Forest Ecology and Management 402 (2017) 265–271

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Potential of selected fungi for biological stump removal of *Eucalyptus* spp.

Lais Gonçalves da Costa[a,*], Juarez Benigno Paes[a], Waldir Cintra de Jesus Junior[b], Victor Fassina Brocco[a], Edson Luiz Furtado[c]

[a] Department of Forest and Wood Science, Federal University of Espírito Santo, Governador Lindemberg, 316, 29550-000 Jerônimo Monteiro, Espírito Santo, Brazil
[b] Lagoa do Sino Academic Department, Federal University of São Carlos, Aracaçu, 18290-000 Buri, São Paulo, Brazil
[c] Department of Vegetal Protection, College of Agronomic Sciences, São Paulo State University, Doutor José Barbosa de Barros, 1780, 18610-307 Botucatu, São Paulo, Brazil

ARTICLE INFO

Keywords:
Fungi selection
Stump decay
Wood chemistry
Wood-decaying fungi

ABSTRACT

This study aimed to evaluate the decay potential of fungi isolated from plantations of *Eucalyptus* spp. for biological stump removal. Therefore, an accelerated decay test was performed with fungi isolated from stumps of *Eucalyptus* spp.: *Phanerochaete chrysosporium*, *Cladosporium* sp., *Penicillium* sp., *Penicillium implicatum*, *Resinicium confertum*, *Trichoderma citrinoviride* and *Acremonium* sp. Chemical analysis of the decayed wood was performed on samples submitted to the most degrading fungi. A field test on eucalyptus stumps, with two methods of inoculation and covering, was performed to evaluate the stumps deterioration by the isolated fungi. *Acremonium* sp., *Cladosporium* sp. and *P. chrysosporium* showed the highest potential in the accelerated decay test. Chemical analysis of the decayed wood indicated the selectivity of fungi for lignin. No significant variations were observed in the field test compared to the control stumps. Biological stump removal shows both environmental and economic potential and needs to be thoroughly studied.

1. Introduction

Plantations of *Eucalyptus* spp. in Brazil have been increasing in recent years. According to the Brazilian Tree Industry – IBÁ (2016), the Gross Domestic Product (GDP) of the Brazilian planted tree industry grew by 3% in 2015, representing 1.2% of all of the wealth generated in the country. Eucalyptus plantations represent 72% of the total area of planted trees.

The multiple uses of eucalyptus wood are one of the factors that contributed to this increase because this wood can be used by the building, furniture, pulp and paper and energy industries. According to Andrade et al. (2012), fast growth and short rotations have consolidated the genus as a wood supply source.

With the increasing development of the planted tree industry, it is necessary to update the techniques that are used during the exploitation of forest plantations to produce less expensive and more eco-friendly alternatives. The removal of stumps of *Eucalyptus* spp. after harvesting is generally performed by a mechanical process. This process is costly and generates environmental damage, such as soil compaction and displacement of the soil organic layer, and there are also some areas that are not easily accessible, which makes mechanical stump removal difficult. Moreover, damage to machine parts and implements can occur (Dias and Arroja, 2012; Dedecek and Gava, 2005; Alonso et al., 2007; Coder, 2014).

Thus, biological stump removal appears to be a promising alternative to mechanical stump removal (Silva et al., 2010; Negrão et al., 2014). However, the natural process of biological degradation of stumps is slow. For several years, such material remains unchanged, making the use of this process unfeasible (Coder, 2014).

On the other hand, according to Andrade et al. (2012), the use of selected fungi significantly increases the degradation of stumps of *Eucalyptus* spp. Moreover, this process leads to a reduction in nutrient export, microbiota maintenance and a reduction in the negative impacts on soil, thus contributing to the sustainability of planted forests (Alonso et al., 2007).

Among the fungi with this potential, wood-decaying fungi present the greatest potential for use. Known as white-, brown- and soft-rot fungi, they are responsible for the majority of wood decay and use enzymatic and non-enzymatic systems to degrade cellulose, hemicellulose and lignin of the wood cell wall (Zabel and Morrell, 1992; Schmidt, 2006; Tian et al., 2010; Ryss et al., 2011; Bari et al., 2015).

Therefore, according to the aforementioned factors, selection of fungi that accelerate the natural decay process of stumps that remain after forest extraction is very important. Thus, this study aimed to evaluate the decay potential of fungi isolated from plantations of *Eucalyptus* spp. for biological stump removal.

* Corresponding author.
E-mail address: lais-costa1@live.com (L.G.d. Costa).

http://dx.doi.org/10.1016/j.foreco.2017.07.054
Received 31 May 2017; Received in revised form 31 July 2017; Accepted 31 July 2017
Available online 08 August 2017
0378-1127/

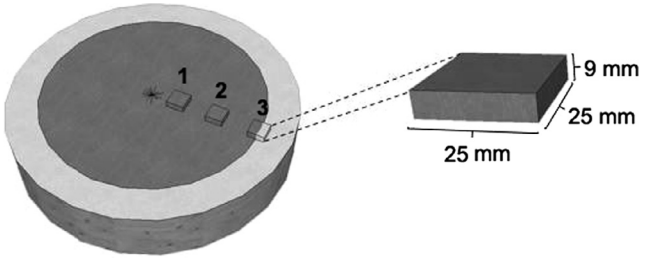

Fig. 1. Wood positions from which samples were obtained and their respective dimensions. Key: 1: inner heart wood; 2: middle heartwood; 3: heartwood-sapwood boundary.

