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Original Article

Behavior of the Brown-rot Fungus *Gloeophyllum trabeum* on Thermally-modified *Eucalyptus grandis* Wood

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ABSTRACT

In this study, we aimed evaluate the behavior of the brown-rot fungus *Gloeophylum trabeum* and white-rot fungus *Pycnoporus sanguineus* on thermally-modified *Eucalyptus grandis* wood. To this end, boards from five-year-eleven-month-old *E. grandis* trees, taken from the Duratex-SA company stock, were thermally-modified between 180 °C and 220 °C in the Laboratory of Wood Drying and Preservation at Universidade Estadual Paulista - UNESP, Botucatu, Sao Paulo state Brazil. Samples of each treatment were tested according to the ASTM D-2017 (2008) technical norm. The accelerated decay caused by the brown-rot fungus *G. trabeum* was compared with the decay caused by the white-rot fungus *P. sanguineus*, studied by Calonego et al. (2010). The results showed that (1) brown-rot fungus caused greater decay than white-rot fungus; and (2) the increase in temperature from 180 to 220 °C caused reductions between 28.2% and 70.0% in the weight loss of *E. grandis* samples incubated with *G. trabeum*.

Keywords: decay resistance, G. trabeum, heat-treated wood, eucalypts, rot fungi.

Comportamento do Fungo de Podridão Parda *Gloeophyllum trabeum* na Madeira de *Eucalyptus grandis* Modificada Termicamente

RESUMO

O objetivo deste estudo foi avaliar o comportamento do fungo de podridão parda *Gloeophylum* trabeum e do fungo de podridão branca *Pycnoporus sanguineus* sobre a madeira de *Eucalyptus* grandis modificada termicamente. Tábuas de árvores de *E. grandis* com cinco anos e 11 meses de idade, da empresa Duratex-SA, foram modificadas termicamente entre 180 °C e 220 °C no Laboratório de Secagem e Preservação de Madeiras da UNESP, Botucatu-SP, Brasil. Corpos de prova de cada tratamento foram testados, de acordo com a norma técnica ASTM D-2017 (2008). O apodrecimento acelerado causado pelo fungo de podridão parda *G. trabeum* foi comparado com o do fungo de podridão branca *P. sanguineus*, estudado por Calonego et al. (2010). Os resultados mostraram que (1) o apodrecimento causado pelo fungo de podridão parda foi maior que o de podridão branca e (2) o aumento da temperature de 180 para 220 °C ocasionou reduções de 28,2% a 70,0% na perda de massa dos corpos de prova de *E. grandis* incubados com o *G. trabeum*.

Palavras-chave: resistência ao apodrecimento, *G. trabeum*, madeira tratada termicamente, eucalipto, fungos apodrecedores.

1. INTRODUCTION

The mechanism of wood degradation differs fundamentally between brown and white rot fungi. In general, the brown-rot fungi selectively removes cellulose and hemicelluloses compounds, whereas the white-rot fungi causes degradation of all cell wood components (Oliveira et al., 1986; Barreal, 1998). However, the white-rot fungus *Pycnoporus sanguineus* (L.) Murrill is a good producer of phenoloxidase, and preferentially degrades lignin (Esposito et al., 1993).

According to Oliveira et al. (1986), Barreal (1998) and Kleman-Leyer et al. (1992), the whiterot fungus attacks the surfaces of the microfibrils resulting in a progressive erosion of the polymers of wood. Yet, the brown-rot fungus completely cleaves the amorphous regions of the cellulose microfibrils, and subsequently, promotes significant loss in wood weight because of degradation in the crystalline region of the cellulose. At advanced stages of decay, the structural polysaccharides are quantitatively removed, and a modified lignin residue remains.

In general, the brown-rot fungi, including the species Gloeophyllum trabeum (Pers.) Murrill, produce extracellular hydrogen peroxide (H₂O₂) and oxalic acid (H₂C₂O₂), which react with the iron ions present in lignocelluloses materials. The hydroxyl radicals produced by the Fenton's reaction were suggested to explain the cleavage of long chain cellulose molecules into small fragments. Thus, there is an increase of porosity of the cell wall allowing penetration of cellulolytic enzymes, which increase the decay on wood (Goodell et al., 1997; Xu & Goodell, 2001; Arantes & Milagres, 2009; Watanabe et al., 2010; Aguiar & Ferraz, 2011). Dutton et al. (1993) showed that the brown-rot fungi secrete large amounts of oxalate in culture medium reducing the pH of the substrate compared with the white-rot fungi, which do not reduce the pH of medium.

