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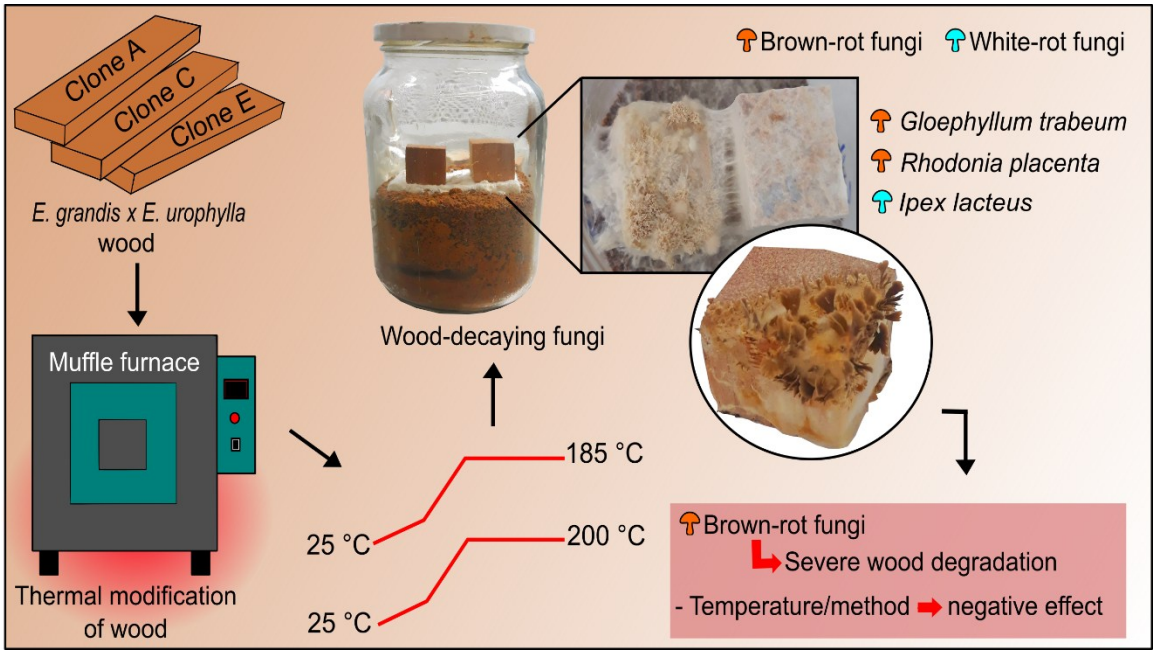
DOI: 10.15376/biores.20.4.10051-10068

GRAPHICAL ABSTRACT



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Eucalyptus hybrids exhibit rapid growth, and their wood is widely used in construction, furniture production, reconstituted panels, energy, pulp, and paper manufacturing. However, they are commonly affected by decay fungi, which reduce their durability, jeopardizing the integrity of structures and the safety of individuals. Therefore, this study aimed to evaluate the effects of thermal treatment on the biological resistance of 14-year-old hybrid *Eucalyptus* clone wood. Chemical analyses were conducted on the wood (lignin and holocellulose), and tests with brown rot fungi (*Gloeophyllum trabeum* and *Rhodonia placenta*) and white rot fungi (*Irpex lacteus*) were performed over 12 weeks, along with colorimetric variable assessments. It was concluded that brown rot fungi caused more severe damage to the wood, also influencing its coloration. Under the conditions studied, a temperature of 200 °C promoted wood deterioration of the clones by xylophagous fungi. Regarding resistance classes, the clones were classified as highly resistant (A, C, and E) to the fungus *I. lacteus*, regardless of heat treatment. For the fungi *G. trabeum* and *R. placenta*, the clones detected as highly resistant (A and C) were those exposed to a temperature of 185 °C.

DOI: 10.15376/biores.20.4.10051-10068

Keywords: Biological tests; Colorimetry; Lignocellulolytic fungi; Planted forests

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INTRODUCTION

Wood is a natural, renewable, hygroscopic, anisotropic, and heterogeneous material. It exhibits variations among species, within the same species, and even within a single tree (Tsoumis 1991). It is the primary product extracted from forests (natural or planted) and serves as a versatile material that can be used for various purposes, such as construction, pulp and paper production, energy, furniture, and reconstituted wood panels. However, its intended use depends on its characteristics and technological properties (Yan *et al.* 2023; Baul *et al.* 2024; Blanchet *et al.* 2024; Garcia *et al.* 2024). These properties can potentially be modified through chemical, mechanical (densification), and thermal treatments (Broda *et al.* 2024).

These treatments aim to improve wood properties and diversify its applications, particularly for wood derived from planted forests, which generally have low resistance to xylophagous organisms and less attractive coloration. However, given the high population demand, planted forests play a significant role in meeting consumer needs while minimizing the impact of exploitation on native forests. In Brazil, according to the Brazilian Tree Industry (IBÁ 2023), planted forests cover approximately 9.94 million hectares, of which 7.60 million hectares are dedicated to *Eucalyptus*, which has adapted well to the country's edaphoclimatic (factors of soil and prevailing climate) conditions.

The superiority of *Eucalyptus* clones favors the selection of these genetic materials, among which *E. grandis* × *E. urophylla* is widely used in commercial plantations, especially in subtropical (Lötter *et al.* 2023) and tropical regions. This hybrid combines advantageous characteristics of each parental species, notably its rapid growth (Brisola and Demarco 2011) and tolerance to adverse environmental conditions, such as drought (Ferraz *et al.* 2024). Some authors also indicate that its genome exhibits high expression of genes responsible for metabolic pathways related to starch and sucrose metabolism, the biosynthesis of antioxidant compounds (flavonoids), and defense mechanisms in plant-pathogen interactions (Shen *et al.* 2023). However, one of the challenges to the use of wood for industrial purposes is related to attacks by xylophagous organisms. This can be observed for the hybrid *E. grandis* × *E. urophylla*, whose natural resistance can vary between clones, especially if there are different genotypic proportions in relation to the parental species (Silva *et al.* 2025).