Table 1
Degradation class of decay fungi..
Source: Adapted from ASTM D – 2017 (2005)

Mass loss (%)	Residual mass (%)	Class of degradation
0–10	90–100	Slightly degrading
11–24	76–89	Moderately degrading
25–44	56–75	Degrading
≥45	≤55	Highly degrading

2. Material and methods

2.1. Accelerated decay test with the isolated fungi

To evaluate the deterioration capabilities fungi, an accelerated decay test using clonal *Eucalyptus grandis* x *urophylla* wood was conducted following the American Society for Testing and Materials – ASTM D2017 (2005). Fungi isolated from *Eucalyptus* spp. stumps, *Phanerochaete chrysosporium*, *Cladosporium* sp., *Penicillium* sp., *Penicillium implicatum*, *Resinicium confertum* and *Trichoderma citrinoviride*, were used. The fungi *Acremonium* sp., isolated by Silva et al. (2014) and cited as basidiomycete 1, was also used.

The isolated fungi were collected in three cities of Espírito Santo State, Brazil: Cachoeiro de Itapemirim (20°46′19″S, 41°18′07″W, 100 m), São José do Calçado (20°55′47″S, 41°37′47″W, 590 m) and Jerônimo Monteiro (20°51′43″S, 41°27′32″W, 800 m), as described by Costa (2014). These fungi were taxonomically classified at the Plant Pathology Department, Seed Pathology and Post-Harvest Laboratory, Federal University of Viçosa, Viçosa–MG, Brazil.

The brown-rot fungi *Gloeophyllum trabeum* (Pers.) Murrill (Madison 617) and *Postia placenta* (Fr.) M.J. Larsen & Lombard (Madison 698) as well as the white-rot fungus *Polyporus fumosus* (Pers.) Fr. (Forintek Canada Corp. 469 A) were compared to the isolated fungi due to their recognized decay potential.

According to ASTM D2017 (2005) procedures, wood samples with dimensions of 2.5 × 2.5 × 0.9 cm (radial × tangential × longitudinal) were prepared in three pith-bark positions: at the heartwood-sapwood boundary and at the middle and inner heartwood (Fig. 1). The test was maintained in a climate controlled room (25 ± 2 °C, with 65 ± 5% relative humidity) for 12 weeks. After 12 weeks, the mass loss was evaluated and compared with the values shown in Table 1.

2.2. Chemical analysis

Chemical analysis of decayed wood was performed with the samples submitted to the most degrading fungi, which were selected in the accelerated decay test. The wood samples were transformed into sawdust following the Technical Association of Pulp and Paper Industry – TAPPI 257 om-92 (1992). Sawdust of wood samples not submitted to fungi attack was used as a control.

For the determination of extractives, 2 g of air-dried sawdust was submitted to a sequence of extractions in ethanol:toluene (1:2), ethanol, and hot water according to TAPPI T264 om-88 (TAPPI, 1996). Klason and acid soluble lignins were measured according to Gomide and Demuner (1986) and Goldschimid (1971), respectively. The total lignin was obtained from the sum of the Klason and acid soluble lignins. The holocellulose content was obtained by the difference of extractives and total lignin.

2.3. Deterioration of stumps by isolated fungi

A field test was conducted in a 7-year-old *Eucalyptus grandis* × *urophylla* plantation located in Providência a rural area of Jerônimo Monteiro, ES, Brazil (20°51′43″S, 41°27′32″W, 800 m).

To verify the decay potential in stumps, the seven isolated fungi were employed. Thus, the remaining stumps from the first harvest without visual signs of fungal colonization were used. The trees of the remaining stumps were harvested 10 days before the field test.

In a laminar flow cabinet, fungal solutions were prepared using liquid culture media (20 g of malt extract in 1 L of sterile distilled water). For each solution, fractions of 50 mL of the fungus developed in Petri dishes were added. The fungal solutions were stored in a climate controlled room (25 ± 2 °C, with 65 ± 5% relative humidity) for a period of 15 days to allow mycelial growth. Afterwards, the solutions were homogenized on a magnetic stirrer and used for the field test.

Two fungal inoculation methods were tested: spraying and inoculation by drilling the stumps. For each inoculation method, stumps covered and uncovered with dark plastic bags were used to verify the coverage effect on creating a suitable environment for fungal development (Fig. 2D).

In inoculation by spraying, a spray bottle containing 25 mL of the fungal solution was used for each stump (Fig. 2A). For inoculation by drilling, a wood boring drill was used (Fig. 2B) to create a hole in the centre of the stump and four holes perpendicularly distributed at the heartwood-sapwood boundary (Fig. 2C). With a syringe, 5 mL of the fungal solution was introduced into each hole.