In evaluating the natural biological resistance of Aspidosperma desmanthum, Parinari excelsa, Mouriri callocarpa, Marmaroxylon racemosum, Peltogyne paniculata and Astronium sp. woods to the brown-rot fungus G. trabeum and white-rot fungus Pycnoporus sanguineus, it was founded that the decay caused by brown-rot fungus was greater. The woods studied presented weight loss between 1.97% and 12.2% when decayed to *G. trabeum*, and between 0.05% and 3.21% to *P. sanguineus* after accelerated decay test of 6 weeks (Alves et al., 2006).

According to Andrade et al. (2012), *Eucalyptus grandis* wood then exposed to the white-rot fungi *Ganoderma applanatum* (Pers.) Pat., *P. sanguineus, Lentinula edodes* (Berk.) Pegler, and *Pleurotus sajorcaju* (Fr.) Singer for 8 weeks, presented weight loss of 34.0%, 29.0%, 27.5%, and 13.0%, respectively.

The biological durability of wood can be increased by impregnation with chemical products, but in general, this technique is not positively regarded. Thus, increasing the biological durability of wood by thermal modification is considered to be more acceptable (Homan et al., 2000).

In general, thermal treatments expose the timber to temperatures approaching 200 °C for several hours, and change the chemical composition of wood. The equilibrium moisture content of wood and the availability of food (hemicelluloses) to fungi are reduced, new molecules that act as fungicides are produced, and there is a cross-linking between the lignin and the polymer from the thermal degradation of cellulose, making the recognition of the substrate by fungi difficult (Weiland & Guyonnet, 2003; Hakkou et al., 2006; Calonego et al., 2010, 2012; Severo et al., 2012).

In the accelerated decay test of *Pinus pinaster* untreated wood and wood that was thermally-modified at 230-260 °C, and then exposed to the brown-rot fungus *Poria placenta* (Fr.) Cooke for 16 weeks, a weight loss of 17.13% and 9.76%, respectively, was verified. The respective weight losses of untreated and treated *Fagus sylvatica* were approximately 22.92% and 5.94% (Weiland & Guyonnet, 2003).

In tests of untreated *Pinus radiata*, *Pinus syslvestris*, *Pseudotsuga menziesii* and *Picea abies* woods exposed to the white-rot fungus *C. versicolor* and brown-rot fungus *C. puteana*, weight losses of 9% and 26%, 4% and 12%, 1% and 9%, and 12% and 19%, respectively, were observed. When the same woods were thermally-treated and exposed to the same rot fungi, weight loss was always less than 4% (Millitz & Tjeerdsma, 2001).

Momohara et al. (2003) thermally-modified *Cryptomerica japonica* heartwood at 150 °C for 24 hours and concluded that the weight loss caused by the brown-rot fungus *Fomitopsis palustris* (Berk. & M.A. Curtis) Gilb. & Ryvarden, for 8 weeks in accelerated laboratory tests, was 30% in untreated wood and 10% in thermally-treated wood.

In the accelerated decay test of untreated *Eucalyptus grandis* wood and wood that was thermally-modified at 180°, 200° and 220 °C, and then exposed to the white-rot fungus *P. sanguineus* for 12 weeks, a weight loss of 34.32%, 28.95%, 23.81% and 6.05%, respectively, was observed (Calonego et al., 2010).

However, there is little information about the effects of thermal treatment on the technological properties of *Eucalyptus* wood (Unsal & Ayrilmis, 2005).

Thus, the aim of this study was to evaluate the behavior of the brown-rot fungus *G. trabeum* and white-rot fungus *P. sanguineus* on thermallymodified *E. grandis* wood.

2. MATERIAL AND METHODS

In this study, we utilized wood from five-yeareleven-month-old *Eucalyptus grandis* trees from the Rio Claro Farm, managed by the Duratex-SA company, located in Lençóis Paulista, Sao Paulo state, Brazil. Six trees were randomly selected from inside the 2.2-ha stand. After felling, the trees were sectioned into 6.0-m logs. The first log from each tree with diameter between 20 and 22 cm was cut into flat saw boards. The boards that contained the pith were cut into 34-mm thick pieces for this study. Subsequently, all of the boards were dried from 75.7% to 10.0% moisture content in a dry kiln with capacity for approximately 2.5 m³ of wood.

