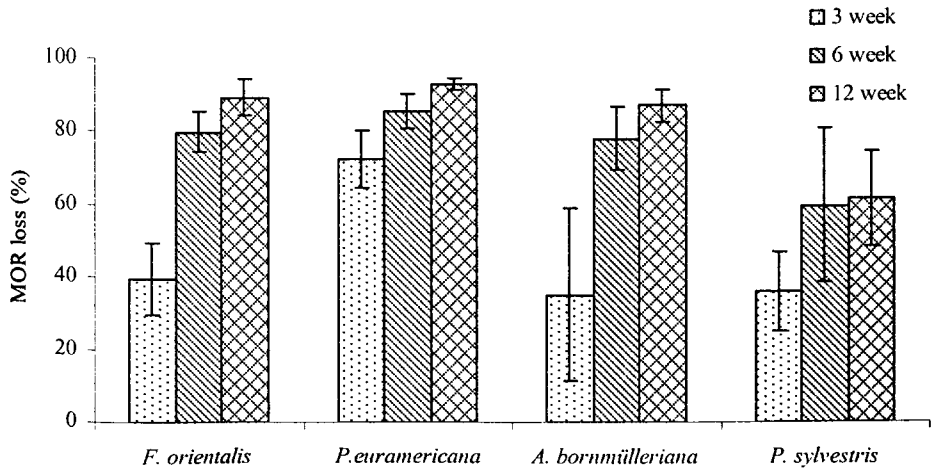

Figure 4. MOR loss in the stakes after decay tests.
Şekil 4. Çürüklük testleri sonrasında meydana gelen eğilme direnci kayıpları.

Figure 5 shows relationship among MOR, mass loss, and oxalic acid accumulation in the stakes after decay tests. The mass losses in wood blocks were two times greater than wood stakes. Measuring mass loss in the stakes in the modified cake pan method might be an inaccurate method compared soil block test method where considerably higher mass losses occurred. The size, shape, and greater volume of stakes might have slowed the progression of fungal colonization (Clausen and Kartal, 2003). In the study, MOR was reduced 72% to 93% by the time mass losses of 10% to 33% occurred. In general, OA production was correlated directly with decreases in pH of the substrates. *S. lacrymans* rapidly lowered the pH to 2.9 after 3 to 12-week decay process in *P. sylvestris, A. bormülleriana*, and *P. euramericana* wood stakes. Highest OA production was observed in the stakes from *P. euramericana* where highest MOR and mass losses occurred. *F. orientalis* and *A. bornmülleriana* stakes also showed MOR losses more than 80%; however, lower OA and mass losses were found in the respective stakes in comparison with *P. euramericana* wood stakes.

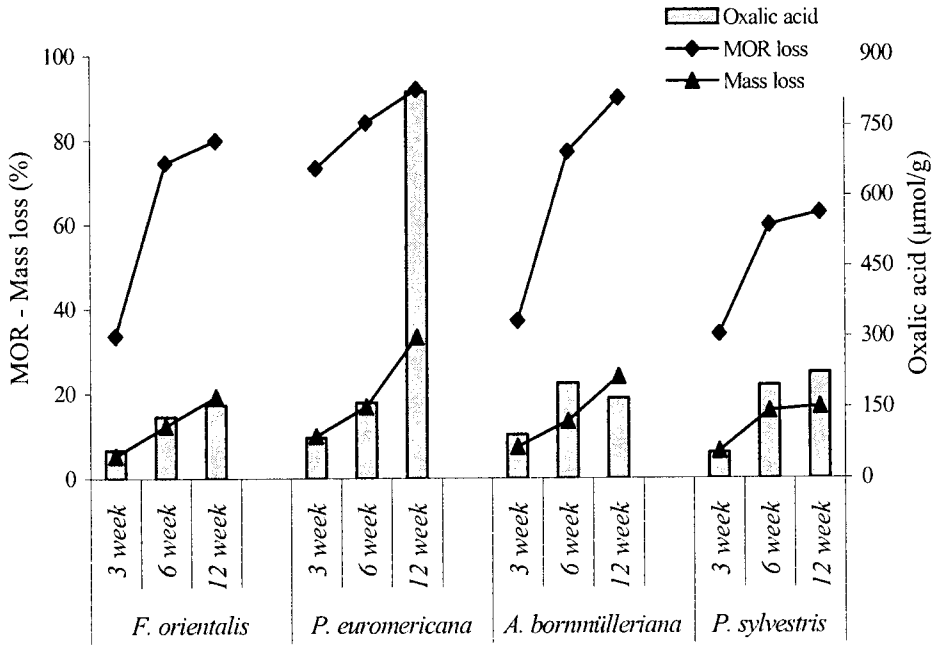

Figure 5. Relationship among MOR, mass loss, and oxalic acid accumulation in the stakes after decay tests.
Şekil 5. Çürüklük testleri sonrasında eğilme direnci örneklerinde direnç kaybı, ağırlık kaybı ve oksalik asit üretimi arasındaki ilişki.