Related to these factors, silvicultural treatments in planted forests can influence the quality of the wood produced and its resistance to pests and insects. Among these practices, studies with genetically modified *Eucalyptus* trees stand out, observing increased insect resistance through the expression of pesticidal proteins (Avisar *et al.* 2024). For *E. grandis* × *E. urophylla* clones, the type of spacing used during planting influences traits of interest, such as growth, productivity, and survival (Stape *et al.* 2022). In this context, clones of *E. grandis* × *E. urophylla* whose silvicultural treatment consisted of a spacing of 5.0 × 4.0 m, with pruning at the age of two years (height of four meters) and at three years (height of seven meters), ending with thinning at nine years, showed resistance to attack by xylophagous fungi influenced by wood density, extractive and ash content (Silva *et al.* 2025).

Despite the numerous advantages of using wood, such as being a natural and renewable resource, low acquisition cost, and high mechanical strength relative to its mass, there are still limitations for it applications. Among these are the presence of knots and dimensions smaller than those demanded by the market (Oliveira *et al*. 2024). An alternative to using large-dimension solid wood is the usage of glued joints, which enables larger lumber pieces to be made from small logs (Varankina *et al.* 2024). Another limitation is its susceptibility to xylophagous organisms, especially when used for furniture, flooring, paneling, and other construction materials (Martha *et al*. 2024).

The most common wood treatment involves the use of chemical products. However, these can be harmful to human health and the environment. Therefore, more sustainable alternatives with reduced environmental impact are being sought (Teacă and Tanasă 2020). Thermal modification is notable for inducing the degradation and transformation of components attractive to wood-eating organisms, such as hemicelluloses and amorphous regions of cellulose (Haseli *et al.* 2024). Hemicelluloses play an important role in the nutrition of wood-eating fungi, and their breakdown reduces the availability of

nutrients essential for the growth of microorganisms. It also affects the hygroscopic behavior of wood and, consequently, fungal proliferation (Candelier *et al*. 2016).

Eucalyptus species attacked by decay fungi (brown and white rot) have a more resistant middle region of the heartwood compared to the transition region, composed of heartwood and sapwood (Medeiros Neto *et al.* 2024). Calonego *et al.* (2010) demonstrated that the use of thermal modification in *E. grandis* wood resulted in increased resistance to attack by the fungus *P. sanguineus* (white rot), in addition to reducing mass loss in wood treated at 180 to 220 °C. Regarding the chemical composition of the wood, it was observed that heat treatment for the species *E. saligna* caused a marked reduction in the sugars arabinose, mannose, galactose, and xylose, in addition to an increase in lignin content and a reduction in extractives (Brito *et al.* 2008).

These same authors also demonstrated increasing mass loss as a function of temperature, reaching 9.6 g when exposed to 180 °C. Heat treatment is also an effective method for increasing dimensional stability and reducing the equilibrium moisture content of wood, as well as promoting darkening and color uniformity (Andrade *et al.* 2024; Haseli *et al.* 2024). A temperature of 150 °C was indicated as ideal for increasing dimensional stability, minimizing mass loss and achieving color uniformity in *E. urophylla* wood (Yang and Jin 2021). Nevertheless, there has been limited research addressing the effect of thermal treatment on the natural durability of eucalyptus clones intended for the production of sawn wood products for structural applications and the furniture industry.

To contribute to the diversification of wood applications and the improvement of resistance to xylophagous organisms, as well as to advance research in this area—which remains underexplored in Brazil—this study hypothesized that thermal treatment is effective in preventing the development of decay fungi in eucalyptus wood. Thus, the study aimed to evaluate the effects of thermal treatment on the resistance of 14-year-old *Eucalyptus* clone wood to decay by fungi.

EXPERIMENTAL

Planting Location and Sampling Process

The planting of *Eucalyptus* clones is located in the municipality of Venda Nova do Imigrante, within the Pindobas Agroindustrial Complex Ltd., in the Southwestern Mountain microregion of Espírito Santo State, Brazil (latitude 20°23'37.1"S, longitude 41°08'29.6"W, and an altitude of 730 m). The climate is classified as tropical highland (Cwb), characterized by summer temperatures below 22 °C with concentrated rainfall and milder winters. The region experiences high precipitation levels due to the influence of the mountains, with annual rainfall ranging from 1,200 to 1,500 mm (Feitoza *et al*. 2010).

The planting information was provided by the company (Silva 2018). The trees used were planted in January 2002 in 40 × 40 × 40 cm pits, with a spacing of 5.0 × 4.0 m. Two fertilizations were carried out: the first at planting, with 400 g of NPK 00-24-00 and micronutrients per pit, and the second at eight months of age, with 100 g of the same formulation. At two years old, pruning was performed up to a height of four meters, and at three years old, up to seven meters. At nine years old, thinning was conducted, leaving 60% of the individuals.

The wood was obtained from three 14-year-old *Eucalyptus* hybrid clones, whose progenitors were the species *Eucalyptus grandis* × *Eucalyptus urophylla*. The proportion of each progenitor varied among the clones, and to maintain their confidentiality, they were generically designated as A, C, and E (Silva 2018).

