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IN VITRO WOOD DECAY OF TEAK (*TECTONA GRANDIS*) BY *RIGIDOPORUS* CFR. *MICROPORUS* (MERIPILIACEAE, POLYPORALES, BASIDIOMYCOTA)

PRUEBA IN VITRO DE LA PODREDUMBRE DE TECA (*TECTONA GRANDIS*) POR *RIGIDOPORUS* CFR. *MICROPORUS* (MERIPILIACEAE, POLYPORALES, BASIDIOMYCOTA)

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ABSTRACT

The use of exotic species like teak for industry demands has increased over the last decades in Central America, however its vulnerability to decay by saprophytic fungi has not been well studied. Among these fungi, *Rigidoporus* spp. have been described as white rotters of dead hardwoods and conifers worldwide. In Costa Rica, *R. microporus* has been found growing on teak stumps. The aim of this study was to determine the effects of this white rot fungus on the chemical, mechanical and physical properties of teak wood from trees of different ages. Six and ten year old sapwood and heartwood samples were used in the assays. Severe anatomical damage and the highest weight and resistance losses were observed on six year old sapwood samples. There was an increase in the quantity of soluble materials in 1% NaOH (relative values) and lignin content in all the samples analyzed, after three months exposure and up to the end of the experiment. Mass loss reduction and increased resistance of wood to compressive strength parallel to the grain were related to both the type of

wood and the age of the tree. Knowledge of the potential damage that this fungus can cause to teak wood might help in a better selection of wood and developing more effective protection measures against decay in the field or in construction wood.

Key words: decay, teak, fungus, *Rigidoporus* cfr. *microporus*, wood, white rot.

RESUMEN

El crecimiento lento y la escasez de especies nativas en Centro América, ha incrementado el uso de algunas especies exóticas, como teca y melina en las últimas décadas, dado su rápido crecimiento y la buena calidad de sus maderas. Un aspecto importante que no ha sido bien estudiado es la vulnerabilidad de estas maderas, en especial la de teca, al ataque de hongos causantes de podredumbre de la madera utilizada en construcción. Varias especies del género *Rigidoporus* han sido comunicadas como causantes de podredumbre blanca en angiospermas y gimnospermas a nivel mundial. En Costa Rica, *Rigidoporus microporus* ha sido reportada en tocones y troncos caídos de teca en plan-

taciones, pero no se han realizado estudios a nivel de laboratorio sobre los efectos que puede causar en las diferentes propiedades de la madera. Esta investigación se llevó a cabo para determinar los cambios que este hongo puede causar en las propiedades anatómicas, físicas, mecánicas y químicas de la madera de teca. Para determinar si existían diferencias en la severidad del ataque con respecto al estado de desarrollo del árbol, se utilizaron muestras de maderas provenientes de árboles con diferentes edades (albura y duramen de árboles de seis y 10 años). Se montaron 262 cámaras de podredumbre para realizar las diferentes pruebas y, se removieron muestras de madera de dichas cámaras cada mes durante seis meses para estudiar los cambios en las diferentes propiedades a través del tiempo. Los cambios más severos en la anatomía, y las pérdidas de peso y de resistencia más altas fueron obtenidos en las muestras de albura de seis años. Se observó un aumento en la cantidad de materiales solubles en 1% NaOH y en los contenidos de lignina en todas las muestras analizadas a partir del tercer mes y hasta el final de la prueba. La reducción en pérdida de masa y el aumento de la resistencia de la madera a la fuerza paralela de comprensión al grano estuvieron relacionadas tanto al tipo de madera como a la edad del árbol de donde esta procedía. Se considera que el conocimiento de los daños potenciales que puede causar este hongo, puede ayudar a realizar una mejor selección de la madera y a desarrollar medidas de protección más efectivas en el campo o a nivel de construcción.

Palabras clave: podredumbre, teca, hongos, *Rigidoporus* cfr. *microporus*, madera, podredumbre blanca.

INTRODUCTION

The reduction and slow growth of native species, especially in tropical areas, have led to increased use of fast-growing species in order to satisfy the demands of the construction industry and for reforestation purposes. Exotic species, like teak (*Tectona grandis*), gmelina (*Gmelina arborea*) and black wattle or mange (*Acacia* spp.) have been selected for their fast growth, wood quality and high yields in Central America (Fonseca 2004; De Camino & Morales, 2013).

