

ORIGINAL ARTICLE

Resistance of acetylated *Jacaranda copaia* wood to termites and decaying fungi attack

Andressa Midori Yamauchi BAUFLEUR¹, Diego Martins STANGERLIN¹, Fernando Nunes GOUVEIA², Anna Sofya Vanessa Silvério da SILVA², José Roberto Victor de OLIVEIRA², Marcelo Fontana da SILVEIRA², Alexandre Santos PIMENTA³, Rafael Rodolfo de MELO^{4*} 

¹ Universidade Federal de Mato Grosso, Programa de Pós-Graduação em Ciências Florestais e Ambientais, Cuiabá, MT, Brazil

² Serviço Florestal Brasileiro, Laboratório de Produtos Florestais, Brasília, DF, Brazil

³ Universidade Federal do Rio Grande do Norte, Escola Agrícola de Jundiá, Macaíba, RN, Brazil

⁴ Universidade Federal Rural do Semi-Árido, Centro de Ciências Agrárias, Mossoró, RN, Brazil

* Corresponding author: rafael.melo@ufersa.edu.br;  <https://orcid.org/0000-0001-6846-2496>

ABSTRACT

The natural durability of the wood is essential for the definition of its use, and this property can be enhanced with the proper chemical treatment of the wood. Thus, the objective of this study was to evaluate the resistance to termites and decay fungi of *Jacaranda copaia* wood chemically modified through acetylation. Five experimental treatments were assessed: acetylation for 2, 4, 6 and 8 hours and a control (non-acetylated). The acetylation was carried out by immersing wood samples in acetic anhydride at 90 °C. Acetylated and control samples were subjected to the action of xylophagous termites (*Nasutitermes* sp.) and decaying fungi (*Gloeophyllum trabeum* and *Trametes versicolor*). The acetylation process significantly increased the resistance of *Jacaranda copaia* wood to the attack of the xylophagous organisms. There was no mass loss after exposure to termites of the wood in any of the acetylation treatments, while in the control wood, mass loss was 9.5%. Regarding the decaying fungi, mass loss occurred in all treatments. Acetylation for 6 and 8 hours were the most efficient chemical treatments, increasing the resistance class of the *Jacaranda copaia* wood to highly resistant.

KEYWORDS: biodeterioration, xylophagous organisms, *Nasutitermes*, *Gloeophyllum trabeum*, *Trametes versicolor*

Resistência da madeira acetilada de *Jacaranda copaia* ao ataque de cupins e fungos apodrecedores

RESUMO

A durabilidade natural da madeira é essencial para a definição de seu uso, e essa propriedade pode ser potencializada com o tratamento químico adequado da madeira. Assim, o objetivo deste trabalho foi avaliar a resistência da madeira de *Jacaranda copaia* modificada quimicamente por acetilação a cupins e fungos apodrecedores. Cinco tratamentos experimentais foram avaliados: acetilação por 2, 4, 6 e 8 horas e um controle (não acetilado). A acetilação foi realizada por imersão das amostras de madeira em anidrido acético a 90°C. Amostras acetiladas e controle foram submetidas à ação de cupins xilófagos (*Nasutitermes* sp.) e fungos apodrecedores (*Gloeophyllum trabeum* e *Trametes versicolor*). O processo de acetilação aumentou significativamente a resistência da madeira de *Jacaranda copaia* ao ataque dos organismos xilófagos. Não houve perda de massa após exposição aos cupins da madeira em nenhum dos tratamentos de acetilação, enquanto na madeira controle a perda de massa foi de 9,5%. Em relação aos fungos em decomposição, ocorreu perda de massa em todos os tratamentos. Os tratamentos químicos mais eficientes foram os de acetilação por 6 e 8 horas, elevando a classe de resistência da madeira de *Jacaranda copaia* para altamente resistente.