For each clone, three trees were selected based on the criteria of straight boles without tortuosity, adequate phytosanitary conditions, and similar diameter at breast height (DBH) to ensure greater sample homogeneity. Central planks were extracted from the second log, measuring 4.20 m in length and 8 to 10 cm in thickness. These were transported to the Department of Forest and Wood Sciences (DCFM) at the Federal University of Espírito Santo (UFES), located in Jerônimo Monteiro, Espírito Santo, Brazil.

Heat Treatment of Wood

For the thermal treatment, wood pieces with dimensions of 2.5 × 10 × 60 cm (thickness × width × length) were obtained and conditioned in a climate chamber at 25 ± 2 °C and 65 ± 5% relative humidity (RH) until reaching the equilibrium moisture content (≈12%) for the application of the treatments. The conditions considered for the thermal treatments were temperature and time. The thermal treatment process was carried out in an electric oven (Linn Elektro®, Germany) with programmable control of temperature (°C) and time (h), and an internal atmosphere containing oxygen (Andrade *et al.* 2024).

For the thermal modification temperatures (185 or 200 ºC), a standard thermal treatment program was established. The ambient temperature (control) was between 25 and 30 °C. The wood samples were exposed inside the oven (ambient temperature) until reaching 100 ºC, with a heating rate of 30 ºC h^{-1}; then from 100 ºC to the final temperature (185 or 200 ºC) with a heating rate of 160 ºC h^{-1}; and finally, they were held at the final constant temperature for three hours (Fig. 1a-b) (Andrade *et al.* 2024).

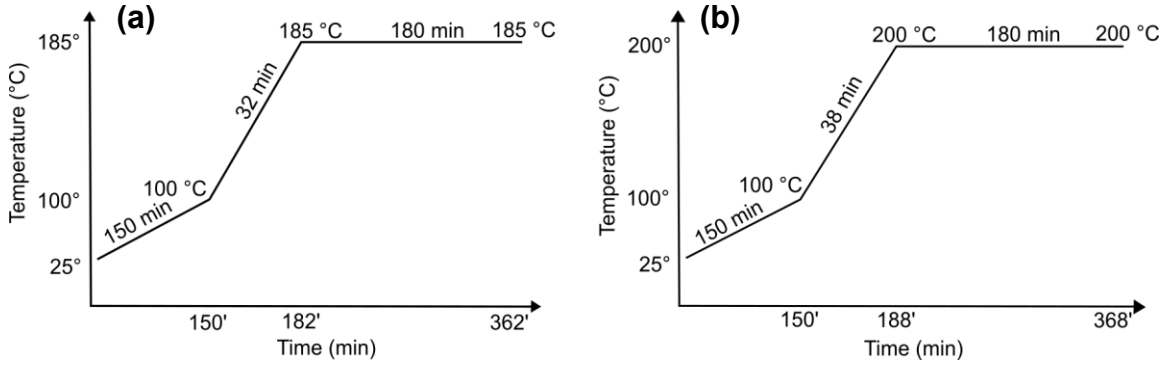

Fig. 1. Characterization of the heating ramp highlighting the exposure time and the heat treatments to which the samples were exposed, with emphasis on the temperatures of (a) 185 °C and (b) 200 °C during the three hours. Source: Andrade *et al*. (2024).

At the end of the treatment, the samples were immediately removed from the oven and cooled in a portable desiccator (Andrade *et al*. 2024). Then they were placed in a climate-controlled room with a temperature of 27 ± 2 °C and relative humidity (RH) of 65 ± 5% until the samples reached equilibrium moisture (≈ 12%) content for the testing.

Chemical Characterization of Wood

When exposed to high temperatures, changes occur in the chemical constituents of wood. Therefore, analyses of total lignin (insoluble and soluble) (Goldschimid 1971; Gomide and Demuner 1986) and holocellulose were performed by difference [100% – (extractives + ash) + total lignin] on thermally treated and untreated (control) wood.

Composite samples were prepared for each clone, with six repetitions per clone, totaling 54 analyses for each chemical component evaluated. The samples were converted into sawdust using a Wiley mill and sieved (40 to 60 mesh). The material was

homogenized, and aliquots were taken for drying in an oven at 103 ± 2 °C until constant weight to obtain the dry moisture content (AS). The samples were kept in a climate-controlled room (≈ 12% moisture) until analyzed.

Tests with Xylophagous Fungi

The test was conducted with cubic samples of 1.9 cm edges, which were placed in glass jars with a capacity of 600 mL. These jars were filled with 300 g of soil, with a water retention capacity of 21.92% and a pH of 7.3, as per the American Wood Protection Association - AWPA E10 (2022). In each jar, samples of brown rot fungi (*Gloeophyllum trabeum* (Pers.) Murrill. (Mad-617) and *Rhodonia placenta* (Fries) Larsen et Lombard (Mad-698-R)) and white rot fungi (*Irpex lacteus* (Fr.) Fr. (Mad-517)) were added, with two samples of different treatments per jar, and 24 repetitions for each fungus, temperature of thermal modification, and clone.

The containers were sterilized at 121 °C (103 kPa) for 30 minutes and then kept in a climate-controlled room at 28 ± 2 °C and 75 ± 5% RH for 84 days (Fig. 2a-f). At the end of the test, mass loss was evaluated and compared with the resistance classes of AWPA E30 (2022) (Table 1).

