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INFLUENCES OF ETHYLENE STIMULATION OF RUBBER TREES *(Hevea brasilliensis)* ON THE EXTRACTIVES AND FUNGAL RESISTANCE OF LUMBER

Keywords:
Ethylene
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Fungi
Resistance
Rubberwood

ABSTRACT: Ethylene stimulation increases the rubber latex yield of live rubberwood (*Hevea brasiliensis*). Lumber samples from ethylene treated rubberwood (TRW) and from untreated rubberwood (URW) were compared mainly for their resistance to fungi, differences in the chemical composition between TRW and URW, and the antifungal activities of their aqueous extracts. The TRW had significantly higher lignin and extractives contents than the URW, but the TRW had comparatively poor resistance to fungal rotting. The white rot fungus *Ganoderma lucidum* and the brown rot fungus *Gloeophyllum striantum* caused in vitro significantly higher mass loss in TRW than in URW. This might be related to the phenolic compounds 2,4-ditert-butylphenol and 4-hydroxy-3,5-dimethoxy-benzaldehyde. The aqueous wood extracts strongly inhibited growth of G. lucidum, with lesser effects on the other fungi tested. Caffeine was detected in the TRW, but not the URW. However, the caffeine degraded so quickly that it had no effect on the 6 and 12 weeks fungal resistances of wood samples.

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INFLUÊNCIA DA ESTIMULAÇÃO COM ETILENO EM SERINGUEIRAS (HEVEA BRASILLIENSIS) SOBRE OS EXTRATIVOS E RESISTÊNCIA DA MADEIRA SERRADA

Palavras chave:
Etileno
Extrativos
Fungos
Resistência
Madeira de seringueira

RESUMO: Estimulação por etileno aumenta a produção de látex em seringueiras (*Hevea brasiliensis*). Amostras de madeira serradas de seringueiras tratadas com etileno (TRW) e de madeira não tratada (URW) foram comparadas quanto sua resistência a fungos, diferenças na composição química entre TRW e URW, e quanto as atividades antifúngicas dos seus extratos aquosos. A TRW tinha significativamente maior conteúdo de lignina e extrativos do que o URW, mas o TRW tinha comparativamente baixa resistência a fungos degradadores. O fungo causador da podridão branca *Ganoderma lucidum* e da podridão parda *Gloeophyllum striantum* causaram *in-vitro* maior perda de massa em TRW do que em URW. Isto pode estar relacionado aos compostos fenólicos 2,4-ditert-Butilfenol e 4-hidroxi-3,5-dimetoxi-benzaldeído. Os extratos aquosos da madeira inibiram fortemente o crescimento de *G. lucidum*, com menores efeitos sobre os outros fungos testados. Cafeína foi detectada em TRW, mas não em URW. No entanto, a cafeína degradou tão rapidamente que não teve efeito nas 6 e 12 semanas de testes de resistências fúngicas nas amostras de madeira.