For each fungus, 16 stumps were inoculated: eight stumps for each inoculation method, four of which were covered with a plastic bag. Eight stumps without the fungal solution that had the same coverage and boring situations were used as a control sample.

After four months, degradation of the stumps was evaluated with a dynamic penetrometer (Andrade et al., 2012). Thus, the depth of the resulting penetration of one impact produced by the penetrometer rod was measured. This evaluation was conducted at four points on the stump surface: three distributed at the heartwood-sapwood boundary and one in the centre of the stump.

A Stress Wave Timer was also used to evaluate the degradation of the stumps using the propagation velocity of stress waves. Therefore, a hammer hit and a transducer were used to generate and receive the signal, respectively. In each stump, six measurements distributed in two perpendicular positions were performed without the presence of bark. The propagation velocity of the stress waves (V) was calculated based on the time (t) it took the wave to travel the distance (d) between the transducers using the following equation: $V = d/t$.

2.4. Analysis and evaluation of the results

A completely randomized two-way factorial design was used for the accelerated decay test and field test with the isolated fungi. The means and standard deviations were used for the chemical analysis of the decayed wood.

The Scott-Knott test ($p \leq 0.05$) was used for the factors and significant interactions detected by the F test ($p \leq 0.05$). The mass loss percentage data (ML) were transformed by arcsin [square root (ML/100)] to meet the necessary conditions for the use of parametric tests.

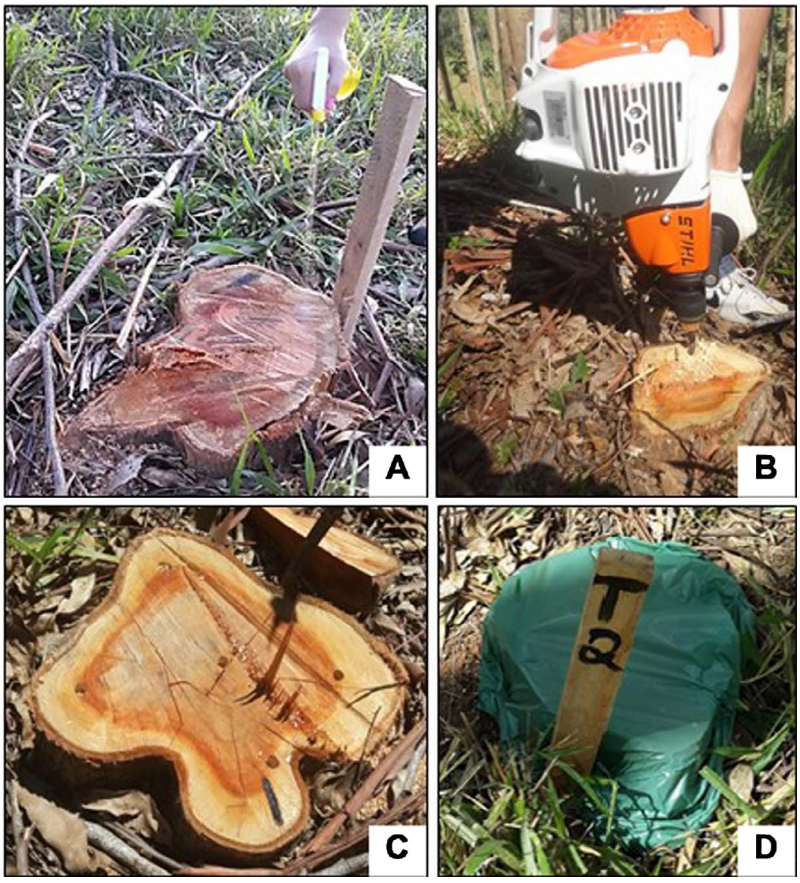

Fig. 2. Assembly steps of the field test: spraying a fungal solution on stumps (A); drilling the stump with a wood boring drill (B); drilled stump (C); and a stump covered with a plastic bag (D).

3. Results and discussion

3.1. Accelerated decay test with the isolated fungi

Significant differences among the fungi, wood position and interaction between the fungi x wood position were indicated by analysis of variance. In the comparison of the mass loss for each fungus among the positions tested (Table 2), the fungi *G. trabeum, P. placenta, P. fumosus*, *P. chrysosporium*, *Penicillium* sp. and *P. implicatum* did not differ among the wood positions, indicating that different positions did not influence degradation by these fungi.

However, the wood positions showed significant differences for the other fungi tested, with the heartwood-sapwood boundary showing the greatest mass loss. Significant differences for all of the positions tested were found only for *Acremonium* sp., which showed a reduction in mass loss in the middle and inner heartwood of 44.31% and 57.88%, respectively, in comparison to the heartwood-sapwood boundary position (Table 2).

The effect of the wood position on the fungal decay potential was observed by Bhat et al. (2005), Moya and Berrocal (2010) and Paes et al. (2015). Generally, degradation by wood-destroying organisms is higher at external positions that contain sapwood. However, for some of the fungi tested, it was observed that the internal positions were deteriorated similarly to the external ones, indicating their potential for the deterioration of stumps.