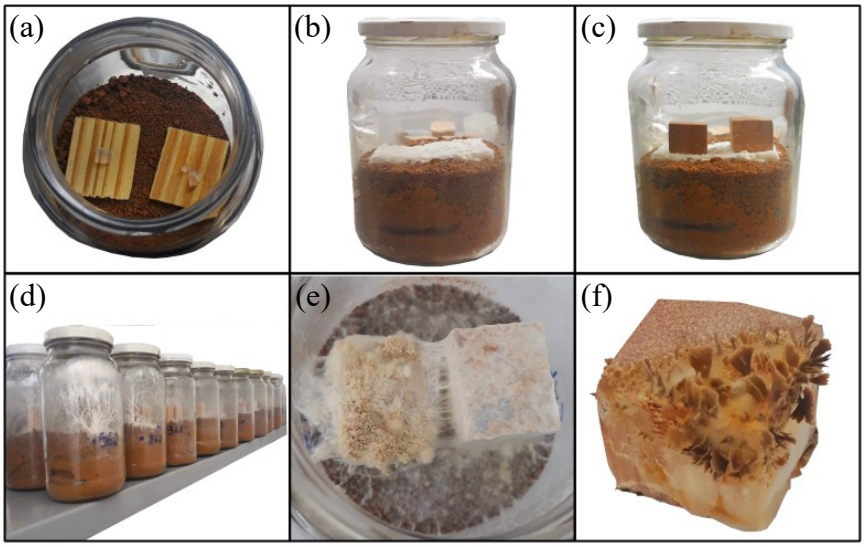

Fig. 2. Tests with xylophagous fungi, (a-b) inoculation and growth of fungi on *Pinus* wood, (c) thermally treated samples placed on *Pinus* wood colonized by fungi, (d) jars in a climate-controlled room, and (e-f) samples deteriorated by test fungi

Table 1. Resistance Classes of Wood Attacked by Xylophagous Fungi

Mass loss (%)	Residual mass (%)	Strength class
0-10	90-100	Highly resistant
11-24	76-89	Resistant
25-44	56-75	Moderately resistant
≥ 45	≤ 55	Not resistant
Source: adapted from AWPA E30 (2022).		

Colorimetric Evaluation of Wood

To measure the wood color, the system of the International Commission on Illumination - CIE (1976) was used, employing a Konica Minolta CM-2500D

spectrophotometer with standard illuminant D65, a 10º observation angle, specular light included, and calibration with black and white standards.

The colorimetric parameters recorded were lightness (L^*), the red-green coordinate or red hue (a^*), and the blue-yellow coordinate or yellow hue (b^*), based on the CIE system (1976). The determination of color changes in the wood was performed before and after the attack by xylophagous fungi, as shown in Eq. 1.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

The total color variations of the tested woods after fungal attack were classified as proposed by Hikita *et al.* (2002), based on levels of visual perception (Table 2).

Table 2. Classification of Total Color Variation (ΔE*) of Wood after Fungal Attack

Colorimetric classification	Total color variation ΔE
Slightly noticeable	0.0 to 4.0
Noticeable	4.1 to 8.0
Very noticeable	Above 8.1
Source: Hikita *et al.* (2002).	

Statistical Analysis of Results

For the chemical analyses of the wood, a completely randomized design was used, with six replicates for each clone and temperature, resulting in 54 analyses for each chemical component evaluated.

For the biological fungal tests, a completely randomized design was also employed, considering three types of fungi, with six replicates for each clone and temperature, totaling 216 samples. In this test, the damage caused by the fungi was not compared among them, as they cause different types of damage to the wood.

Before applying analysis of variance (ANOVA), the assumption of normality was verified using the Lilliefors test ($p < 0.05$), and the homogeneity of variances was checked using the Cochran test ($p < 0.05$). When necessary, data were transformed as recommended by Steel *et al.* (1997) for percentage data and numerical values containing zero. Once these assumptions were met, ANOVA was applied, and factors and interactions identified as significant by the F-test ($p \leq 0.05$) had their means compared using the Scott-Knott test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Chemical Characteristics of Wood

The highest lignin contents were observed in clone A (control and 185 °C) and clones A and C (200 °C). Thermal modification produced different effects on the evaluated clones, with an increase in clone C (200 °C), a decrease in clone A (200 °C), and variations of increase (control and 185 °C) and decrease (185 and 200 °C) in clone C (Table 3 and Table A1). For the *E. grandis* clone, Moura *et al.* (2012) reported higher lignin values in wood thermally modified at 200 °C. Compared to carbohydrates, lignin is thermally more stable, presenting a relative increase in its content after heat treatment. This behavior was observed for eight *Eucalyptus* species (*E. botryoides*, *E. globulus*, *E. grandis*, *E. maculata*, *E. propinqua*, *E. rudis*, *E. saligna*, and *E. viminalis*), whose wood was gradually heated at

temperatures from 160 to 230 °C for 3 h (Lourenço *et al.* 2020). At high temperatures (between 180 and 210 °C), greater condensation of lignin molecules occurs, which are composed of high molecular weight fractions (Kačík *et al.* 2025). Lignin content can also be influenced by the genetic material used, as observed in triploid *E. urophylla*. In this context, the expression of specific genes regulates the biosynthesis (*EuLAC17*) and inhibition (*EuMYB308*) of lignin (Xu *et al.* 2024).

Table 3. Total Lignin and Holocellulose Contents in Wood as a Function of Clones and Thermal Modification Temperature

Clone	Lignin			Holocellulose		
	Temperatures (ºC)			Temperatures (ºC)		
	Control	185	200	Control	185	200
A	33.29 Aa	33.37 Aa	31.19 Ba	52.25 Bc	53.27 Bb	55.38 Ab
C	29.03 Bb	29.33 Bc	30.31 Aa	54.38 Bb	56.4 Aa	55.27 Bb
E	29.11 Bb	30.47 Ab	29.05 Bb	56.54 Ba	55.52 Ba	57.85 Aa
Means followed by the same capital letter, horizontally, and lowercase, vertically, do not differ from each other by the Scott-Knott test ($p > 0.05$).						