PALAVRAS-CHAVE: biodeterioração, organismos xilófagos, *Nasutitermes*, *Gloeophyllum trabeum*, *Trametes versicolor*

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INTRODUCTION

The natural durability of wood is one of its most important characteristics and is directly related to its ability to withstand different deterioration agents. Biotic agents such as fungi, insects, and marine borers are responsible for wood deterioration, as well as abiotic agents such as natural mechanical forces (wind and rain), and physical and chemical agents (Alfredsen *et al.* 2021). Under favorable conditions of humidity and pH, wood becomes susceptible to attack by xylophagous organisms. These organisms focus on the cell wall, which contains essential natural polymers, as a source of nutrition and energy. Xylophagous organisms responsible for the greatest deterioration of wood are fungi and insects (Gouveia *et al.* 2021).

Insect attacks, mainly by termites, are major causes of damage to low-resistance wood, especially that of low-density trees (Melo *et al.* 2015). Termites of the genus *Nasutitermes* Dudley, 1890 (Isoptera, Termitidae, Nasutitermitinae) live underground and have a preference for dry or decaying wood, are among the most abundant insect species in tropical and subtropical regions of the planet (Paes *et al.* 2007; Cruz *et al.* 2014; Pereira *et al.* 2015; Batista *et al.* 2020). Fungi occurring in wood can be divided into mold, stain, and decaying fungi. The latter group is responsible for the deterioration of the wood cell wall, especially fungi of the class Basidiomycetes, responsible for brown and white rot (Emmerich *et al.* 2021; Gouveia *et al.* 2021).

There are several methods to preserve wood from biological damage, including chemical modification of the constituent polymers (Gouveia *et al.* 2021). A chemical treatment that has shown particular efficiency in increasing the durability of wood is acetylation. It is a process based on the reaction of the hydroxylic groups present in the constituents of the wood cell wall, mainly hemicellulose and lignin, with the acetic anhydride reagent. In this way, hydroxylic groups, which are hydrophilic, are replaced by acetate groups that have a hydrophobic characteristic (Rowell 2013; Ibach and Rowell 2021).

The increase in the biological resistance of wood is followed by an increase in its dimensional stability (Rowell 2016; Grace *et al.* 2020). In addition to being water adsorption sites, the hydroxylic groups in cell wall polymers are deteriorated by fungi and termites through their enzymatic systems, making the polymers digestible (Paes *et al.* 2007). Therefore, if these hydroxylic groups are chemically altered, the enzymatic action of wood deteriorating organisms does not occur.

Jacaranda copaia (Aubl.) D. Don (Bignoniaceae) is a species of wide occurrence in the Amazon region (Farias-Singer 2022). Its wood has significant commercial relevance and is frequently obtained from sustainable forest management projects in the region (Melo *et al.* 2019). It produces a light-colored, pinkish-gray wood, which is a very attractive feature for the

production of furniture and accessories (Melo *et al.* 2019). However, the wood of *J. copaia* has undesirable characteristics such as low density, low strength, low dimensional stability and high susceptibility to attack by termites and decaying fungi, which restrict its use to applications with lower added value such as boxes and pallets. In this sense, the use of acetylation could potentially increase the resistance of *J. copaia* wood to biological decay agents, increasing its uses and profitability (Lobato *et al.* 2020). Thus, this study aimed to evaluate the efficiency of different acetylation reaction times on the durability of *J. copaia* wood subjected to attack by xylophagous termites and fungi that cause brown and white rot.

MATERIAL AND METHODS

Raw material

The *J. copaia* wood used in this study was obtained from three trees harvested in an area of native uneven-aged forest management in the municipality of Sinop (11°52.119'S, 55°27.746'W), Mato Grosso state, Brazil, in the transition zone between the Amazon and Cerrado biomes. The local climate is classified as Aw type (tropical savanna climate), characterized by two well-defined seasons, a rainy (October to April) and a dry season (May to September), with low annual thermal amplitude (monthly averages from 24 to 27 °C) and rainfall around 1,974 mm (Souza *et al.* 2013).