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INTRODUCTION

Rubber trees (*Hevea brasiliensis* Muell. Arg) are an important industrial plant that yields natural rubber latex, which is widely used in a variety of products. The tree grows fast and sustains latex and rubberwood lumber based industries. Latex is secreted by specific latex cells that synthesize it in the cytoplasm, and the latex bleeds out when the tree bark is tapped (AN et al., 2014; TUNGNGOEN et al., 2011). Modern rubber plantation farmers stimulate the rubber trees with ethylene gas, which increases the latex yield of trees 10 years or older (LACOTE et al., 2010). At around 25-30 years of age the trees start having poor yields, so they are felled and new ones are planted. While ethylene stimulation increases the latex yield, it also affects the physical, chemical, and mechanical properties of rubberwood, and thereby the value of the lumber (TOEH et al., 2014). The stimulation used required boring holes into the wood, which generated injures and also affected the color of lumber, decreasing its value. These problems were solved with a new generation of ethylene treatment techniques without the need for holes in the wood. Nowadays the stimulating ethylene passes through the wood bark, possible with holes drilled only through the bark layer, and it is supplied from a bag or a tank. Cherdchim and Sudchada (2014) report significantly elevated moisture content in fresh rubberwood exposed to ethylene treatments relative to untreated wood, and also the water permeability was fivefold elevated. Ethylene treatment of live wood also increases water absorption of the lumber, and the penetration rate of boron compounds into treated wood is elevated (YAOWALERD; YINGPRASERT, 2013). Prior reports from our research group show increased penetration rate of urea formaldehyde (UF), and improved bond ability of ethylene treated wood (KHONGAO; PHETARWUT, 2013). A study of physical properties performed at the Suratthani Rubber Research Center (Thailand) showed no significant treatment effects on moisture content or shrinkage swelling, but did not assess effects on chemical properties (SANGSING et al., 2009). Ethylene treatment of *Populus* alba L. induces the cambium to produce more parenchyma, and shorter fibers and vessel elements than in control (JUNGHANS et al., 2004). Ethylene treated *P*. alba showed abnormal growth and stem anatomy, evident in the dimensions of xylem cells and the tissue patterns. Treatment increased the size and number of ray cells (JUNGHANS et al., 2004, LITTLE; EKLUND, 1999). The effects of ethylene treatment of live trees on the chemical properties of wood lumber remain unknown, and the current research aims to address this gap in knowledge.

Hevea brasiliensis wood is highly attractive in the tropical areas, such as Africa and South America, and in Southeast Asia it is particularly cultivated in Thailand, Indonesia and Malaysia. This fast growing plantation tree provides high potential for a sustainable wood products industry. The effects of ethylene stimulating rubber trees, in terms of impacts on wood lumber for indoor and outdoor uses, need to be well understood. Currently poorly understood or known effects include those on the chemical composition, as well as those on the rotting fungal resistance of wood: both are of importance to the wood products industry. In this work, the norm EN 113 and the TAPPI standards were applied to assess the fungal resistance and the chemical composition effects, respectively.

The study is relevant to both rubber plantation farmers and the industries that use rubberwood. The farmers need to balance between the yield from live trees and the value of lumber, while the wood product industry needs to appreciate the effects of ethylene treatment on chemical composition and fungal resistance aspects of wood quality.

MATERIALS AND METHODS

Wood material and its chemical composition

The 20-25 years old *Hevea* trees sampled were PRIM 600 strain, and the ethylene gas stimulation had lasted for 6 years in the case of ethylene treated rubberwood (TRW). Fresh *H. brasiliensis* wood specimens were collected at Tumbon Chaibury, Amphor Chaibury, Suratthani province, Thailand. The extractives and lignin contents were measured following TAPPI T204 om-88 and TAPPI T222 om-02, respectively. The cellulose content was determined with an anthrone assay (MORRIS, 1948), and the hemicellulose content with an orcinon assay (MEJBAUM, 1939). Total hexose and total pentose were spectrophotometrically determined at 620 nm and 665 nm, respectively.

Fungal strains

All fungal strains used in this study were obtained from the collection of the Royal Forest Department, Bangkok, Thailand. Solid culture gel media (2% malt extract and 1% agar) was placed in the middle of each Petri dish with one mycelium-overgrown agar plug per plate, of either one of the white-rot fungi *Ganoderma lucidum* and *Schizophyllum commune*, or one of the

brown-rot fungi *Gloeophyllum sepiarium* and *Gloeophyllum striantum*. The inoculated plates were sealed with Parafilm (Laboratory Film, Chicago, USA) and grown for 7 days at 25oC, for use in the EN 113 (1996) wood duration test (BRAVERY, 1978).

Wood block decay test

A mini block duration test of *H. brasiliensis* solid wood was carried out according to the European standard EN 113 (1996). The wood blocks were separately obtained from the border in between the sapwood and the heartwood: the wood was cut into small blocks of 30 × 10 × 5 mm (longitudinal × tangential × radial). The aqueous extractions from such wood blocks were by Soxhlet with distilled water for 6 hours, following TAPPI Test Method T 204 om-88 (1998). In the EN 113 duration test, each type of wood sample (TRW or URW) and each fungus were tested in a full factorial design. For one such test, six wood blocks were laid on a plastic net holder on fungal mycelium, grown on malt extract medium. Controls without fungi on the malt extract agar were treated analogously. The samples were exposed to the fungi for 6 weeks or 12 weeks, until the total loss of wood mass was gravimetrically determined as the difference of the initial dry mass and the final dry mass. The results are expressed as relative mass loss %.