In the comparison of mass loss for each wood position, there were no significant differences among the fungi *G. trabeum*, *P. placenta* and *Acremonium* sp. at the heartwood-sapwood boundary position (Table 2).

Table 2
Comparison between the average mass loss due to the wood position and tested fungi with their respective degradation classes.

Fungus	Mass loss (%) Wood position					
	H-S boundary	Degrad. class[a]	Middle heartwood	Degrad. class	Inner heartwood	Degrad. class
G. trabeum	32.62 aA	D	31.84 aA	D	37.02 aA	D
P. placenta	32.77 aA	D	33.46 aA	D	34.63 aA	D
P. fumosus	20.25 bA	MD	17.31 bA	MD	19.76 bA	MD
Acremonium sp	28.52 aA	D	15.88 bB	MD	12.01 cC	MD
P. chrysosporium	11.30 cA	MD	10.31 cA	SD	11.14 cA	MD
Cladosporium sp.	10.20 cA	SD	2.57 dB	SD	3.06 dB	SD
T. citrinoviride	7.41 dA	SD	2.67 dB	SD	2.10 dB	SD
R. confertum	5.73 dA	SD	2.48 dB	SD	2.62 dB	SD
Penicillium sp	0.69 eA	SD	0.97 eA	SD	0.41 eA	SD
P. implicatum	0.55 eA	SD	0.27 eA	SD	0.79 eA	SD

Means followed by the same lowercase (column) or uppercase (line) letter do not differ (Scott-Knott $p > 0.05$).
[a] SD: Slightly degrading, MD: Moderately degrading, D: Degrading.

The mass loss caused by *Acremonium* sp. was similar to that of the wood-decaying fungi with recognized potential (*G. trabeum* and *P. placenta*), making it a promising fungus for the degradation of stumps. Silva et al. (2014) studied the potential of *Acremonium* sp. (basidiomycete 1) to decay eucalyptus wood and found an average mass loss of 28.43% at the sapwood position, which was similar to the results found in this study.

Among all of the fungi isolated in this study, *P. chrysosporium* and *Cladosporium* sp. showed the best performances and did not differ from each other at the heartwood-sapwood boundary position, with mass losses that were 65.51% and 68.87% lower compared to *P. placenta* (Table 2), respectively.

At the middle heartwood position, the fungi *G. trabeum* and *P. placenta* again caused the greatest mass loss, differing from the other fungi. Deteriorations caused by *P. fumosus* and *Acremonium* sp. were similar and were greater than that caused by *P. chrysosporium*. Compared to *Acremonium* sp., *P. chrysosporium* showed a reduction of 35.07% in its decay potential (Table 2).

G. Trabeum and *P. placenta* caused the highest mass loss in the inner heartwood, followed by *P. fumosus*. At the same wood position, *Acremonium* sp. showed a decay potential that was 7.24% higher than that of *P. chrysosporium*; however, no significant differences were found (Table 2). Silva et al. (2014) found an average mass loss of 5.48% in eucalyptus heartwood deteriorated by *Acremonium* sp. (Basidiomycete 1), which is lower than the value found in this study.

In addition to *Acremonium* sp., *P. chrysosporium* also showed decay potential, since their average mass losses did not vary among the wood positions.

It was also found that *G. trabeum* and *P. placenta* were classified as degrading at all positions. *Acremonium* sp. was considered to be degrading only at the heartwood-sapwood boundary position and was classified as moderately degrading at the other wood positions. The fungus *P. chrysosporium* was also classified as moderately degrading at the boundary and inner positions, while it was classified as slightly degrading at the middle heartwood position (Table 2).

The low degradation caused by the fungus *T. citrinoviride* can be explained by the fact that the decay test conducted is specific for brown- and white-rot fungi and the fungi of this genus are reported in the literature as causing soft-rot (Shrestha et al., 2009). Therefore, it would be necessary to conduct specific tests for these fungi to obtain their effective decay potential.

3.2. Chemical analysis

The fungi *R. confertum*, *Acremonium* sp., *P. chrysosporium*, *Cladosporium* sp. and *T. citrinoviride* showed the highest mass loss in the accelerated decay test, and the test samples decayed by these fungi were used in the chemical analysis, which indicated changes in the chemical composition of decayed wood (Table 3).

In the control samples, the highest proportion of extractives was obtained at the heartwood-sapwood boundary position. According to Hillis (1971), the higher content found in this region is caused by fresh biochemical changes of reserve substances.

An increase in the total extractive content was observed at the heartwood-sapwood boundary position that was decayed by *Acremonium* sp., *P. chrysosporium* and *Cladosporium* sp. compared to the control samples. This increase (32%) can be explained by the highest mass loss caused by these fungi, promoting a higher amount of breakdown and increasing the solubility of the major components of wood.

In addition to changes in the major components of wood, biological degradation of wood can also increase the content of soluble extractives due to the increase of free sugars, which is caused by the enzymatic action of fungi on hemicellulose (Silva et al., 2014). These authors also stated that this increase can vary with the deterioration level and type of fungi tested. Istek (2006) and Malakani et al. (2014) also found an increase in wood extractives in response to a fungal attack, attributing this fact to the breakdown of cell wall constituents, increasing the solubility of extractives by the employed solvents.