2.1. Thermal treatments of boards

The six dried boards were planed to 32-mm thick and cut into smaller pieces measuring 0.60 m in length. Regions with cracks and knots were discarded. One of these smaller pieces was kept in its original condition (untreated wood), and the other pieces were reserved for the thermal treatments (thermally-modified wood). The material was placed in a thermal modification oven with programmable control. The treatment proceeded in steps from an initial temperature of 100 °C to 180 °C, and then to 200 °C and 220 °C over a period of 2.5 hours, according to the application of patent developed by Severo & Calonego (2009). Following the end of the thermal treatment, the oven was turned off and the wood pieces were kept inside. The pieces were allowed to cool naturally.

2.2. Accelerated laboratory tests of decay resistance of wood

The test to assess the attack of brown-rot fungus on the thermally-modified *Eucalyptus grandis* wood was conducted according to the standards presented in ASTM D-2017 (2008) norm. This procedure was performed simultaneously in the material inoculated with the white-rot fungus *P. sanguineus* studied by Calonego et al. (2010).

The samples were cut to create wood perfectly oriented in relation to the three anatomical planes (radial, tangential and longitudinal), and were approximately 40 mm from the pith of each piece of wood. The samples were sawn into test blocks measuring 25 by 25 by 9 mm in size, with the 9 mm dimension in the grain direction.

Although the ASTM D-2017 (2008) standard show that the necessary number of samples to characterize the decay resistance of wood is six, eighteen samples obtained from six boards were used to characterize each treatment (untreated wood and three other thermally-modified woods), totaling seventy-two samples by fungus tested.

In preparation for the test, the wood samples were dried in a drying oven at 103 ± 2 °C, until constant weight was reseached. As recommended by the ASTM D-1413 (2007) norm, the initial oven-dry weight (WI) of each test block was determined.

The soil-block test was prepared in 725 mL cylindrical culture bottles using 300 g of soil with a water holding capacity of 29%. After filling the bottles with distilled water, a feeder strip of *Pinus* sp. was added. Subsequently, the bottles were sterilized at 121 \pm 1 °C for 1 hour.

The culture bottles were then inoculated with the brown-rot fungus *G. trabeum* (collected from mycology collection of the INPA, Manaus, Amazonas state, Brazil, and identified as ID 408).

After sterilization, the test blocks were placed in the culture bottles with the cross-section face of the feeder strip facing down. The culture bottles were incubated in an incubation chamber in the dark to promote the growth of the fungus at 26.7 ± 1 °C and $70 \pm 4\%$ relative humidity for 12 weeks.

At the end of the exposure period, the test blocks were removed from the culture bottles and any surface fungus growth was carefully brushed off. The blocks were then dried in drying oven at 103 ± 2 °C once again until constant weight was reseached. This weight was determined as the final oven-dry weight (WF) of each test block.

The percent weight losses in the individual test blocks from before and after exposure to the decay fungi were then calculated. The percent weight losses in the test blocks provide a measure of the relative decay susceptibility of the untreated and thermallymodified *Eucalyptus grandis* wood.

3. RESULTS AND DISCUSSION

The weight loss data of *Eucalyptus grandis* wood caused by the action of the brown-rot fungus *G. trabeum* was normally distributed and analysis of variance with a randomized block design was therefore used, taking into account the thermal treatments, as well as Tukey's test at 5% significance level for the comparison of the means.

The values for the amount of weight loss in untreated *Eucalyptus grandis* wood, found in Table 1, were 50.33% and 34.32% for the samples incubated with the brown-rot and white-rot fungi, respectively.

These results are similar to those cited by Andrade et al. (2012), who characterized the biological resistance of *Eucalyptus grandis* wood and concluded that material inoculated with the whiterot fungus *P. sanguineus* showed a weight loss of 29.0%, after 8 weeks in the accelerated test decay.

However, as shown in Table 1, the resistance of *E. grandis* wood to the brown-rot fungus *G. trabeum* was smaller than to the white-rot fungus studied by Calonego et al. (2010). According to Dutton et al. (1993), these variations were expected because the brown-rot fungi secrete large amounts of oxalate in culture medium, reducing the pH of the substrate during growth, and the amount of oxalate produced by white-rot fungi was not enough to reduce the pH of the medium during growth.