Fungal growth inhibition by the wood extracts

Antibiotic activities of the wood extracts (150 μl wood extract mixed with 1 ml dimethyl sulfoxide DMSO per 1 g dried wood) were tested in 90 mm diameter Petri plates containing 20 ml solid growth medium (2% malt extract and 1% agar), against spore suspensions of *G. lucidum*, *S. commune*, *G. sepiarium* and *G. striantum*. Spores and mycelial debris were collected from each fungal culture grown for 7 days on 20 ml similar growth medium, by scraping them off from the aerial mycelium with a spatula and collecting into 10 ml of sterile H2O. Spores (oidia) or small mycelial fragments were separated from larger mycelial fragments by filtration through sterile glass wool in a bell-shaped funnel. The number of oidia and the number of small hyphal fragments after filtration in solution was then determined with a hematocytometer (type Thoma-chamber: 0.05 mm depth and 0.0025 mm^2 per each small square of the hematocytometer counting area). Spore and hyphal suspensions were adjusted to a concentration of 10^4 spores or mycelial pieces/ml solution. Of each fungal cell suspension 0.5 ml was evenly spread in a Petri dish over the entire growth medium, and a 5 mm diameter hole was made with a cork borer in the center of the medium. Then 75 μl of a wood extract in DMSO was added into each such hole, and when the medium had completely absorbed the extract, another 75 μl was added. The controls were similar agar plates with plated fungal cells and 150 μl DMSO added without wood extract. Three replicate plates were used for each experimental condition. The plates were cultured at 25oC, and the diameters of inhibition zones without any fungal growth around the holes were measured after 5 days of incubation.

Wood extract characterization

Wood particles of *H. brasiliensis* were prepared with wood grinder (Polymix PX-MFC 90 D, Kinematica AG, Switzerland) and sieving the wood particles following the methods presented in Müller et al. (2009). Wood particles (10 g) were extracted in a Soxhlet apparatus with 450 ml boiling water for 6 hours, following TAPPI Test Method T 204 om-88 (1988). Each extract was dissolved in DMSO, with 1 ml DMSO per 1 g extracted wood. One half (5 ml) of the DMSO solution with extracts was used in separate 1.5 ml portions for extraction in chloroform with a separating funnel that contained 50 ml water, 50 ml chloroform and, as a catalyst, 0.5 ml phosphoric acid. After short mixing, the pH of the water phase was measured and the pH adjusted to about 2 with HCl. Then, the mixture was shaken for 10-15 min prior to finally separating the chloroform and water phases. Chloroform extraction was once repeated with 20 ml fresh chloroform. The chloroform fraction was concentrated and evaporated to dryness in a rotatory evaporator at 40°C. The wood extractives from chloroform were dissolved in DMSO with 1 ml DMSO per 1 g extracted wood. These extracts were analyzed by GC-MS (Gas Chromatography Mass Spectrometry, Trace GC Ultra/ISQ MS, Thermo Scientific Inc., USA) to determine the chemical constituents.

Statistical analyses

The mass loss results were subjected to analysis of variance (ANOVA) using Fisher's least significant difference (LSD) and Duncan's test for multiple comparisons (SPSS 8.0 for Windows; USA). The mass losses within one experiment duration were contrasted between each fungal strain and the control treatment without fungus.

RESULTS AND DISCUSSION

The chemical composition of *H. brasiliensis*, in relation to wood degradation by fungi

The chemical composition of *H. brasiliensis* in terms of cellulose, hemicellulose, lignin and extractives is shown in Table 1, with (TRW) and without (URW) ethylene gas treatment of the live tree.

TABLE 1 Chemical compositions of ethylene treated rubber wood (TRW) and untreated rubber wood (URW). Different superscripts within a single column indicate statistically significant ($p<0.05$) differences based on analysis of variance (ANOVA).