In order to mitigate the problems brought on by deforestation in Costa Rica, the Government established the National Forestry Fund in 1980 and promoted plantations of the above-mentioned species. Teak was one of the main species introduced in Costa Rica, and by 2010 there were 9291.12 ha (22963.72 acres) of teak from a total of 28337.73 ha (70038.88 acres) of plantations (Sage & Quirós 2001; Arias *et al.*, 2005, Murillo & Guevara, 2013, de Camino & Morales, 2013) in the country.

The diseases that affect this exotic tree have been studied in Costa Rica (Arguedas *et al.* 2013); however, the effects of saprophytic fungi on construction wood have received little attention. The aim of this study was to determine the effects of a white rot fungus (*Rigidoporus* cfr. *microporus*) previously collected on teak on the chemical, mechanical and physical properties of wood from teak trees of different ages. Knowledge of the potential damage of teak wood by this fungus, which is commonly found on the field, could help to improve control strategies to preserve the wood from decay.

MATERIAL AND METHODS

Wood samples and fungal strain

The wood samples and the fruiting bodies of *Rigidoporus* cf. *microporus* were obtained from a teak plantation in Aguas Zarcas, San Carlos, Alajuela, Costa Rica (10°34'36'' N, 84°23'17'' y 84°21'38'' W). A dikaryotic strain of *R. cf. microporus* was isolated and maintained on 2% MEA. Mycelia discs were removed from the peripheral growth zone and used to inoculate the wood samples (blocks) within the decay chambers. Sapwood (S) and heartwood (H) blocks were used as wood sources as well as sawdust from 6 and 10 yr. old trees (age factor), all these materials were prepared by the company, following standard procedures (ASTM 2000a). All the experiments were carried out at the Forest Resources Laboratory, Institute of Engineering Research, and at the School of Biology, University of Costa Rica.

Assays

Decay chambers were prepared according to the ASTM D-2017-05 (ASTM 2005). Two hundred and sixty two decay chambers were prepared with 658 sterilized blocks (198 chambers with 3 blocks each for wood decay, and physical and chemical studies, and 64 with 1 block each for mechanical analysis). A set of uninoculated sterilized wood blocks were used as control. The chambers were incubated at 27°C in the dark and samples were removed monthly for the physical tests for a period of 6 months. Samples for chemical, mechanical and anatomical studies were removed after 3 and 6 months.

A total of 594 blocks were sampled for the decay, physical and chemical test. Before placing them into the decay chambers, their initial dry weight was determined. Three hundred sixty blocks were inoculated for physical analysis (2 wood sources x 2 age factors x 6 months x 15 inoculated blocks), and 176 blocks used as control. In all cases, the removed blocks were oven dried at 65 ± 1°C for a week until achieving constant weight. Wood biomass losses were determined on the basis of the initial and final dry weight.

Light and electron microscopy studies were carried out with 3 and 6 months decay wood blocks (2), following the methodology recommended by Carpio (1992) and Karnovsky (1965).

Wood density was obtained according to ASTM D-2395-02 (ASTM 2002). The mechanical test of maximum compressive strength parallel to the grain was carried out at the third and sixth month. A total of 64 blocks were used for this test (2 wood sources x 2 age factors x 2 sampling months x 8 inoculated blocks). The maximum stress sustained by the samples was determined following ASTM D-143-94 (ASTM 2000b).

Chemical quantification of wood components was performed in order to determine the fungal effect on the xylem cell walls (insoluble lignin on acid and 1% NaOH solubles). Chemical assays were carried out according to procedures of the Forest Resources Laboratory, University of Costa Rica (Blanco 1998). The tests were repeated 2 or 3 times with samples exposed to the fungus for 3 or 6 months (a total of 56 blocks). Control tests were carried out with sawdust from 6 and

10 yr. old trees. The Klason lignin chemical analysis and the 1% NaOH soluble analyses were conducted following LPF – QM – 04 y LPF – QM – 08 procedures of the Forest Resources Laboratory, University of Costa Rica (Blanco 1998).

Statistical analysis

Wood biomass loss was analyzed using the STATISTICA program (Anonymous 1995), and the Post-Hoc Comparison of Means test LSD (0.05 and 95% confidence levels) was used for comparisons among treatments and for statistical significance (Sokal & Rohlf 1981).