Wood acetylation process

A set of 240 test specimens with dimensions of 2.5 cm x 2.5 cm x 1.0 cm were obtained from the three trees (80 per tree), from the heartwood of tangentially unfolded boards from basal logs (up to 2 m in length). The test specimens were placed in a laboratory oven and conditioned at 60 °C until dry weight. Then, the samples were divided into five groups (48 test specimens per group). Of these, four groups were subjected to acetylation and one group remained untreated as a control. The control samples were immersed in a non-acetylated medium for mass gain comparison.

The four acetylation treatments consisted in subjecting the wood samples to reaction with acetic anhydride for 2, 4, 6, and 8 hours. The reaction was conducted in 1,000-mL glass flasks containing the samples immersed in the reactant and kept in a hot-water bath at 90 °C.

After chemical processing, the samples were washed with ethyl alcohol to remove all the acetic anhydride, and were then oven-dried at 60 °C under continuous ventilation, until reaching the dry condition (constant mass). The drying temperature was chosen to prevent potential thermal degradation of polyose and wood extractives at higher temperatures (Figueiredo *et al.* 2019). After drying, the weight and dimensions of the samples were precisely determined with an analytical scale and digital pachymeter, respectively. The weight gain caused by the acetylation was determined by the

ratio between the weight of each test specimen before and after chemical treatment.

Resistance to termite attack

The termite attack test was carried out according to a method similar to that used by Melo *et al.* (2015). In a plastic water tank with a capacity of 500 L, a 10-cm layer of sterilized sand was inserted, which was kept saturated with water throughout the experiment, to prevent the termites from dying. For the termite attack assessment, we used eight test specimens for each treatment. The test specimens of all treatments were arranged horizontally in randomized blocks on a ceramic plate placed on the sand layer so that they were not in direct contact with the sand and were submitted to simultaneous attack by the termites. A colony of *Nasutitermes* sp. termites was installed on the test specimens. The termites were collected from one nest in a field near Universidade Federal do Mato Grosso (UFMT), Sinop campus (11°50'53"S, 55°38'57"W).

The nest was approximately 50 cm in length and 30 cm in diameter and was completely removed from a tree branch and placed inside the water tank, to include all the castes present in the nest. The experiment was set up in an air-conditioned room, with a temperature of $25 \pm 5^\circ\text{C}$ and relative humidity of $65 \pm 10\%$. Each replicate was exposed to the action of termites for 40 days. After the exposure, the test specimens were cleaned with a soft-bristled brush and again subjected to drying in an oven until the anhydrous weight was obtained (constant weight), and the mass loss was determined subtracting from the initial weight.

Resistance to fungal attack

The accelerated decay test was conducted in the Wood Biodegradation Sector of the Forest Products Laboratory (LPF) in Brasília, Brazil, based on the ASTM D 2017 standard (ASTM 2014). The test specimens were exposed to the action of white-rot fungus, *Trametes versicolor* (L.) Lloyd (Mad 697), and brown-rot fungus, *Gloeophyllum trabeum* (Pers.) Murrill (Mad 617), both belonging to the collection of the LPF. For each fungus species, we used 20 test specimens per treatment.

Small disks (1 cm²) with mycelia of the fungi were grown in 1-L Erlenmeyer flasks (one for each fungus) containing 200 mL of 3% liquid malt medium that were stirred for five days at room temperature at 150 rpm. Subsequently, the flasks were placed in an incubator at $25 \pm 1^\circ\text{C}$ and $73 \pm 2\%$ relative humidity, where they remained for four weeks. After this period, the solutions were homogenized in a blender for inoculation. The test specimens were dried at 60°C in a forced-air oven until they reached constant weight. This temperature was defined to avoid potential thermal degradation of polyose and wood extractives at higher temperatures (Stangerlin *et al.* 2013). The test specimens were then sterilized in an autoclave at 121°C for 30 min.