The increase in lignin content in thermally modified wood is caused by the degradation of chemical constituents, primarily hemicelluloses. This was observed by Medeiros *et al*. (2024) in *Pinus* wood treated at temperatures exceeding 200 °C, with cell wall contraction and lumen diameter reduction due to the breaking of hydroxyl groups in cellulose and hemicellulose polysaccharides. The authors also stated that this contributes to increased mass loss and reduced roughness and hydrophobicity. Kučerová *et al*. (2024) described an antagonistic relationship between lignin and holocellulose contents in *Pinus* wood, where thermal treatment increases lignin content due to the reduction in holocellulose content, particularly when wood is exposed to 260 °C.

In the present study, the highest holocellulose values were observed for clone E (control and 200 °C). However, at a temperature of 185 °C, the values for clones C and E were similar and higher than those for clone A. An increase in holocellulose content was noted in the 185 to 200 °C range for clones A and E, whereas clone C showed an increase (control and 185 °C) followed by a decrease between 185 and 200 °C. This variation in the chemical composition of wood among clones at the tested temperatures occurs because they are biologically distinct materials, with variable proportions of genes from each parental species (*E. grandis* and *E. urophylla*) (Silva *et al.* 2025), with clone specifications being confidential (Andrade *et al.* 2024).

Overall, the holocellulose content was lower than the values reported by Santos *et al.* (2010) and Frederico (2009), which were 65% and 69%, respectively. This difference can be attributed to genetic improvement and the specific intended use of the wood. The clones used in this research were enhanced to meet the requirements of the sawn timber sector, whereas the clones studied by the cited researchers were aimed at pulp production.

Biological Assays with Wood-Decaying Fungi

For the fungus *G. trabeum*, mass loss increased with the temperature rise for all clones (Table 4). The control and clone E exhibited the highest mass loss. Some authors have reported mass loss for *E. grandis* ranging from 0.5%, 25%, and 60%, respectively (Modes 2010; Batista 2012).

The *P. elliottii* wood used as a reference by AWPA E10 (2022) showed a mass loss of 47.7%. Brown-rot fungi tend to prefer conifer woods (Vivian *et al.* 2015). Consequently, the mass loss was higher for the reference wood compared to the *Eucalyptus* clones studied under all tested conditions. The mass loss caused by *G. trabeum* indicates that the culture used was vigorous and capable of causing significant wood degradation.

Table 4. Mass loss and Colorimetric Variation caused by *G. trabeum* Attack as a Function of Clones and Thermal Modification Temperatures

Mass Loss (%)				Colorimetric Variation (ΔE)*			
Clone	Control	185 °C	200 °C	Clone	Δ*E*	Temperature (ºC)	Δ*E*
A	0.71 Bb	2.79 Bc	38.63 Aa	A	9.32	Control	9.31
C	1.87 Cb	12.42 Bb	28.55 Ab	B	8.88	185	9.37
E	18.5 Ba	19.15 Ba	40.44 Aa	C	10.97	200	10.12
Means followed by the same capital letter, horizontally, and lowercase, vertically, do not differ from each other by the Scott-Knott test ($p > 0.05$). *Not significant by the F test ($p > 0.05$).							

At a temperature of 185 °C, clone E showed the highest mass loss, followed by clone C. At 200 °C, clones A and E were the most consumed. Thermal treatment was not effective against deterioration caused by *G. trabeum*, instead facilitating fungal attack. During thermal modification, chemical transformations may have occurred in the toxic substances of the clones, leading to susceptibility to fungal attack (Esteves *et al*. 2021). An increase in holocellulose content was also observed after thermal modification. This may have favored fungal degradation of heat-treated wood, considering the attractiveness of this structural component to xylophagous fungi (Vidholdová *et al.* 2022). In Fig. 3, the damage caused by the fungus can be observed.

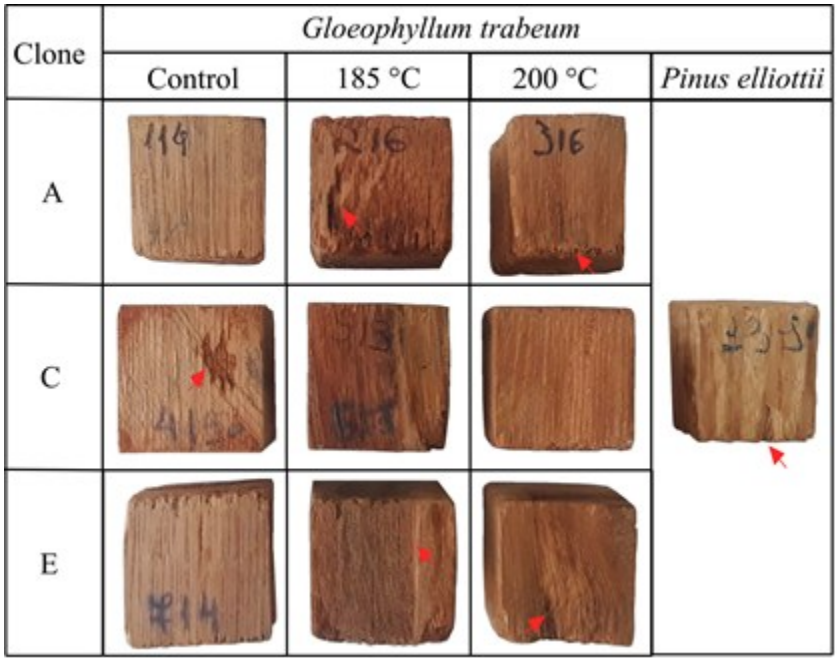

Fig. 3. Wood samples of *Eucalyptus* and *Pinus* subjected to attack by the fungus *G. trabeum*. Red arrows point to eroded tissue.