In a study by Clausen and Kartal (2003) MOR showed the most rapid decline of mechanical properties tested in cake pan method using wood stakes. In their study, stakes showed a 6:1 ratio of strength loss to weight loss after 4 weeks incubation and MOR was reduced 19% by the time 3% weight loss had occurred. In a study by Curling et al. (2001), the effect of hemicellulose degradation on strength properties of wood was studied by exposing southern yellow pine stakes to *Gloeophyllum trabeum*. Their results showed a ratio of strength to weight loss of approximately 40:1.

Figures 6, 7 and 8 represent relationships between mass loss and oxalic acid accumulation, MOR and mass loss, and MOR loss and oxalic acid accumulation in the stakes after decay tests. In general good correlations were observed between mass loss and oxalic acid accumulation and MOR and mass losses in the stakes. However weaker correlation was found for MOR loss and oxalic acid production.

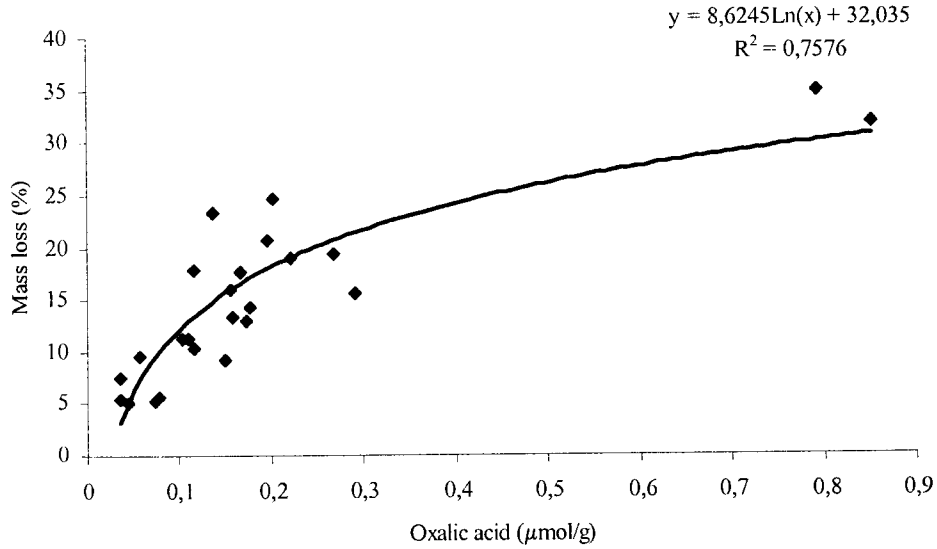

Figure 6. Relationship between mass loss and oxalic acid accumulation in the stakes after decay tests.
Şekil 6. Çürüklük testleri sonrasında eğilme direnci örneklerinde ağırlık kaybı ve oksalik asit üretimi arasındaki ilişki.

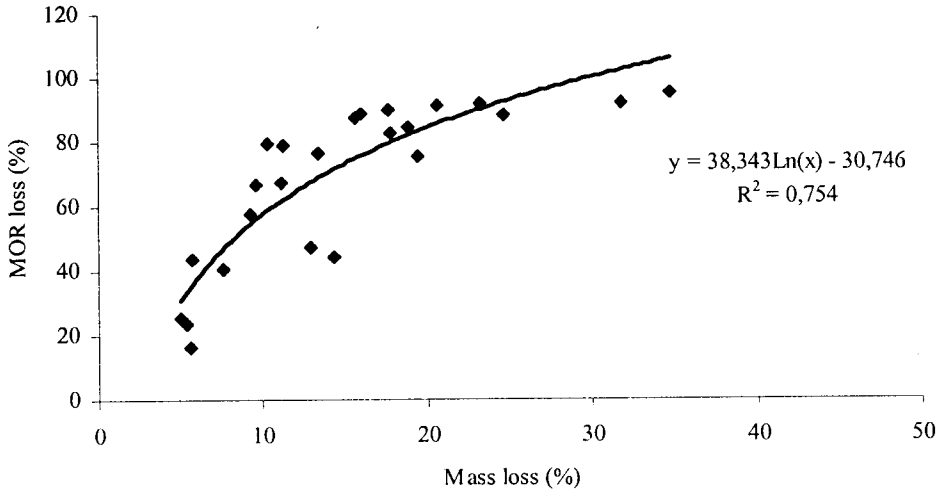

Figure 7. Relationship between MOR and mass loss in the stakes after decay tests.
Şekil 7. Çürüklük testleri sonrasında eğilme direnci örneklerinde direnç ve ağırlık kayıpları arasındaki ilişki.