For each test specimen, a 250 mL screw-capped glass flask was prepared with a layer composed of 130 g of soil from the B horizon (subsoil). The soil was previously sieved and prepared according to the procedures of the standard ASTM D 1413 (ASTM 2005). The soil layer inside the flask was moistened until reaching 130% of water retention capacity and the pH was adjusted to 6.0 with lime. A support plate (3 mm thick x 29 mm wide x 35 mm long) of wood was placed in each flask: hardwood *Cecropia* sp. for exposure to white-rot, and softwood *Pinus* sp. for exposure to brown-rot, according to the attack preference of each fungus (Stangerlin *et al.* 2013).

The flasks were sterilized in an autoclave at 130°C for 30 min. After cooling to room temperature, the wood plates were inoculated with 3 mL of fungal solution and the flasks were carefully closed and placed in an incubation chamber at $25 \pm 1^\circ\text{C}$ and $73 \pm 2\%$ relative humidity until the complete mycelial growth of the fungi (support plate completely covered). After four weeks, the flasks were opened, a test specimen was placed in each one, and the flasks were closed and returned to the incubation chamber, where they were kept for 16 weeks. After this period, the test specimens were gently removed from the flasks so that no part of the specimen was detached. The test specimens were again dried at 50°C in a forced-air oven until they reached constant weight, which was recorded as the final weight. Mass loss of each specimen was determined as the difference between the initial and final weight. The efficiency of each acetylation treatment was evaluated by classifying the test specimens according to their resistance after the accelerated decay assay, based on the classification proposed by ASTM D - 2017 (ASTM 2014): highly resistant (HR) = mass loss 0 – 10%; resistant (R) = mass loss 11 – 24%; moderately resistant (MR) = mass loss 25 – 44%; nonresistant (NR) = mass loss above 45%.

Statistical analysis

Distribution normality and homogeneity of variance of the data were ascertained with the Shapiro-Wilk test and Levene test, respectively. Mass gain after the acetylation process and mass loss after the attack of the xylophagous termites was compared among treatments using analysis of variance and pairwise comparison of means by the post-hoc Scott-Knott test at 95% probability. Mass loss data from the accelerated decay assay were compared among treatments for each fungus species separately using the Scott-Knott test, since the data did not present normal distribution, at 95% probability.

RESULTS

All acetylation treatments promoted a mass gain of the test specimens, confirming the efficiency of acetylation (Table 1). The mass gain varied from 18.1 to 21.6%.

The average mass loss after the termite attack varied significantly among treatments. Mass loss for the control

was 9.5%, while there was no mass loss in the acetylation treatments (Table 2).

In general, mass loss varied significantly between treatments for both decaying fungi for *T. versicolor* and *G. trabeum*, with all treatments showing lower values than the control (Table 3).

DISCUSSION

Mass gain

Our results for mass gain after acetylation were similar to those by Ajdinaj *et al.* (2013), Blanco and Alfaro (2014), Dong *et al.* (2016), Rowell (2016), and Fodor *et al.* (2017), who determined values ranging from 8.3 to 24.8% for acetylation of different wood species. Mass gain is an indicator of the intensity of the acetylation, as this gain is directly related

to the weight of the reactant added to the wood structure during the chemical treatment (Rowell 2013). The increase in mass occurs because the molecular weight of the acetate group (59 g mol⁻¹) is higher than that of the hydroxyls (17 g mol⁻¹), which are substituted during the acetylation reaction (Hunt *et al.* 2018).

The two-hour and four-hour treatments presented the lowest mass gain, indicating that time directly influences the proportion of mass gain. The quantity of substituted hydroxyl groups is related to the degree of acetylation (Figueiredo *et al.* 2019). However, the degree of substitution of hydroxyl groups increases with reaction time until a certain level, after which it remains constant (Gröndahl *et al.* 2013). This explains why there was no significant difference between the means of the six- and eight-hour treatments, indicating that these longer reaction times did not increase the mass gain under the conditions employed in this study.