Regarding color variation (Table A2), no significant differences were observed between clones and tested temperatures. However, this fungus caused changes in the wood's color (Table 4), which can be classified as "very perceptible" according to Hikita *et al*. (2002) (Table 2). Brown rot fungi degrade wood more rapidly compared to soft and white rot fungi, resulting in greater color variations in shorter time intervals (Brischke and Meyer-Veltrup 2016). Kučerová *et al.* (2024) reported that the lightness of *Pinus* wood decreased with increased temperature and exposure time, becoming more evident at temperatures above 150 °C. These authors also associated wood color changes during thermal treatments with shifts in the concentrations of chemical components such as holocellulose, lignin, and total extractives.

Mass loss (control and 200 °C) due to *I. lacteus* attack showed no statistical differences between clones (Tables 5 and A3). At 185 °C, clone E exhibited the highest mass loss. Thermal treatment did not affect the mass loss of clones A and C. However, for clone E, an increase (control to 185 °C) and subsequent decrease (185 to 200 °C) were observed. For *P. elliottii* samples used as a reference by AWPA E10 (2022), mass loss was 3.78%, which was lower than clone E (185 °C) alone. White rot fungi cause less degradation in coniferous woods (Zabel and Morrell 1992; Schmidt 2006). Brocco (2019) observed that *P. taeda* (yellow pine) wood treated with teak extract and subjected to artificial weathering experienced mass loss caused by *I. lacteus* of approximately 20%. Since this study used the same mycelium from the same culture used in Brocco's study, this fungus was confirmed for causing significant deterioration to the tested woods.

Table 5. Mass Loss and Color Variation Caused by *I. lacteus* Attack as a Function of Clones and Thermal Modification Temperatures

Mass Loss (%)				Colorimetric Variation (Δ*E*)			
Clone	Control	185 °C	200 °C	Clone	Δ*E*	Temperature (°C)	Δ*E*
A	1.62 Aa	0.96 Ab	1.44 Aa	A	5.02 b	Control	7.40 a
C	0.68 Aa	1.82 Ab	1.03 Aa	C	6.90 a	185	6.41 a
E	0.70 Ba	4.69 Aa	1.17 Ba	E	6.97 a	200	5.08 b
Means followed by the same capital letter, horizontally, and lowercase, vertically, do not differ from each other by the Scott-Knott test ($p > 0.05$).							

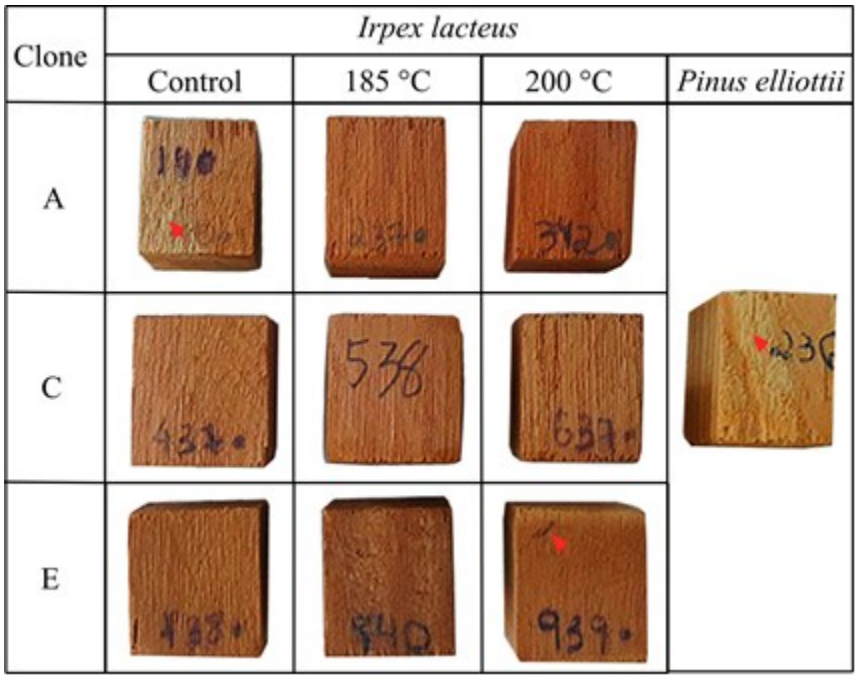

Fig. 4. *Eucalyptus* and *Pinus* wood samples subjected to attack by the fungus *I. lacteus*. Red arrows point to eroded tissue.

Damage caused by the fungus *I. lacteus* was observed in samples with low wood consumption, regardless of the situation and wood tested or reference (*Eucalyptus* and *Pinus*) (Fig. 4).

For the color variation results, no significant interaction was observed between the tested clones and temperatures. The lowest color variation values were recorded for clone A and the 200 ºC temperature. All tested conditions were classified as perceptible changes according to Hikita *et al.* (2002) (Table 2).

Regarding deterioration caused by *R. placenta* (Table 6), mass loss in the control did not differ statistically among clones (Table A4). At 185 and 200 ºC, clones A and E exhibited the highest mass loss. For all clones, thermal treatment increased mass loss. This phenomenon was previously observed and discussed in the context of the chemical composition of the fungus *G. trabeum* (Table 4), which, like *R. placenta*, is also a brown-rot fungus affecting wood.

