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Biological Resistance of *Eucalyptus* Wood Treated with Chromated Copper Borate to Fungi Decay

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Abstract

The objective of this study was to evaluate the efficiency of a preservative treatment with Chromated Copper Borate (CCB) in different concentrations and autoclave pressure time on the biological resistance of *Eucalyptus urophylla* x *Eucalyptus grandis* (called urograndis). Urograndis fence posts were submitted to industrial autoclaving in nine treatments as a function of CCB concentration and pressure time: 1.5% (30, 60 and 90 minutes); 2.0% (30, 60 and 90 minutes); and 2.5% (30, 60 and 90 minutes). Then the obtained specimens were submitted to an accelerated laboratory decay test. The industrial preservative treatment increased the resistance of the wood to decay and the concentration of 1.5% CCB and 30 minutes of autoclave pressure can be applied to treat urograndis wood without compromising its resistance to the attack of white-rot and brown-rot fungi.

Keywords: Accelerated decay test, CCB concentration, autoclave pressure time.

1. INTRODUCTION

Species of the *Eucalyptus* genus are used in supplying raw material for various industrial segments such as cellulose and paper, energy, and solid products such as lumber and fence posts. In the year 2017, an area of 313.60 thousand hectares of planted forests was destined to serve the solid wood market (*INDÚSTRIA BRASILEIRA DE ÁRVORES - IBÁ*, 2018), with emphasis on *Eucalyptus* fence posts, which are used in civil and rural construction projects.

Eucalyptus' wood used in civil and rural construction, especially in ground contact as fence posts, presents low natural durability (Oliveira et al., 2017) due to its anatomical and chemical characteristics (Oliveira et al., 2012). Knowledge of the natural resistance of wood to attack by xylophagous organisms is important for recommending the most appropriate use, reducing unnecessary expenses on replacing parts and decreasing the impacts on the environment (Paes et al., 2013).

In this sense, a set of measures and procedures must be implemented in using wood as fence posts or in another use in contact with the soil, such as industrial preservative processes (Valle et al., 2013) to increase resistance to wood decay xylophagous organisms (Vidal et al., 2015), especially regarding rotting fungi, which are the main cause for wood damage, causing devaluation and reducing the service life of the product (Kelley et al., 2002; Vivian et al., 2015; Lopes et al., 2017).

Among the preservatives used in industrial processes in Brazil, water-soluble substances such as Chromated Copper Arsenate (CCA) and Chromated Copper Borate (CCB) stand out. Although CCA is the most widely used product in Brazil (Vidal et al., 2015), its use in wood impregnation can cause health damage due to exposure to arsenic, and therefore the use of CCB can not only reduce environmental risks, but also health problems, since boron is less toxic compared to arsenic (Gallio et al., 2017).

Some factors involved in the process and the preservative product influence the final quality of treated fence posts, such as temperature (oil-soluble), pressure time and concentration of the preservative product (Vivian et al., 2012; Gallio et al., 2017).

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The most common industrial process for wood treatment in Brazil employs 60 min as pressure time and 2.0% product concentration (Valle et al., 2013; Amaral et al., 2014; Lopes et al., 2017; Gallio et al., 2017). However, with the high cost of preservative coupled with the rotation time of wood pieces inside the autoclave, it is essential to evaluate alternative times and concentrations that provide better autoclaving conditions aiming at lower costs in producing treated wood.

The accelerated laboratory decay test with white, brown and soft rot fungi are one of the main techniques for assessing wood resistance to attacks by xylophagous organisms and can be considered the first stage to determine the efficiency of wood preservatives (Ramos et al., 2006; Paes et al., 2007; Vivian et al., 2015). The objective of this study was to evaluate the efficiency of preservative treatments with CCB at different concentrations and pressure times in the biological resistance of *Eucalyptus urophylla* x *Eucalyptus grandis* wood subjected to white-rot and brown-rot fungi under controlled conditions.