Table 1. Mass gain of *Jacaranda copaia* wood samples after acetylation during different treatment times. Values are the mean ± standard deviation of 48 samples per treatment.

| Acetylation time (hours) | Mass gain (%) |
|--------------------------|----------------|
| Control | 00.00 ± 0.00 c |
| 2 | 18.06 ± 1.07 b |
| 4 | 18.70 ± 1.43 b |
| 6 | 21.57 ± 5.19 a |
| 8 | 21.62 ± 0.72 a |

Means followed by the same letters are statistically equal at 95% probability by the Scott-Knott test

Table 2. Mass loss of *Jacaranda copaia* wood samples treated with acetylation and subjected to the attack of *Nasutitermes* sp. termites. Values are the mean ± standard deviation of eight samples.

| Acetylation time (hours) | Mass loss (%) |
|--------------------------|---------------|
| Control | 9.50 ± 5.04 a |
| 2 | 0.00 ± 0.00 b |
| 4 | 0.00 ± 0.00 b |
| 6 | 0.00 ± 0.00 b |
| 8 | 0.00 ± 0.00 b |

Means followed by the same letters are statistically equal at 95% probability by the Scott-Knott test

Table 3. Mass loss of *Jacaranda copaia* wood samples treated with acetylation and subjected to the accelerated fungal decay test with white-rot fungus, *Trametes versicolor*, and brown-rot fungus, *Gloeophyllum trabeum*. Values are the mean ± standard deviation of 20 samples.

| Acetylation time (hours) | <i>Trametes versicolor</i> | | <i>Gloeophyllum trabeum</i> | |
|--------------------------|----------------------------|------------------|-----------------------------|------------------|
| | Mass loss (%) | Resistance class | Mass loss (%) | Resistance class |
| Control | 56.51 ± 4.32 Ac | NR | 35.19 ± 8.09 Bb | MR |
| 2 | 11.83 ± 2.79 Ab | R | 13.05 ± 5.87 Aa | R |
| 4 | 10.38 ± 2.67 Ab | HR | 12.08 ± 5.89 Aa | R |
| 6 | 2.67 ± 1.57 Ba | HR | 9.50 ± 2.64 Aa | HR |
| 8 | 1.17 ± 0.57 Ba | HR | 10.29 ± 4.91 Aa | HR |

Resistance class (ASTM 2014): HR = highly resistant (mass loss 0 – 10%); R = resistant (mass loss 11 – 24%); MR = moderately resistant (mass loss 25 – 44%); NR = nonresistant (mass loss above 45%). Means followed by the same capital letter in rows, or lowercase letters in columns, are statistically equal at 95% probability by the nonparametric Scott-Knott test.

Resistance to termite attack

Acetylation decreased the susceptibility of the wood to deterioration by termites, since they tend to prefer less dense wood (Figueiredo *et al.* 2019). As the acetyl groups that reacted with the wood theoretically should not be toxic to termites, the resistance of modified wood seems attributable to its unpalatability to the termites (Imamura and Nishimoto 1986).

All our experimental treatments completely prevented termite attack, as reflected by the absence of mass loss of the test specimens. In *Picea jezoensis*, *Larix leptolepis* and *Pseudotsuga menziesii*, wood acetylation resulted in a 20% mass gain and respective efficiency in preventing termite attack of 34.3, 50 and 32.1% (Imamura and Nishimoto 1986). Moreover, wood panels produced with acetylated wood were resistant to the action of subterranean termites (*Coptotermes gestroi*) and dry wood termites (*Cryptotermes cynocephalus*) in laboratory assays (Hadi *et al.* 1995; Ibach and Rowell 2021).

Resistance to decaying fungi

Based on experimental results, Hunt *et al.* (2018) proposed four mechanisms that explain the increased resistance of

modified wood to fungi: (1) acetylated hemicelluloses do not serve as a source of nutrients for fungi; (2) fungal degradation enzymes that break down wood polymers are inhibited by the modification through acetylation; (3) fungal degradation enzymes are unable to enter the cell wall because the micropores are blocked by the modification, and (4) diffusion within the cell wall is inhibited because the modification decreases the equilibrium moisture content of the wood.