Wood	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Extractive content (%)
TRW	42ab	17ab	20a	21a
URW	45a	30a	12b	13b

TRW had significantly higher lignin and extractives contents than URW, while no significant difference was observed in the cellulose and hemicelluloses contents. The results from Little and Savidge (1987) were supported by our experiment, that ethylene-generating compounds affected cambium and promote the formation of wood on high lignin and extractive content.

Fungal growth effectively degrades dead wood in the nature, especially by consuming the cell wall components such as cellulose, hemicellulose, and lignin. The primary organisms that decompose wood in the forest (and also lumber wood, restricting its service life) are basidiomycete decay fungi. Naturally, some wood species are more durable because of substances toxic to fungi and insects, such as extractives and lignin. Lignin and extractives might both play roles as wood chemicals conferring resistance to fungi, or contrariwise, they might induce fungal enzymes to degrade wood and encourage wood degradation (SJOESTROM, 1993; CHERCCHIM, 2010; EATON; HALE, 1993; JAYASHREE et al., 2011).

Activity of URW and TRW wood extracts against the fungal decay of *H. brasiliensis* wood

To test the influences of wood extractives on select wood decaying fungi, 30 x 10 x 5 mm (longitudinal x tangential x radial) mini wood blocks of *H. brasiliensis*, after aqueous extraction, were used in EN 113 tests, determining the wood mass losses separately caused by the basidiomycete white rot fungi, *G. lucidum*or *S. commune*,or the brown rot fungi *G. sepiarium*or *G. striantum* . Statistically significant effects of extractives on

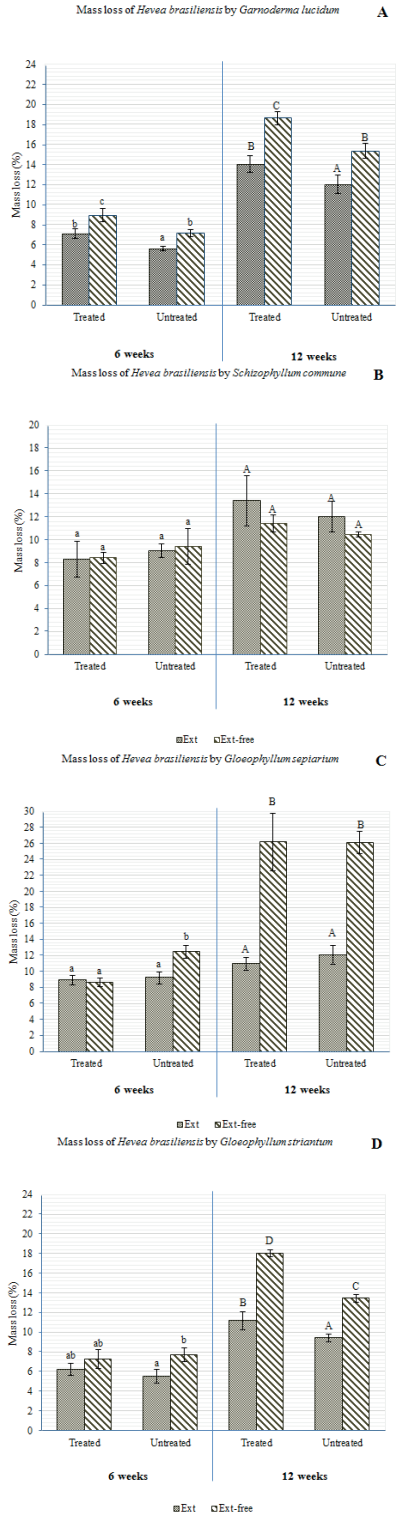


FIGURE 1 Total mass loss of *H. brasiliensis* wood blocks at 6 weeks and at 12 weeks of incubation, with A) *G. lucidum*, B) *S. commune*, C) G. *sepiarium*, and D) *G. striantum*. Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW. Wood post extraction is labeled Ext-free, and non-extracted wood is labeled Ext. Different superscripts on the chart indicate statistically significant ($p<0.05$) differences according to analysis of variance (ANOVA).

the loss of wood were seen with *G. lucidum*, *G. sepiarium*and *G. striantum* , but not with *S. commune* (Figure 1).