RESULTS

Wood decay assay (anatomical studies)

Rigidoporus cfr. *microporus* showed a rapid growth on MEA; hyphae with clamp connections were observed after a week and the mycelia covered the wood samples in 3-4 weeks. The infection process started on the multiseriate rays formed by parenchyma (fig. 1). After invading and degrading this tissue, hyphae penetrated the vessel elements through the bordered pits (fig. 2). During the first 3 months, a strong tissue disruption was observed as a loose arrangement of fibers, due to ray degradation and separation, but fibers did not show signs of deterioration (fig 3). After 6 months, severe damage was observed on 6 and 10 yr. old S blocks; 10 yr. old H samples were almost not degraded at all, but wall erosion furrows on the vessel elements were observed in all the samples (fig. 4).

Physical Assay

Wood characteristics

Six and ten yr. old samples (HS) exhibited a moisture content of 10.0 %, and an average density of 0.54 g/cm³ with minimal differences between type of wood and age [lowest (0.49 g/cm³) and highest (0.61 g/cm³) in 6 and 10 yr. old (H), respectively].

Weight Loss (on dry weight basis)

The greatest weight loss was obtained in 6 yr. old (S) and varied from 1.3 to 8.0%. Weight loss in 10 yr. old (S) and 6 yr. old (H) blocks was similar at the end of 6 months (5.8% and 6.0%, respectively). Minimal weight losses were obtained with 10 yr. old (H) (1.6% after 6 months of exposure). A comparison of mass weight losses in the different treatments after 3 and 6 months, revealed no statistically significant differences between 10 yr. old (S) and 6 yr. old (H) (tables 1-2).

Mechanical assay

Maximum compressive strength parallel to the grain test

Previous tests carried out on control samples (6 and 10 yr. S and H blocks) showed that the maximum resistance to compressive strength was in the range of 600-814 kg/cm².

This test was repeated with blocks exposed to fungal decay for 3 and 6 months. A loss of resistance was observed in all treatments over time due to the removal of the cell wall components. Greater losses were obtained

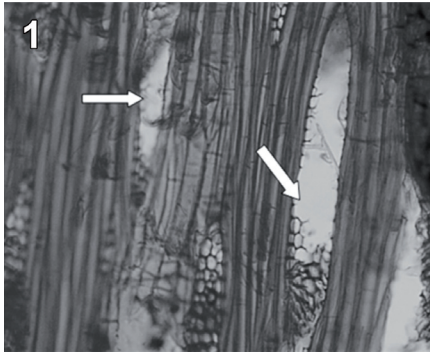


Fig.1. Tangential section (100 X) of teak wood block after 3 months of exposure. Arrows show degraded radial parenchyma.

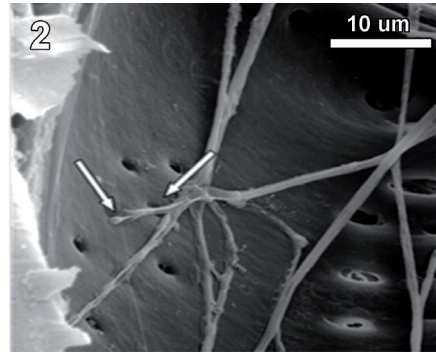


Fig. 2. Hyphae penetration through vessel element bordered pits (arrows).

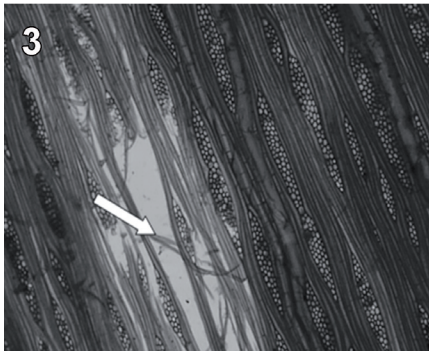


Fig. 3. Tangential section (40 X) of teak wood block after 3 months of exposure. Arrows show tissue disruption observed as a loose arrangement of fibers.

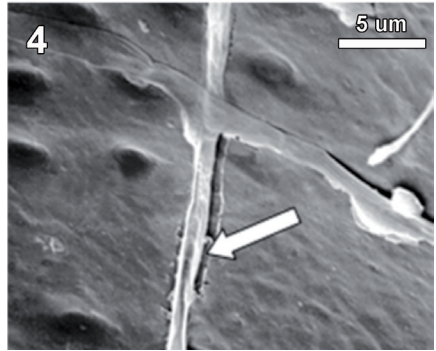


Fig. 4. A 6 yr. old heartwood vessel showing wall erosion furrow.

in 6 yr. old S with values ranging from 544 kg/cm² to 486 kg/cm² in 3 and 6 month exposure, respectively (control: 600 kg/cm²) (table 3).

Loss of resistance of the wood exposed to decay was statistically significant compared to control samples at 3 and 6 month exposure.