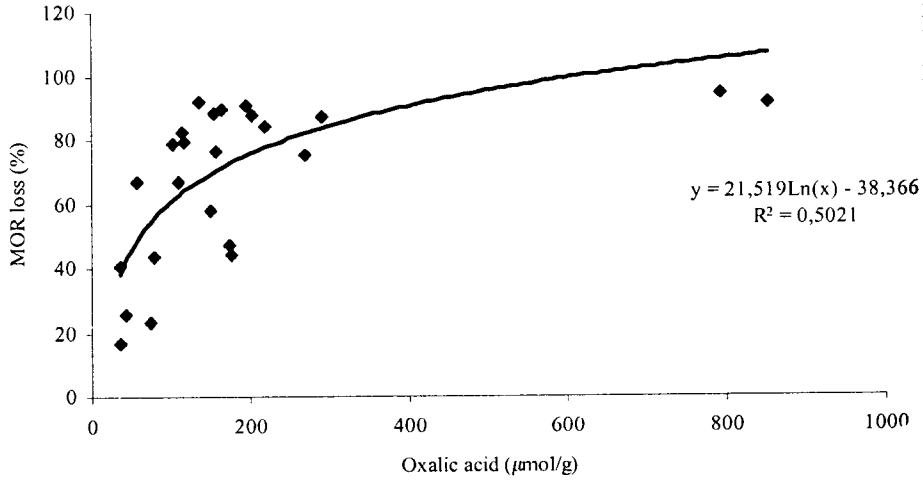

Figure 8. Relationship between MOR and oxalic acid accumulation in the stakes aft decay tests.
Şekil 8. Çürüklük testleri sonrasında eğilme direnci örenklerinde direnç kayıpları v oksalik asit üretimi arasındaki ilişki.

5. Conclusions

This study evaluated decay capacity of the dry wood fungus, *S. lacrymans* in various wood species measuring mass losses, OA production and strength losses in wood specimens. In the study, untreated wood specimens were exposed to the fungus in both soil block tests and modified soil bed tests. In the soil block tests, more mass losses in the wood blocks were observed in the stakes subjected to soil bed tests. Amount of OA production by the fungus over the wood blocks was greater than the stakes. MOR losses in the stakes were much more apparent than mass losses to determine initial steps of the decay by the fungus. Further studies are in progress to evaluate copper tolerance of *S. lacrymans* considering OA production and different wood species treated with copper-containing wood preservatives.

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Kuru Çürüklük Mantarı *Serpula lacrymans* Tarafından Odunda Meydana Getirilen Çürüklük, Oksalik Asit Üretimi ve Direnç Kaybının İncelenmesi

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Özet

Kuru çürüklük mantarı *Serpula lacrymans* (Wulfen:Fr.) Schroeter, dünyanın ılıman bölgelerinde önemli ekonomik kayıplara neden olan odun tahripçisi mantarlardan biri olarak kabul edilmektedir. Bu çalışma, dört farklı ağaç odunlarından 2 farklı boyutta hazırlanan örneklerde uygulanan 2 farklı çürüklük testinde *S. lacrymans*'ın bir izolasyonunun oluşturduğu çürüklük kapasitesini incelemektedir. Çürüklük sürecinde *S. lacrymans* tarafından örneklerde oluşturulan ağırlık kayıplarının yanı sıra, direnç kayıpları ve oluşan oksalik asit miktarı da belirlenmiştir. Modifiye edilmiş toprak yatak (soil bed) denemeleriyle karşılaştırıldığında, toprak blok (soil block) denemelerinde odun örneklerinde daha yüksek ağırlık kayıpları meydana geldiği görülmüşütür. Eğilme direnci deneme örneklerinde meydana gelen direnç kayıpları, ağırlık kayıplarına gore çok daha ayırt edicidir. Çürüklük denemelerinde kullanılan örneklerde hem ağırlık kaybı ile oksalik asit üretimi hem de eğilme direnci kaybı ile oksalik asit üretimi arasında iyi bir ilişki olduğu belirlenmiştir. Bakır esaslı emprenye maddelerine karşı *S. lacrymans* mantarının emprenyeli odun örneklerinde bakır toleransı kapasitesinin belirlenmesine yönelik çalışmalar sürdürülmektedir.

Anahtar Kelimeler: *Serpula lacrymans*, oksalik asit, ağırlık kaybı, eğilme direnci kaybı, çürüklük

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