The extracted *H. brasiliensis* samples had lost resistance to fungal decay (Figure 1), especially against the brown rot fungi *G. sepiarium* and *G. striantum* (Figure 1C, 1D), and the white rot fungus *G. lucidum* (Figure 1A), but not against the white rot fungus *S. commune* (Figure 1B). Of the four fungal species, *G. sepiarium* incurred the most decay of *H. brasiliensis* wood at 12 weeks of incubation. Water-extracted wood showed in 12 weeks of incubation with *G. sepiarium* 26.16±3.58% (TRW) and 26.09±1.36% (URW) of mass loss, compared to only 10.92±0.84% (TRW) and 12.05±1.18% (URW) of mass loss in non-extracted samples incubated with this fungus. *S. commune* showed no significant differences in mass loss between the four sample types tested: in all cases, the mass loss of *H. brasiliensis* wood at 12 weeks of incubation was around 12% (Figure 1B).

These wood decay tests indicate that the extractives hindered fungal decay of *H. brasiliensis* wood, so the water extracts were tested for in vitro activities against the fungi *G. lucidum*, *S. commune*, *G. sepiarium* and *G. striantum* growing on 2% malt extract plus 1% agar plates (Figure 2). Among these cases, the mycelial growth of *G. lucidum* was the most inhibited by the aqueous wood extracts, and its growth inhibition zone had about 34 mm diameter on day 5. The least sensitive fungi were *S. commune* and *G. striantum*. The growth inhibition zone of *G. sepiarium* on day 5 of incubation was about 12 mm in diameter (Table 2) showing middling sensitivity to the extracts (between *S. commune* and *G. striantum*, and the most sensitive *G. lucidum*). The sensitivity to wood extracts correlated across the fungi with their ability to decay *H. brasiliensis* wood shown in Figure 1. Figure 2 illustrates the growth inhibition of the white rot fungus

TABLE 2 Growth inhibition by diffusion of wood extracts into a gel growth medium (2% malt extract + 1% agar) on which fungi were grown for 5 days at 25°C (*G. lucidum, S. commune, G. sepiariumand G. striantum*).

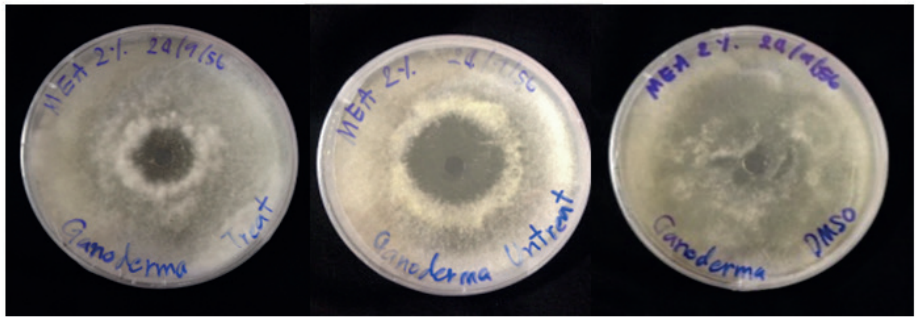
Fungus	Growth inhibition zone (mm in diameter) [a]		
	TRW	URW	DMSO control [b]
G. lucidum	24.00±2.94	34.33±0.94	0.00±0.00
S. commune	0.00±0.00	0.00±0.00	0.00±0.00
G.sepiarium	12.33±1.25	12.00±0.82	0.00±0.00
G. striantum	0.00±0.00	0.00±0.00	0.00±0.00

[a]Data are means of three replicates, and standard deviations are presented [b] Wood extracts were added into punched holes within agar plates in a solution of in total 150 µl DMSO, while the controls were similarly treated but with 150 µl plain DMSO.

G. lucidum by the wood extracts. A statistically significant difference was observed between extracts from URW and TRW, while the control treatments showed no inhibition zone (Table 2).