2. MATERIAL AND METHODS

2.1. Sample collection, preparation and preservative process

Ten *Eucalyptus urophylla* S.T. Blake x *Eucalyptus grandis* W. Mill ex Maiden hybrid (called urograndis) trees were selected at seven years old from a plantation located in the municipality of Nerópolis (16º18'28.85"S and 49º13'3.80"W) in the state of Goiás, Brazil. The trees were cut down, debarked and obtained wood logs along the trunk, with a length of 2.20 meters, and denominated fence posts. Four logs were cut per tree, totaling 40 round pieces.

The urograndis fence posts were dried for 60 days until reaching 20% humidity, and were divided into two groups: the treated and untreated. Four fence posts were selected in their original state for the untreated group (or control specimens), without receiving preservative treatment.

The fence posts in the treated group were submitted to the industrial preservative treatment with Chromated Copper Borate (CCB) (MOQ OX 50, Montana Química, SA) with 50% of active ingredients in an autoclave of 15 meters in length and 1.60 meters diameter.

Full-cell process (Bethell method) was applied, with an initial vacuum of 600 mmHg for 30 min, and 12 kgf cm^{-2} of pressure; and a final vacuum of 600 mmHg for 15 minutes at concentrations of 1.5; 2.0 and 2.5%, and pressure times of 30; 60 and 90 min, totaling nine treatments (3 concentrations x 3 pressure times; four fence posts per treatment).

The concentrations and pressure times used in this study were defined as within the commonly used parameters in wood treatment plants in Brazil (Amaral et al., 2014; Gallio et al., 2017; Lopes et al., 2017). The fence posts were dried for 30 days after the preservative process at the different concentrations and times for the fixation of the product used in the impregnation.

2.2. Accelerated laboratory decay test

The accelerated decay test was conducted at the Wood Biodegradation and Preservation Laboratory (*Laboratório de Biodegradação e Preservação da Madeira*), of the Forest Products Laboratory (*Laboratório de Produtos Florestais - LPF*), located in Brasília, Brazil, according to the American Society for Testing and Materials - ASTM D- 2017 (2005) and ASTM D-1413 (2007).

The test preparation included fungi cultivation, preparing the soil samples and flasks for developing the culture in an aseptic environment, as well as evaluating the mass loss caused by the tested fungi.

2.2.1. Fungi cultivation

Two fungi were used for this experiment: white-rot *Trametes versicolor* (L.) Lloyd (mad 697), and brown-rot *Gloeophyllum trabeum* (Pers.) Murrill (mad 617), from the *LPF* fungus collection. The culture medium used for fungi growth was prepared with deionized water and malt extract (30g of the extract/ 1000 ml of water). The solution was sterilized in an autoclave at 121 ºC with a pressure of 1 kgf cm^{-2} for 30 minutes and then maintained in the incubator at 27 ºC and 70% relative humidity for seven days to verify the occurrence of contamination.

The fungi were harvested aseptically in a laminar flow hood after ultraviolet light action for 5 min. Inoculates of approximately 1 cm^2 containing mycelia, were added to the culture medium and routed to a shaker table at 100 rpm for 72 h. The fungi then remained in the incubator for four weeks until their full development.

2.2.2. Sample preparation

Wood specimens from the fence posts sapwood region were obtained, in dimensions 2.0 x 2.0 x 1.0 cm (radial x tangential x longitudinal). Forty specimens were collected from each conditions (nine treated: three concentrations x three pressure times and one untreated); twenty for exposure to the *Gloeophyllum trabeum* fungus, and twenty for *Trametes versicolor*, totaling 400 wood specimens.

The specimens were sanded to remove burrs and small barbs and placed in an oven at 50 ºC (± 2 ºC), with forced

ventilation until reaching constant mass. This procedure was performed on an electronic scale with an accuracy of 0.0001g. At the end of this step, the initial mass of the specimens was registered and then used to calculate the mass loss.

2.2.3. Soil and flask preparation

Soil preparation and flasks were conducted according to ASTM D 1413 (2007) using glass vials with a screw cap of 250 mL capacity. Next, 130 g of red latosol (horizon B) was added to each flask from the *Fazenda* Água *Limpa* of the *Universidade de Brasília* (UnB), Brazil. This soil was sieved in a 30 mm aperture mesh, its pH was corrected to 6 ± 0.5, and 65 mL of distilled water was added to each flask to achieve the water retention capacity and soil moisture content established in the standard.