According to our mass loss values, the control samples were classified as nonresistant to *T. versicolor* and moderately resistant to *G. trabeum*. After acetylation, the wood became resistant to the attack of the *G. trabeum* after treatment for two hours and highly resistant after longer treatment times. Regarding *T. versicolor*, the wood became resistant after two and four hours of acetylation and highly resistant after six and eight hours. Therefore, the weight gain was directly related to the increase in the biological resistance to decaying fungi. Wood density and porosity are factors that affect natural durability of wood, with less dense and more porous wood offering lower resistance to decay by fungi (Panshin and De Zeeuw 1980). An accelerated decay test of acetylated *Pinus echinata* wood showed that a weight gain of 17% was enough to completely prevent fungal attack (Goldstein et al. 1961).

The efficiency of the fungal attack increased due to the chemical alterations that occurred in the constituents of the wood through acetylation, which improved the dimensional stability of the wood and prevented the enzymatic action of the xylophagous fungi. The increase in the level of acetylation is directly proportional to the resistance to fungal attack (Rowel 2013), as also evidenced in our results when mass loss decreased with increasing acetylation time, with higher significance for white rot. The fungus that causes white rot demands higher moisture content than that of brown rot to reach optimal attack (Zabel and Morrell 2020). This can explain the accentuated decrease in the fungal vigor of *T. versicolor* compared to *G. trabeum* after the acetylation process. The mass loss resulting from *T. versicolor* attack was 55.3%, reaching near zero for the weight gain of 21% in the 8-hour treatment. The same mass loss efficiency was observed for a weight gain of only 12% after acetylation for *Betula maximowiczii* Regel wood decayed by the brown-rot fungus *Tyromyces palustris* (Berk. et Curt.) Murr. (FFPRI 0507) (Ohkoshi 1999).

When evaluating the natural resistance of the wood of 43 Mexican hardwood species, Torelli and Cufar (1994) found that, for some samples, the mass loss caused by *T. versicolor* was higher than that recorded for *G. trabeum*. Similarly, we observed a lower mass loss to brown rot attack (35.2%), than to white rot attack (56.5%) for the control. The lower mass loss to brown rot may be linked to the preference of this fungus for coniferous species (Zabel and Morrell 2020), being less efficient in the deterioration of hardwood species

such as *J. copaia*. In addition, white-rot fungi attack the polysaccharides and lignin present in the cell wall indistinctly, while brown rot fungi attack only polysaccharides (Stangerlin et al. 2013). However, we observed higher mass loss after *G. trabeum* attack than after *T. versicolor* attack in all acetylated treatments. Brown-rot fungus were also reported to be more resistant to acetylation compared to white-rot fungi on wood of *Pinus densijiora* Sieb. et Zucc., *Albizia falcata* (L.) Fosberg and *Fagus crenata* Blume, with a mass loss decrease by up to 25.7%, reaching 9.5% for a mass gain of 21% for a 6-hour acetylation treatment, but there was no significant difference between treatments (Takahashi 1996). There was no mass loss for a 20% mass gain in acetylated wood of *B. maximowiczii* submitted to attack of the white-rot fungus *Coriolus versicolor* (L. ex. Fr.) Quel. (FFPRI 1030) (Ohkoshi (1999).

CONCLUSIONS

The acetylation process increased the biological resistance of *Jacaranda copaia* wood. The acetylated wood samples did not undergo attack by xylophagous termites, while the attack by decaying fungi decreased significantly compared to the untreated samples. In general, the six- and eight-hour acetylation treatments were more effective in improving the biological resistance of *J. copaia* wood. Further studies should address whether the same promising results can be achieved with the acetylation of larger and thicker wood pieces of *J. copaia*, as well as the applicability to other Amazonian woods with low natural durability.

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