Changes in extractives caused by fungi in the middle and inner heartwood were lower compared to control samples, which may be explained due to the lowest mass loss at these wood positions, except for with the fungus *P. chrysosporium*, which caused an increase in the content of extractives at these positions (Table 3). As shown in Table 2, only *P. chrysosporium* showed no significant differences at these wood positions; therefore, its behaviour with regard to the extractives was similar at the different positions tested.

In terms of the total lignin content, no variations among the positions tested were observed in the control samples. Overall, a decrease in the total lignin content was observed at the heartwood-sapwood boundary position, where the highest mass loss occurred. The lowest values of the total lignin content at this position were observed in wood decayed by *R. confertum*, *Acremonium* sp., and *P. chrysosporium*, with reductions of 10.95, 10.61, and 13.21%, respectively, compared to control samples, while *Cladosporium* sp. and *T. citrinoviride* promoted reductions of 5.64 and 8%, respectively, compared to control samples (Table 3).

Table 3
Chemical composition of samples after fungal attack in an accelerated decay test.

Fungus	Wood positions	Mass loss (%)	Total extractives (%)	Total lignin (%)	Holocellulose (%)
R. confertum	H-S bound.	5.73	2.57 (0.02)	28.37 (0.39)	70.34 (1.43)
	Middle heart.	2.48	2.76 (0.05)	32.18 (2.06)	65.06 (2.10)
	Inner heart.	2.62	3.10 (0.03)	32.47 (0.28)	64.43 (0.31)
Acremonium sp.	H-S bound.	28.52	4.82 (0.67)	28.48 (0.33)	66.71 (1.00)
	Middle heart.	15.88	2.78 (0.03)	32.08 (0.18)	65.14 (0.15)
	Inner heart.	12.01	2.04 (0.30)	30.67 (0.30)	67.30 (0.61)
P. chrysosporium	H-S bound.	11.30	4.85 (0.42)	27.65 (0.47)	67.50 (0.05)
	Middle heart.	10.31	5.41 (0.56)	29.92 (1.75)	64.67 (2.22)
	Inner heart.	11.14	5.71 (0.42)	30.97 (0.18)	63.32 (0.38)
Cladosporium sp.	H-S bound.	10.20	4.66 (0.07)	30.06 (1.13)	65.28 (1.20)
	Middle heart.	2.57	2.41 (0.49)	32.61 (0.65)	64.98 (0.16)
	Inner heart.	3.06	2.81 (0.22)	31.57 (0.28)	65.61 (0.49)
T. citrinoviride	H-S bound.	7.41	3.76 (0.04)	29.31 (0.23)	66.93 (0.20)
	Middle heart.	2.67	2.44 (0.40)	34.06 (1.31)	63.50 (1.70)
	Inner heart.	2.10	2.69 (0.49)	32.73 (1.89)	64.58 (2.38)
Control	H-S bound.	–	3.61 (0.67)	31.86 (1.96)	64.53 (2.62)
	Middle heart.	–	2.99 (0.15)	31.71 (0.89)	65.30 (0.75)
	Inner heart.	–	2.21 (0.19)	33.03 (0.03)	64.63 (0.02)

* Numbers in parentheses are standard deviations.

Table 4
Comparison between the average penetration due to fungi and treatments tested.

Fungus	Penetration (cm) Inoculation methods			
	Drilling		Spraying	
	Covered stumps	Uncovered stumps	Covered stumps	Uncovered stumps
P. implicatum	1.11 bB	1.05 bB	1.25 aA	1.26 aA
R. confertum	1.18 bA	1.11 bA	1.26 aA	1.18 aA
Acremonium sp.	1.23 aA	1.30 aA	1.26 aA	1.20 aA
P. chrysosporium	1.15 bB	1.15 bB	1.34 aA	1.25 aA
Cladosporium sp.	1.30 aA	1.21 aB	1.14 bB	1.18 aB
Penicillium sp.	1.21 bA	1.21 aA	1.10 bA	1.12 aA
T. citrinoviride	1.30 aA	1.17 aB	1.06 bB	1.27 aA
Control	1.17 bA	1.21 aA	1.27 aA	1.26 aA

Means followed by the same lowercase (column) or uppercase (line) letter do not differ (Scott-Knott $p > 0.05$).

The lowest values of the total lignin content at all of the tested positions was obtained by *P. chrysosporium* compared to control samples, indicating its preference for lignin. According to El-Gammal et al. (1995) and Istek et al. (2005), the fungus *P. chrysosporium* has special enzymes that are highly selective for lignin, while cellulose is slightly changed. Istek et al. (2005) studied the degradation of *Abies bornmülleriana* and *Fagus orientalis* by the white-rot fungus *P. chrysosporium* and found significant losses in the lignin content. Compared to control samples these losses represented 15% and 23% reductions, respectively.