Next, a wooden plate (3 x 29 x 35 mm) was laid on the soil to support the mycelial development in each vial. Pinus (*Pinus* spp.) was used in flasks inoculated with brown rot fungus, and Embaúba (*Cecropia* spp.) was used in flasks with white-rot fungi. After receiving the plates, the vials were autoclaved at 120 °C for 45 minutes and taken to an incubator (RH 75 ± 2% and 25 ± 2 °C) for 10 days to check for possible contamination.

2.2.4. Fungi inoculation

The culture media with each of the already developed rotting fungi were aseptically homogenized from an adaptation of ASTM D 2017 (2005) and ASTM D 1413 (2007) in a blender, and then 3 ml of this solution were inoculated into the support board and soil with an automatic pipette. The flasks were returned to the incubator for four weeks, the time required for the fungi properly develop on the carrier plate. After this period, the samples were sterilized under the already mentioned conditions, and after being naturally cooled were placed on the support plate colonized by the fungus. The vials were then returned to the incubator where they remained for up to 16 weeks.

2.2.5. Evaluation of mass loss

After the exposure period to the fungi in the incubator, the specimens were removed from the vials. The adhered mycelium was removed with a toothbrush with soft bristles. The wood specimens were taken to a greenhouse and kept under the already described conditions, where they remained until reaching constant mass and their final mass was registered.

The conditions (nine treated: three concentrations x three pressure times and one untreated) proposed in this study were evaluated for the mass loss variation after the accelerated rotting test, calculated by the difference between the initial mass (before the fungal attack) and the final mass (after the fungal attack). The conditions were classified according to the average mass loss of the wood specimens, according to ASTM D 2017 (2005) (Table 1).

Table 1. Resistance classes of wood to fungi rot.

Resistance class	Mass loss (%)	Residual mass (%)
Highly resistant (HR)	0 – 10	90 – 100
Resistant (R)	11 – 24	76 – 89
Moderately resistant (MR)	25 – 44	56 – 75
Non-resistant (NR)	> 45	> 55

Source: ASTM D 2017 (2005)

The data distribution by the Shapiro-Wilk test, outliers by the Box-plot method, and the heterogeneity of the variance by the Bartlett and Levene tests ($p < 0.05$) were performed. The data presented distribution normality and homogeneity of variance, and Analysis of Variance (ANOVA) and Tukey test ($p < 0.05$) was applied to verify the condition effect (treated and untreated), CCB concentration, autoclave pressure time and interactions.

3. RESULTS AND DISCUSSION

The analysis of variance indicated a significant effect of the condition (treated and untreated) on the mass loss of urograndis wood (Table 2). The preservative treatment with CCB increased the wood's resistance to the fungi attack.

Some studies have also found a higher resistance of wood to degrading agents when submitted to preservative treatment, evidencing lower mass loss when treated in controlled laboratory trials (Lazarotto et al., 2016; Lopes et al., 2017; Xuan et al., 2017).

There were no significant differences between concentrations (1.5 to 2.5%), regardless of the type of rot (Table 2). The reduced mass loss with the 1% increase in the concentration of the CCB product applied to the fence posts was only 0.14% for *Gloeophyllum trabeum* and 0.41% for *Trametes versicolor*, indicating that the concentration of 1.5% can be applied to impregnate urograndis wood by autoclaving, without compromising its resistance to attack by white and brown rot fungi.

When evaluating the mass loss of wood treated at different concentrations (2.0, 4.0 and 6.0%) of CCB subjected to fungal deterioration, Gallio et al. (2017) also found satisfactory values for all concentrations, including the lowest used (2%) with a mass loss of 1.23%.

Table 2. Wood mass loss in the presence of *Gloeophyllum trabeum* (GT) and *Trametes versicolor* (TV) by condition and CCB concentration.