Several authors have reported that the brown-rot fungus *G. trabeum* produce extracellular hydrogen peroxide (H_2O_2) and oxalic acid $(H_2C_2O_2)$, which

Treatment	N		Moisture content (%)			
			Before test		After test	
	P. sanguineus	G. trabeum	P. sanguineus	G. trabeum	P. sanguineus	G. trabeum
Untreated	21	18	9.2 a	9.2 a	244.3 a	172.7 a
180 °C	21	18	7.5 b	7.5 b	226.9 ab	161.6 a
200 °C	21	18	6.5 c	6.4 c	200.0 b	121.0 b
220 °C	21	18	4.8 d	4.5 d	126.5 c	96.2 b
Treatment	Soil-block test					
	P. sanguineus		G. trabeum			
	Weight loss %	Reduction by Reduction by thermal Weight loss % thermal modification type of fun		(Increase) by ingus %		
		%		%		
Untreated	34.32 a		50.33 a		(46.7) *	
180 °C	28.95 ab	15.7	36.12 b	28.2	(24.8) ^{NS}	
200 °C						
	23.81 b	30.6	23.51 c	53.3	1.3	NS

Table 1. Attack of brown-rot and white-rot fungi on thermally-modified Eucalyptus grandis wood.

Where: N - repeat number of samples; *P. sanguineus* according to Calonego et al. (2010); different letters - significant difference by Tukey's test at 95% probability between treatments; * - significant difference by "F" test at 95% probability between fungi; same letters and NS - non-significant difference.

react with the iron ions present in lignocellulosic materials by the Fenton's reaction and cause the cleavage of long chain cellulose molecules into small fragments. Thus, there is an increase of porosity of the cell wall allowing penetration of cellulolytic enzymes which increase the decay on wood (Goodell et al., 1997; Xu & Goodell, 2001; Arantes & Milagres, 2009; Watanabe et al., 2010; Aguiar & Ferraz, 2011).

These explanations are in agreement with Alves et al. (2006), who evaluated the natural biological resistance of various tropical woods to the brown-rot fungus *G. trabeum* and white-rot fungus *P. sanguineus* and concluded that the decay caused by brown-rot fungus was greater.

However, the objective of this study was also to evaluate the resistance of thermally-modified *E. grandis* wood to brown-rot fungus in comparison with white-rot fungus.

The visual features of samples submitted to the action of the brown-rot and white-rot fungi are shown in Figure 1, and the values for the amount of weight loss and the average moisture content of *E. grandis* wood are presented in Table 1.

These evaluations indicated that although the thermal treatment reduced some of the mechanical properties of wood (Calonego et al., 2012), there was a significant improvement in the decay resistance of *Eucalyptus grandis* wood by the increase in treatment temperatures. These results were expected because Homan et al. (2000), Millitz & Tjeerdsma (2001), Momohara et al. (2003), Weiland & Guyonnet (2003), Hakkou et al. (2006), and Calonego et al. (2010) found that thermal treatment at high temperatures increased the decay resistance of wood of other species and/or other fungi.

The decrease in weight loss caused by decay fungi (see Table 1) has already been explained by several authors, among them Weiland & Guyonnet (2003), Hakkou et al. (2006), Calonego et al. (2010) and Severo et al. (2012), because of changes in the chemical composition of wood, mainly the unavailability of food (hemicelluloses) to the fungi, the production of new molecules that act as fungicides, and the cross-linking between lignin and the polymer from the thermally degraded cellulose.

Verifying the effects of the thermal treatment on the decay caused by the white-rot fungus *P. sanguineus* and by the brown-rot fungus *G. trabeum*, it was possible to verify that the decay caused by brown-rot fungus was greater in all temperatures of thermal treatment.

These results can be explained by the ability of brown-rot fungi to secrete hydroxide complexes and oxalic acid able to cleave long chain cellulose



where: W.R.F. is white-rot fungi *Pycnoporus sanguineus*; B.R.F. is brown-rot fungi *Gloeophylum trabeum*.

Figure 1. Aspects of test blocks from untreated and thermally-modified *Eucalyptus grandis* wood after decay by brown-rot and white-rot fungi.

molecules into small fragments, increasing the porosity of the cell wall, the penetration of cellulolytic enzymes, and the decay of wood (Goodell et al., 1997; Xu & Goodell, 2001; Arantes & Milagres, 2009; Watanabe et al., 2010; Aguiar & Ferraz, 2011).

4. CONCLUSIONS

This study shows that the decay caused by brown-rot fungus was greater than that caused by white-rot fungus. However, in verifying the effects of thermal modification on the decay resistance of *E. grandis* wood, it was possible to conclude that there was a decrease between 28.2 % and 70.0 % in the weight loss of wood exposed to the brown-rot fungus *Gloeophylum trabeum*.

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