Table 6. Mass Loss and Color Variation Caused by the Attack of *R. placenta* Based on Clones and Thermal Modification Temperatures

Mass loss (%)				Colorimetric variation (Δ*E*)			
Clone	Control	185 °C	200 °C	Clone	Control	185 °C	200 °C
A	4.35 Ca	22.23 Ba	36.80 Aa	A	9.50 Aa	4.78 Bb	8.51 Aa
C	7.83 Ba	5.87 Bb	23.33 Ab	C	7.47 Ba	7.31 Ba	8.58 Aa
E	11.29 Ba	25.67 Aa	33.06 Aa	E	10.11 Aa	9.89 Aa	11.82 Aa

Means followed by capital letters, horizontally, and lowercase letters, vertically, do not differ from each other according to the Scott-Knott test ($p > 0.05$).

For deterioration caused by *R. placenta* (≈ *Postia placenta*), Batista (2012) observed a decrease in mass loss (control - 180 ºC) for *E. grandis*, contrary to the results of this study. For *P. elliottii*, used as a reference, a mass loss value of 55.5% was obtained, which was higher than the values recorded for the tested *Eucalyptus* clones (control, 185, and 200 °C). This result indicates the vigor of the fungal culture used in this study. The referenced author found a similar value for *Pinus*, with a mass loss of 54%.

In Fig. 5, the damage caused by the fungus on the samples is evident, revealing the consumption of both test and reference wood (*Eucalyptus* and *Pinus*). For clone E (185 to 200 °C), the breakage of some samples after drying was observed, indicating greater fungal attack and wood consumption.

For the control and 200 °C temperature, no differences were observed in the colorimetry of the wood after the attack by *R. placenta* (Table 6). It was also observed that at 185 °C, clones C and E exhibited the greatest color variation. No thermal treatment effect was observed on the color variation of clone E. However, for clone A, there was a decrease (control to 185 °C) and an increase (185 to 200 °C). For clone C, there was an increase in the color variation of the wood with the thermal treatment. According to the classification by Hikita *et al.* (2002), clone A (control) and clone E (control, 185 and 200 °C) achieved values of color variation classified as highly perceptible. Meanwhile, the other situations were classified as perceptible.

The clones A, C, and E studied in this research were selected from those used by Silva (2018), which showed the least deterioration caused by wood-destroying organisms among the evaluated clones. This explains some results in which the untreated (control) wood exhibited biological resistance to the tested wood-destroying organisms.

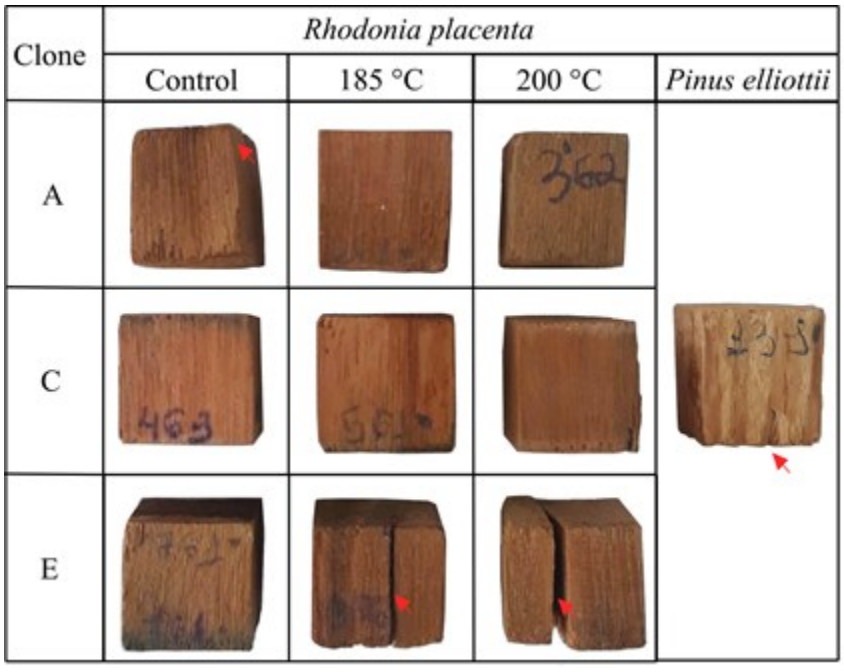

Fig. 5. Wood samples of *Eucalyptus* and *Pinus* subjected to the attack of the fungus *R. placenta.* Red arrows point to eroded tissue.

Decay Resistance Classes

Based on the mass loss (ML, %) of the samples corresponding to the *E. grandis* × *E. urophylla* clones, it was observed that clones A, C, and E presented different levels of

resistance to the attack of the fungi *G. trabeum*, *I. lacteus*, and *R. placenta*, according to the classification established by the AWPA E30 (2022) standard, as detailed in Table 1. In the control treatment, clones A and C were classified as highly resistant (ML = 0-10%) to the three rot fungi analyzed. For the fungus *I. lacteus*, regardless of the clones and temperatures used, the treatments were classified as highly resistant. Regarding the fungus *G. trabeum*, samples treated at 185 °C were classified as highly resistant (clone A) and resistant (clones C and E; ML = 11-24%). A decrease in resistance to moderate (ML = 25-44%) was also observed in samples treated at 200 °C for all three clones. Clones exposed to the fungus *R. placenta* after treatment at 185 °C were classified as highly resistant (clone C), resistant (clone A), and moderately resistant (clone E). At 200 °C, the clones were classified as resistant (clone C) and moderately resistant (clones A and E).