Condition	Concentration (%)	Mass loss GT (%)	Resistance class	Mass loss TV (%)	Resistance class
Treated	1.5	0.81 (0.24) a	HR	1.41 (0.63) a	HR
	2.0	0.74 (0.18) a	HR	1.10 (0.25) a	HR
	2.5	0.67 (0.19) a	HR	1.00 (0.25) a	HR
Untreated	-	19.10 (1.85) b	R	53.00 (2.98) b	NR

The averages are followed by the standard deviation. Means followed by the same letter in the column do not differ statistically.

Evaluating the degree of resistance of urograndis wood according to ASTM D 2017 (2005), it was possible to determine that all the concentrations reached the minimum required to classify them as highly resistant, meaning that the mass loss of wood was less than 10%. It is evidenced that the lower concentration used (1.5%) gave the urograndis wood high resistance, with a residual mass of 99.2% - 98.6% for *Gloeophyllum trabeum* and *Trametes versicolor*, respectively.

Trametes versicolor caused the greatest mass losses: the wood untreated samples submitted to this fungus showed a reduction of more than 50% of their mass and were classified as non-resistant. On the other hand, the wood samples from the untreated fence posts were classified as resistant to the mass loss caused by *Gloeophyllum trabeum*.

The higher mass loss caused by the action of the *Trametes versicolor* fungus can be explained since white-rot fungi develop better in hardwoods (such as eucalyptus), while brown-rot fungi develop more in conifers (Vivian et al., 2015). White-rot fungi attack cellulose, hemicellulose and lignin, while brown-rot fungi only attack cellulose and hemicellulose, leaving lignin intact (Lelis et al., 2011).

The mean mass loss of the treated wood at different times (30, 60 and 90 min) and without the preservative treatment during the accelerated rotting test with the white-rot and brown-rot fungi are shown in Table 3.

Significant differences were verified between the times (30 to 90 minutes) for both types of rot. There was a reduction in the mass loss with a 60 minute pressure increase of the preservative treatment in 0.39% for *Gloeophyllum trabeum* and 0.57% for *Trametes versicolor*.

For the urograndis wood treatment by autoclaving, the results obtained in this study suggest that despite having the largest mass loss, the 30 minute condition does not compromise the wood's resistance to the white and brown-rot fungi attack. Scientific papers, which have evaluated the wood mass loss, treated with CCB by rot fungi in laboratory tests considering different pressure times are scarce, making it difficult to compare the data obtained in these studies.

In Figure 1 it is possible to observe the interaction between the CCB concentration and the autoclave pressure time in the mass loss of urograndis wood subjected to the accelerated rotting test in the presence of *Gloeophyllum trabeum*. Higher mass losses are associated with lower autoclave pressure times and lower CCB concentrations, although they are not always significant (Tables 2 and 3).

Higher mass losses were observed at all concentrations where the pressure in the autoclave was applied for 30 minutes (1.05, 0.94, and 0.83% to 1.5, 2.0, and 2.5% respectively), while lower losses were found for all pressure time concentrations of 90 minutes (0.60, 0.59, and 0.47% for 1.5, 2.0 and 2.5%, respectively).

Despite the higher percentages of mass loss found in the conditions under which the wood was subjected for less time and with lower concentrations, all the woods exposed to *Gloeophyllum trabeum* are classified as highly resistant according to ASTM D 2017 (2005), as previously discussed.

For urograndis wood exposed to *Trametes versicolor* in the accelerated decay test (Figure 2), the mean mass loss varied from 0.93% (2.5% to 90 minutes) to 2.19% (1,5% to 30 minutes). This result shows the same behavior as that observed for brown-rot fungus, but with a lower mass loss difference in the concentrations of 2.0-2.5% and in the pressure times of 60-90 min. Despite the differences noted in the mass loss percentages, all applied conditions gave high resistance to urograndis wood in the presence of *Trametes versicolor* fungus.

Table 3. Wood mass loss in the presence of *Gloeophyllum trabeum* (GT) and *Trametes versicolor* (TV) by condition and autoclave pressure time.