Stress on 10 yr. old S varied from 752 kg/cm² to 692 kg/cm² in 3 and 6 month exposure respectively (control: 760 kg/cm²). Loss of resistance compared to control blocks was statistically significant after a sixth month decay (table 4).

Six yr. old H blocks showed changes related to the applied stress that varied between 553

Table 1. Media comparison (DMS) of mass weight losses (%) of the different treatments after 3 months exposure. S-6 [sapwood 6 yr. old], H-6 [heartwood 6 yr. old], S-10 [sapwood 10 yr. old], H-10 [heartwood 10 yr. old]

Treatment	S-6	H-6	S-10	H-10
Mean	6.314	3.918	4.799	1.435
S-6		0.0001*	0.031*	0*
H-6	0.001*		0.194	0.001*
S-10	0.031*	0.194		0.00001*
H-10	0*	0.001*	0.00001*	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

Table 2. Media comparison (DMS) o mass weight losses (%) of the different treatments after 6 months exposure. S-6 [sapwood 6 yr. old], H-6 [heartwood 6 yr. old], S-10 [sapwood 10 yr. old], H-10 [heartwood 10 yr. old].

Treatment	S-6	H-6	S-10	H-10
Mean	7.962	5.992	5.768	1.621
S-6		0.0001*	0.00001*	0*
H-6	0.0008*		0.631	0*
S-10	0.00001*	0.631		0*
H-10	0*	0*	0*	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

Table 3. Compressive strength parallel to grain (kg/cm²) of 6 yr. old sapwood exposed to *Rigidoporus cf. microporus*: control (S-6-0), 3 months (S-6-3), 6 months (S-6-6).

Treatment	S-6-0	S-6-3	S-6-6
Mean	599.691	543.611	486.52
S-6-0		0.0301*	2.2122 x 10 ⁻⁵ *
S-6-3	0.0301*		0.049*
S-6-6	2.2122 x 10 ⁻⁵ *	0.049*	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

Table 4. Compressive strength parallel to grain (kg/cm²) of 10 yr. old sapwood exposed to *Rigidoporus* cfr. *microporus*: control (S-10-0), 3 months (S-10-3), and 6 months (S-10-6).

Treatment	S-10-0	S-10-3	S-10-6
Mean	760.528	751.619	692.104
S-10-0		0.761	0.011*
S-10-3	0.761		0.088
S-10-6	0.011*	0.088	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

kg/cm² and 557 kg/cm² at 3 and 6 month exposures (control: 624kg/cm²). Loss of resistance was statistically significant at the sixth month of exposure (table 5).

Variation in the applied stress on 10 yr. old H ranged from 749 kg/cm² to 750 kg/cm² at 3 and 6 months of exposure, respectively (control: 814 kg/cm²). Variations were not statistically significant compared to control samples (table 6).

Chemical assay

There was an increase in the quantity of soluble materials in 1% NaOH (relative values) and lignin content in all the samples analyzed at the end of the 3-month experiment, which continued increasing until the end of the 6 month period (table 7).

DISCUSSION

The genus *Rigidoporus* is characterized by its monomitic hyphal system and simple septate generative hyphae (Gilbertson & Ryvarden 1987); nevertheless, *Rigidoporus* cfr. *microporus* developed clamp connections in culture, a peculiar character

that also has been reported in *Rigidoporus lineatus* (Bakshi *et al.*, 1963) but is not found on fruiting bodies.

The parenchyma tissue was first invaded during the initial stages of decay where the fungus utilized the starch and probably other components that can be degraded easily and provide the necessary energy for its establishment. The strong degradation of those cells but not of fibers observed in the early stages of decay has also been mentioned by Schwarze *et al.* (2000) and Fernandes *et al.* (2005). At the end of the experiment, some of the blocks showed the typical white-rot appearance described by other authors (Gilbertson & Ryvarden 1987, Ramsden *et al.*, 2002; Schmidt, 2006; Schwarze *et al.*, 2000).

Teak has been considered to be a very resistant wood to pathogens in plantations, natural forests or during storage (Bhat & Florence, 2003; Bhat *et al.*, 2005, Kokutse *et al.*, 2006; Thulasidas & Bhat, 2007). This resistance has been attributable to the high percentage of extract contents (organic compounds), especially that found in old heartwood walls (for example, naphthoqui-

Table 5. Compressive strength parallel to grain (kg/cm²) of 6 yr. old heartwood exposed to *Rigidoporus* cfr. *microporus*: control (H-6-0), 3 months (H-6-3), 6 months (H-6-6).