The natural resistance of wood to attack by wood-boring organisms varies, even among clones of the same species, as observed in studies with the hybrid *E. grandis* × *E. urophylla* intended for sawmills (Silva et al. 2025). The authors also observed that sapwood was degraded to a greater extent by the brown-rot fungus *Neolentinus lepideus* and the white-rot fungus *Trametes versicolor*. For the fungus *G. trabeum*, mass loss ranged from 7 to 16% and for *R. placenta*, from 17 to 28%. The resistance classification of the six clones studied ranged from resistant to highly resistant for *G. trabeum* and moderately resistant to resistant for *R. placenta* (Silva *et al.* 2025).

Regarding the middle heartwood, seven *Eucalyptus* species were classified as highly resistant (*E. camaldulensis*, *E. urophylla*, *E. robusta*, *E. saligna*, *E. pellita*, and *Corymbia citriodora*) and moderately resistant (*E. grandis*) to wood-decaying fungi. In the transition region between heartwood and sapwood, these species were classified as resistant, except for *E. saligna* and *E. camaldulensis*, which were classified as highly resistant. A greater mass loss was also observed in the middle heartwood for *E. grandis* and in the transition region for *E. grandis*, *E. urophylla*, and *E. pellita* (Medeiros Neto *et al.* 2024).

Thermal modification of *Eucalyptus* wood is an efficient strategy to increase its natural resistance to attack by xylophagous organisms (mainly fungi) through thermal degradation and the transformation of components more susceptible to fungal deterioration (Haseli *et al.* 2024). This can be observed in studies with *E. saligna*, which showed greater resistance to the fungus *G. trabeum* at temperatures ranging from 120 to 180 °C, similar to that of the present study at 185 °C. The studies also demonstrated resistance to the fungus *P. sanguineus* and soft rot at temperatures above 120 °C. The percentage of mass loss for *E. saligna* was higher when affected by the fungus *P. sanguineus* (9.46 to 35.49%) compared to *G. trabeum* (0.73 to 9.79%) (Brito *et al.* 2019). For *E. grandis*, the most efficient temperature for attacking *P. sanguineus* ranged between 180 and 220 °C (Calonego *et al.* 2010).

CONCLUSIONS

1. For the total lignin content, the thermal treatment resulted in a decrease in the value for clone A and an increase for clone C. Meanwhile, clone E exhibited the highest lignin value at 185 °C. In clones where there was an increase in lignin, a decrease in holocellulose values was observed.

2. Brown rot fungi caused more significant damage to the wood of the tested clones, especially at 200 °C, indicating that thermal modification had an undesirable effect on the biological resistance of the wood to this class of fungi. The fungus *I. lacteus* (white rot) showed minimal influence on the woods, regardless of the situation (clone and temperature) or reference wood (*Pinus*).
3. All the fungi tested caused color variations in the wood. The fungus *I. lacteus* (white rot) caused perceptible changes, while the brown rot fungi (*G. trabeum* and *R. placenta*) caused greater alterations, which were classified as highly perceptible.
4. Thermal modification, mainly at 200 °C, resulted in greater susceptibility of the wood of *E. grandis* × *E. urophylla* clones to decay by xylophagous fungi, under the conditions studied. However, regarding resistance classes, the clones were classified as highly resistant (A, C, and E) to the fungus *I. lacteus*, regardless of the heat treatment. For the fungi *G. trabeum* and *R. placenta*, the highly resistant clones (A and C) were exposed to a temperature of 185 °C.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001, and the National Council for Scientific and Technological Development - Brazil (CNPq) - Process 304721/2018-2 for the scholarships and support to the research (respectively), and to the Complexo Agroindustrial Pindobas Ltda., Brazil, for donating the wood used in the research.

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Article submitted: April 25, 2025; July 11, 2025; Revised version received: August 22, 2025; Accepted: August 31, 2025; Published: October 3, 2025.
DOI: 10.15376/biores.20.4.10051-10068

APPENDIX

Table A1. Summary of Variance Analyses for Wood Chemistry

Variation Source	Degree of Freedom	Mean Squares	
		Lignin	Holocellulose
Clone	2	56.37**	41.19**
Temperature	2	3.57**	14.66**
Clone x Temperature	4	6.08 **	7.61**
Residue	45	0.71	1.17
Total	53		
**significant ($p \leq 0.05$), nsnot significant ($p > 0.05$).			

Table A2. Summary of Variance Analyses for Biological Tests with *G. trabeum* Fungus

Variation Source	Degree of Freedom	Mean Squares	
		Mass loss	Colorimetric variation
Clone	2	845.67**	21.75ns
Temperature	2	4343.14**	2.96ns
Clone x Temperature	4	200.00**	13.59ns
Residue	45	20.65	11.11
Total	53		
**significant ($p \leq 0.05$), nsnot significant ($p > 0.05$).			

Table A3. Summary of Variance Analyses for Biological Tests with *I. lacteus* Fungus

Variation Source	Degree of Freedom	Mean Squares	
		Mass loss	Colorimetric variation
Clone	2	5.24**	21.96**
Temperature	2	11.69**	24.33**
Clone x Temperature	4	9.85**	4.69ns
Residue	45	1.60	3.92
Total	53		
**significant ($p \leq 0.05$), nsnot significant ($p > 0.05$).			

Table A4. Summary of Variance Analyses for Biological Tests with *R. placenta* Fungus

Variation Source	Degree of Freedom	Mean Squares	
		Mass loss	Colorimetric variation
Clone	2	608.71**	13.21**
Temperature	2	2444.02**	25.71**
Clone x Temperature	4	212.63**	29.35**
Residue	45	53.44	8.12
Total	53		
**significant ($p \leq 0.05$).			