Condition	Time (minutes)	Mass loss GT (%)	Resistance class	Mass loss TV (%)	Resistance class
Treated	30	0.94 (0.21) a	HR	1.54 (0.48) a	HR
	60	0.72 (0.16) ab	HR	0.99 (0.25) b	HR
	90	0.55 (0.18) b	HR	0.97 (0.25) b	HR
Untreated	-	19.10 (1.85) c	R	53.00 (2.98) c	NR

The averages are followed by the standard deviation. Means followed by the same letter in the column do not differ statistically.

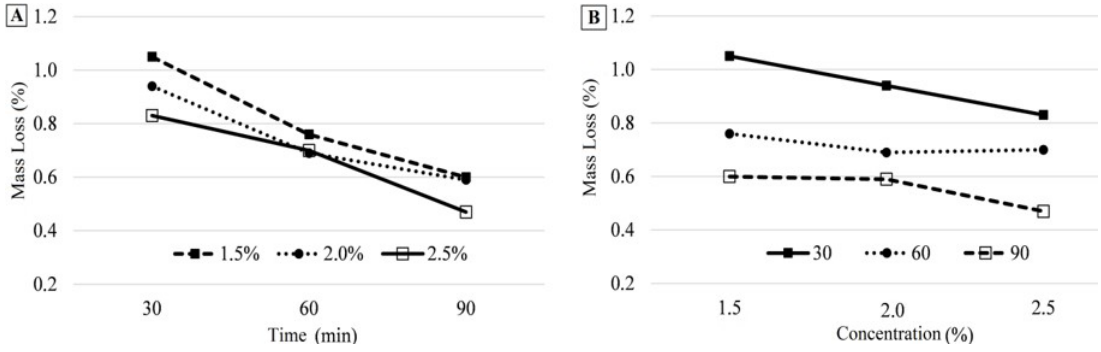

Figure 1. Interaction between time (A) and CCB concentration (B) in the urograndis wood mass loss in the presence of *Gloeophyllum trabeum*.

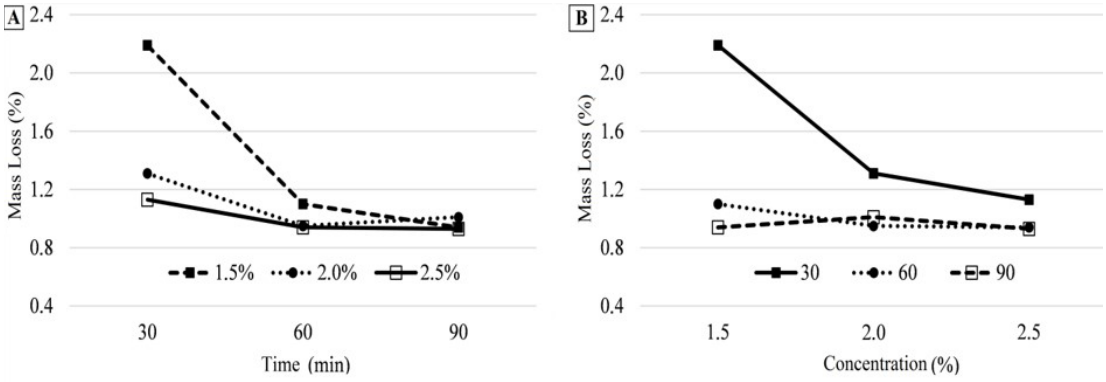

Figure 2. Interaction between time (A) and CCB concentration (B) in the urograndis wood mass loss in the presence of *Trametes versicolor*.

The results obtained in this study show that the application of CCB increases the resistance of urograndis wood to deterioration by fungi. Supplementary studies, such as the exposure of CCB treated wood in a field test in ground and the evaluation of boron leaching, as well the economic feasibility, are recommended for the recommendation of replacing Chromated Copper Arsenate (CCA) by CCB in the wood treatment plants.

4. CONCLUSIONS

The industrial treatment with Chromated Copper Borate increased the *Eucalyptus urophylla* x *Eucalyptus grandis* wood resistance to white and brown-rot fungi.

The application of 1.5% Chromated Copper Borate concentration and 30 minutes of pressure in the autoclave can be applied in treating *Eucalyptus urophylla* x *Eucalyptus grandis* wood without compromising its resistance to attack by *Trametes versicolor* and *Gloeophyllum trabeum* fungi.

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