Silva et al. (2014) studied the decay potential of two basidiomycetes isolated from 5- to 6-year-old decayed eucalyptus stumps and found 10% and 2% reductions in the lignin content of *E. grandis* × *urophyla* sapwood and heartwood, respectively, which is close to the results found in this study for *Acremonium* sp.

No reduction in the holocellulose content was observed compared to control samples, indicating the selectivity or preference of the isolated fungi for lignin. According to Ferraz et al. (2008) and Jawjit et al. (2007), fungi with this selectivity can be used for biopulping, which consists of treating wood chips used in the paper industry with lignin-degrading fungi. This biological pre-treatment increases the susceptibility of lignin to solubilization by pulping liquor, facilitates defibrillation and pulp refining, and results in savings of reagents and energy.

3.3. Deterioration of stumps by isolated fungi

3.3.1. Decay evaluation using a dynamic penetrometer

Significant differences of the penetration depth values among the fungi tested and the interaction between fungi and inoculation methods were indicated by analysis of variance. In the comparison between fungi inoculated in drilled and covered stumps (Table 4), only *Acremonium* sp., *Cladosporium* sp. and *T. citrinoviride* promoted penetration averages that were significantly higher than the control samples, which did not differ from each other. For these fungi, the levels of penetration achieved in the stumps were 5%, 11% and 11%, respectively, which were higher than the control stumps.

For the drilled and uncovered stumps, the highest depth achieved by the dynamic penetrometer was for stumps inoculated with *Acremonium* sp. The average depth reached was 7.4% higher compared to the control stump, although there was no significant difference. Similar to the above observation, in the treatments in which fungi were inoculated by spraying, in both covered and uncovered stumps, the higher average penetration did not differ from control samples (Table 4).

Comparing the inoculation methods for each fungus tested, higher averages for *P. implicatum* and *P. chrysosporium* were achieved in inoculation of stumps by spraying and were not influenced by the coverage conditions. These averages were 16.2% (*P. implicatum*) and 12.6% (*P. chrysosporium*) higher compared to the treatment with drilled stumps. This fact can be explained by the higher contact area covered by the spraying method, while the other inoculation method remained concentrated near the holes.

No significant differences were found between the treatments for the fungi *R. confertum*, *Acremonium* sp., and *Penicillium* sp. and control stumps. For *Cladosporium* sp., treatment with drilled and covered stumps obtained the highest penetration average, which was 10.5% higher compared to the other treatments employed. Regarding the fungus *T. citrinoviride*, treatments with drilled and covered stumps as well as with sprayed and uncovered stumps were statistically similar and superior to the other treatments (Table 4).

Regardless of the treatment used, a deeper penetration was expected when the stumps were covered as this treatment presents a suitable environment for fungal development. However, deeper penetration was only observed in the drilled stumps inoculated with *Acremonium* sp., *Cladosporium* sp. and *T. citrinoviride*.

Andrade et al. (2012) used a dynamic penetrometer to evaluate the potential of isolated fungi for biological stump removal and observed that stumps inoculated with the fungus *Pycnoporus sanguineus*, which is known for its degradation capability, showed similar results compared to control samples. However, when the stumps were covered, the fungi employed were able to reduce resistance to the penetrometer impact, indicating that the use of a cover provided a suitable environment for fungal development.

3.3.2. Decay evaluation using a stress wave timer

No significant differences were found in statistical analysis for stress wave propagation. The averages of the propagation velocities of stress waves ($m\ s^{-1}$) are presented in Table 5. Overall, despite the presence of fungal colonization in the stumps (Fig. 3), the changes promoted by the treatments tested were similar to those of the control stumps. Inoculated stumps were expected to be less resistant than control samples after four months of exposure, and the higher propagation velocity of stress waves will be able to confirm their degradation once the stress wave method is capable of detecting sensitive changes in wood decayed by fungi (Teles, 2014).

The propagation velocity of the stress waves in the decayed wood tends to be higher than in healthy wood. When propagated in wood, the waves move along the damaged tissue and its empty spaces, determining its internal condition. Thus, an increase in propagation velocity directly reflects a loss in mechanical strength of wood, indicating increased decay (Zabel and Morrell, 1992; Teles, 2014).

According to Reis and Reis (1997) and Coder (2014), because it is a biological material, the remaining stump is subject to the variation of

Table 5
Comparison between the average propagation velocity of stress waves due to the fungi and treatments tested.

Fungus	Propagation velocity of stress waves ($m\ s^{-1}$) Inoculation methods			
	Drilling		Spraying	
	Covered stumps	Uncovered stumps	Covered stumps	Uncovered stumps
P. implicatum	348.34 (27.55)	310.69 (61.62)	347.46 (10.74)	310.80 (55.50)
R. confertum	295.72 (62.81)	346.98 (88.56)	302.30 (19.45)	324.97 (36.18)
Acremonium sp.	304.90 (15.75)	320.63 (25.47)	308.72 (25.40)	337.66 (19.14)
P. chrysosporium	319.32 (6.89)	342.69 (59.50)	289.85 (38.47)	313.24 (95.06)
Cladosporium sp.	365.73 (54.97)	377.99 (68.08)	364.50 (43.47)	301.48 (33.25)
Penicillium sp.	279.57 (23.47)	292.63 (22.73)	291.85 (16.48)	332.22 (66.21)
T. citrinoviride	321.58 (34.87)	295.55 (28.68)	360.35 (61.92)	292.75 (46.34)
Control	374.27 (25.39)	287.73 (2.40)	364.70 (92.66)	340.06 (40.66)

* Numbers in parentheses are standard deviations.