Treatment	H-6-0	H-6-3	H-6-6
Mean	624.333	553.304	557.462
H-6-0		0.084	0.048*
H-6-3	0.084		0.925
H-6-6	0.048*	0.925	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

Table 6. Compressive strength parallel to grain (kg/cm²) of 10 yr. old heartwood exposed to *Rigidoporus* cfr. *microporus*: control (H-10-0), 3 months (H-10-3), and 6 months (H-10-6).

Treatment	H-10-0	H-10-3	H-10-6
Mean	814.311	748.754	750.417
H-10-0		0.094	0.058
H-10-3	0.094		0.970
H-10-6	0.058	0.970	

*Significant difference between treatments (STATISTICA Program, Pos-Hoc Comparison of Means test LSD, 0.05 and 95% confidence levels).

Table 7. Percentages of solubles in 1% NaOH and lignin content in the different treatments (3 and 6 month exposures).

Wood	Soluble in 1% NaOH (%)	Soluble in 1% NaOH (%)	Differences (% relative values)	Lignin (%)	Lignin (%)	Differences (%)
Type/Age	3 months	6 months		3 months	6 months	
6 yr. sapwood	9.71	10.99	13.18	28.75	33.45	4.7
10 yr. sapwood	9.92	10.90	9.87	27.82	31.33	3.51
6 yr. heartwood	10.67	11.72	9.84	26.71	28.13	1.42
10 yr. heartwood	13.41	13.84	3.20	25.40	29.00	3.6

none and tectoquinone) (Chaves & Fonseca 1991; Fonseca, 2004; Rivero & Moya, 2006; Thulasidas & Bhat, 2007; Rowell, 2013).

Rivero and Moya (2006) and Thulasidas and Bhat (2007) mentioned that 6 and 8 yr. old teak showed less resistance to fungal or insect attacks due to reductions of organic compounds as compared with older trees. Kokutse *et al.* (2006) obtained < 20% mass loss in heartwood samples exposed to *Antrodia* sp. and *T. versicolor* and < 7% when exposed to *Pycnoporus sanguineus* and *Gloeophyllum trabeum*. Another study by Moya *et al.* (2009) reported weight losses on heartwood of < 11% in decay tests with *Trametes versicolor* and *Pycnoporus sanguineus*; however, weight losses in sapwood were up to 50% with *T. versicolor* and $\leq 35\%$ with *Pycnoporus sanguineus*.

It is worth mentioning that none of the fungi used in those previous decay studies has been isolated from teak wood. Our study is the first one that utilized a fungus that grows on teak, and the overall small wood biomass lost (1.3-8%) confirmed the natural resistance of teak to fungal degradation.

Donoso and Veloso (1968) pointed out that there is a tendency in high-density woods to initially resist fungal infection, but during the decay process, the resistance decreases or disappears. In our study, the lack of a strong variation in wood density in the different treatments (control and decay samples) made it impossible to relate this factor to the resistance or susceptibility of teak wood to decay.

The results obtained from the compressive strength parallel to grain test and weight losses showed that age was an important

factor determining resistance to compression and decay, probably due to the increase in lignified walls as mentioned by Rivero & Moya (2006). The loss of resistance over time was due to the removal of the cell wall components by white rot fungi, as pointed out by Schmidt (2006).

Variations in chemical contents revealed that the fungus has a preference for low-weight molecular carbohydrates, since the solubles in 1% NaOH increased in all the treatments. This suggests that low-weight molecular carbohydrates in wood increased due to fragmentation of polymer chains in wood walls (cellulose and hemicellulose). The increase in lignin content was related to modifications of the chemical structure of the wood. This could be interpreted as an increase in cellulose and hemicellulose degradation that caused a numerical increase in lignin content (Schmidt, 2006).

CONCLUSIONS

Rigidoporus cfr. *microporus* is a potential wood rotter of teak wood, and can cause undesirable changes in the chemical, mechanical and physical properties of teak wood. The decay risk is related to the type of wood and the age of the tree.

Silvicultural practices recommend that the first thinning in teak plantations should be done after four years (Fonseca, 2004). According to our results, the wood obtained from these first thinnings will be more vulnerable to decay, especially the sapwood. Therefore, it is highly recommended to protect it to prevent decay. Wood from 10 yr. harvest trees or older ones will be less vulnerable to decay and therefore more desirable for construction purposes.

Knowledge of the potential damages that this fungus can cause to teak wood might contribute to a better selection of wood and to the development of more effective protection measures against decay in the field or in construction wood.

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