Fig. 3. Visual signs of fungal colonization in the inoculated stumps.

its resistance to wood-destroying organisms, which varies with environmental conditions and the characteristics of the wood. The death of a stump has a fundamental importance in its deterioration process and may be linked to soil and climate conditions. These authors emphasize that the reduction of the physiological vigour of the remaining stumps is more intense in poor soils and dry seasons.

The above-mentioned authors also highlight that the physiological vigour of the remaining stumps can last after the tree is cut and can induce the growth of adventitious points. While physiological activity is still present, a number of defence mechanisms are active, which can delay the appearance of pests and progress of deterioration until the stump vigour is reduced.

Regardless of the treatments tested, the presence of sprouting was observed in some stumps during the field test, indicating their physiological vigour. Due to the above facts, the development and advancement of deterioration in stumps may have been impaired, requiring more exposure time than used in this study to allow stump death or the use of techniques and products to accelerate the death in the remaining stumps.

4. Conclusions

Among the fungi isolated from eucalyptus stumps, *Acremonium* sp. showed the best potential, similar to fungi with recognized deterioration capabilities. Although not as expressive, the isolated fungi *P. chrysosporium* and *Cladosporium* sp. also showed a decay potential.

The selectivity of fungi isolated by lignin was observed, highlighting their potential for use in studying biopulping. Although the visual presence of fungi was observed in the stumps, no significant variations were observed in the field test. The short period of exposure and physiological vigour of the stumps may have contributed to this result and should be observed for further studies. Biological stump removal shows both environmental and economic potential and needs to be thoroughly studied.

References

Alonso, S.K., Silva, A.G., Kasuya, M.C.M., Barros, N.F., Cavallazzi, J.R.P., Bettucci, L., Lupo, S., Alfenas, A.C., 2007. Isolation and screening of wood white rot fungi from *Eucalyptus* spp. forests with potential for use in degradation of stumps and roots. Rev. Árvore 31, 145–155. http://dx.doi.org/10.1590/S0100-67622007000100016.

de Andrade, F.A., Calonego, F.W., Severo, E.T.D., Furtado, E.L., 2012. Selection of fungi for accelerated decay in stumps of *Eucalyptus* spp. Biores. Technol. 110, 456–461. http://dx.doi.org/10.1016/j.biortech.2012.01.069.

American Society for Testing and Materials – ASTM D-2017, 2005. Standard method of accelerated laboratory test of natural decay resistance of woods. Annu. Book ASTM Stand. 410, 1–5.

Bari, E., Nazarnezhad, N., Kazemi, S.M., Tajick Ghanbary, M.A., Mohebby, B., Schmidt, O., Clausen, C.A., 2015. Comparison between degradation capabilities of the white rot fungi *Pleurotus ostreatus* and *Trametes versicolor* in beech wood. Int. Biodeterior. Biodegrad. 104, 231–237. http://dx.doi.org/10.1016/j.ibiod.2015.03.033.

Bhat, K.M., Thulasidas, P.K., Maria Florence, E.J., Jayaraman, K., 2005. Wood durability of home-garden teak against brown-rot and white-rot fungi. Trees – Struct. Funct. 19,

654–660. http://dx.doi.org/10.1007/s00468-005-0429-0.
Coder, K. D., 2014. Arboriculture Series: Stump Removal in Landscapes. Univ. of Georgia, Warnell School of Forestry and Natural Resources, Publication WSFNR14-8, 27pp.
da Costa, L.G., 2014. Selection of fungi with capacity of degradation in stumps of *Eucalyptus* spp. for use in biological stump removal. Federal University of Espírito Santo.
Dedecek, R.A., Gava, J.L., 2005. Influence of soil compaction on eucalyptus coppice productivity. Rev. Árvore 29, 383–390. http://dx.doi.org/10.1590/S0100-67622005000300005.
Dias, A.C., Arroja, L., 2012. Environmental impacts of eucalypt and maritime pine wood production in Portugal. J. Clean. Prod. 37, 368–376. http://dx.doi.org/10.1016/j.jclepro.2012.07.056.
El-Gammal, A.A., Nagleb, Z.A., Helmy, S.M., 1995. Ultrastructural aspects of lignocellulose degradation by some microorganisms. Polym. Degrad. Stab. 50, 199–202.
Ferraz, A., Guerra, A., Mendonça, R., Masarin, F., Vicentim, M.P., Aguiar, A., Pavan, P.C., 2008. Technological advances and mechanistic basis for fungal biopulping. Enzyme Microb. Technol. 43, 178–185. http://dx.doi.org/10.1016/j.enzmictec.2007.10.002.
Goldschimid, O., 1971. Ultraviolet spectra. In: Lignins: Occurrence, Formation, Structure and Reactions. J. Wiley, New York, pp. 241–298.
Gomide, J.L., Demuner, B.J., 1986. Determinação do teor de lignina em material lenhoso: método Klarson modificado. O Pap. 47, 36–38.
Hillis, W.E., 1971. Distribution, properties and formation of some wood extractives. Wood Sci. Technol. 5, 272–289. http://dx.doi.org/10.1007/BF00365060.
Indústria Brasileira de Árvores (IBÁ), 2016. Ibá report 2016. Base Year 2015.
Istek, A., Sivrikaya, H., Eroglu, H., Gulsoy, S.K., 2005. Biodegradation of *Abies bornmülleriana* (Mattf.) and *Fagus orientalis* (L.) by the white rot fungus *Phanerochaete chrysosporium*. Int. Biodeterior. Biodegrad. 55, 63–67. http://dx.doi.org/10.1016/j.ibiod.2004.07.002.
Istek, A., 2006. Effect of *Phanerochaete chrysosporium* white rot fungus on the chemical composition of *Populus tremula* L. Cellulose Chem. Technol. 40 (6), 475–478.
Jawjit, W., Kroeze, C., Soontaranun, W., Hordijk, L., 2007. Options to reduce the environmental impact by eucalyptus-based Kraft pulp industry in Thailand: model description. J. Clean. Prod. 15, 1827–1839. http://dx.doi.org/10.1016/j.jclepro.2006.10.003.
Malakani, M., et al., 2014. Influence of fungal decay on chemi-mechanical properties of beech wood (*Fagus orientalis*). Cellulose Chem. Technol. 48 (1–2), 97–103.
Moya, R., Berrocal, A., 2010. Wood colour variation in sapwood and heartwood of young trees of Tectona grandis and its relationship with plantation characteristics, site, and decay resistance. Ann. For. Sci. 67, 109–118. http://dx.doi.org/10.1051/forest/2009088.
Negrão, D.R., da Silva Júnior, T.A.F., Passos, J.R. de S., Sansígolo, C.A., Minhoni, M.T. de A., Furtado, E.L., 2014. Biodegradation of Eucalyptus urograndis wood by fungi. Int. Biodeterior. Biodegrad. 89, 95–102. http://dx.doi.org/10.1016/j.ibiod.2014.01.004.
Paes, J.B., Brocco, V.F., Moulin, J.C., Motta, J.P., Alves, R.C., 2015. Efeitos dos extrativos e da densidade na resistência natural de madeiras ao térmita *Nasutitermes corniger*. Cerne 21, 569–578. http://dx.doi.org/10.1590/01047760201521041849.
Reis, G.G. dos, Reis, M. das G.F., 1997. Fisiologia da brotação de eucalipto com ênfase nas suas relações hídricas. Piracicaba.
Ryss, A.Y., Kulinich, O.A., Sutherland, J.R., 2011. Pine wilt disease: a short review of worldwide research. For. Stud. China 13, 132–138. http://dx.doi.org/10.1007/s11632-011-0205-8.
Shrestha, P., Khanal, S.K., Pometto, A.L., van Leeuwen, J.H., 2009. Enzyme production by wood-rot and soft-rot fungi cultivated on corn fiber followed by simultaneous saccharification and fermentation. J. Agric. Food Chem. 57, 4156–4161. http://dx.doi.org/10.1021/jf900345n.
TAPPI, 1992. T 257 om-92: sampling and preparing wood for analysis, in: Test Methods 1992–1993. Tappi Technology Park, Atlanta.
TAPPI, 1996. T 264 om-88: preparation of wood for chemical analysis. In: Test Methods 1996–1997. Tappi Technology Park, Atlanta.
Schmidt, O., 2006. Wood and Tree Fungi: Biology, Damage, Protection, and Use. Springer-Verlag, Berlin doi: 10.1007/3-540-32139-X.
da Silva, G.A., Marino, R.H., Lopes, M.E.G., Almeida, T.Á., Costa, Â.C.F., Martins, M.V.G., 2010. Evaluating the degradation potential of white rot fungi. Rev. Bras. Ciências Agrárias 5, 225–231.
da Silva, L.F., Paes, J.B., de Jesus Junior, W.C., Oliveira, J.T. da S., Furtado, E.L., Alves, F.R., 2014. Deterioration of *Eucalyptus* spp. wood by xylophagous fungi. Cerne 20, 393–400. http://dx.doi.org/10.1590/01047760201420031462.
Teles, R.F., 2014. Nondestructive tests to evaluate the perfomance of treated amazonian woods. Universidade de Brasília.
Tian, B.-Y., Huang, Q.-G., Xu, Y., Wang, C.-X., Lv, R.-R., Huang, J.-Z., 2010. Microbial community structure and diversity in a native forest wood-decomposed hollow-stump ecosystem. World J. Microbiol. Biotechnol. 26, 233–240. http://dx.doi.org/10.1007/s11274-009-0165-5.
Zabel, R.A., Morrell, F.L., 1992. Wood Microbiology: Decay and Its Prevention. Academic Press, San Diego.