Lignin is built up of phenyl-propane units that are major building block of wood extractives. Both the lignin and the extractives protect a live tree against microbiological damage or insect attacks (SJOESTROM, 1993; HAYGREEN; BOWYER, 1994). Functionally, brown rot fungi selectively degrade cellulose and hemicellulose, mostly leaving alone the lignin. White rot fungi mainly degrade lignin and somewhat affect cellulose and hemicellulose. These fungi secrete enzymes to destroy the wood, and the enzyme secretion by the fungi might be induced by specific phenolic compounds in the extractives and lignin of wood (CHERDCHIM, 2010; DORADO et al., 2001; DE SOUZA et al., 2005; THURSTON, 1994).

In our experiments, the white rot fungus *G. lucidum* and the brown rot fungus *G. striantum* (Figures 1A and 1D) caused significantly higher mass losses to TRW than to URW wood. On the other hand, Table 1 shows significantly higher lignin and extractive contents in TRW than in URW and



TRW URW Control

FIGURE 2 Growth inhibition of a fungus by H. brasiliensis wood extracts. The G. lusidum fungus was incubated for 5 days at 25oC on gel plates (2% malt extract + 1% agar) to which 150 µl wood extract in DMSO was added into a hole in the middle. The aqueous extract from 0.15 g of H. brasiliensis wood was used per plate.

these contents should improve the wood resistance against fungi. However, both lignin and extractives might also induce the fungi to secrete enzymes that degrade wood. Overall, these functions might not be dominant in determining fungal resistance, which for example correlates with water absorption. Restricted water absorption also restricts the growth of biological agents, and thereby contributes to biological resistance (ROWELL, 1983). Prior results (CHERDCHIM; SUDCHADA, 2014) suggest that ethylene stimulation increases the water permeability and absorbance of rubber wood, and also increases the areal number density of pits and the average vessel diameter. Aside from effects on the transport and absorbance of water in wood, the porosity (or bulk density) is likely affected**.** The increased pit density and enlarged vessel diameter in TRW may facilitate penetration by fungal mycelia, contributing to wood degradation.

GC-MS identification of compounds in *H. brasiliensis* extracts

The aqueous extracts in chloroform-purified form were subjected to a GC-MS analysis, in order to identify individual compounds (Figure 3).

In the analysis by GC-MS, in total 11 compounds were found in chloroform-purified aqueous extracts (with identification confidence better than 95%).

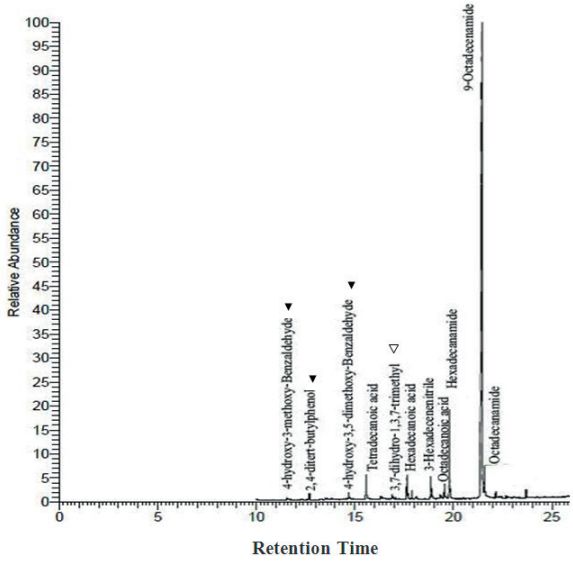


FIGURE 3 GC-MS analysis of the chloroform fraction of aqueous extracts from TRW H. brasiliensis. The filled arrows indicate those compounds common with the chloroform fraction of extracts from URW, while the open arrows indicate those compounds only found in TRW extracts that are expected to inhibit fungal growth. (see TABLE 3).

Between the extracts from URW and TRW, 10 compounds were shared while one compound was only found in TRW, namely caffeine: 3,7-dihydro-1,3,7-trimethyl (compound 5 in Table 3).

Table 3 shows phenolic compounds and caffeine, which are of interest for inhibiting fungal growth. On comparing extracts from URW and TWR, striking differences are observed for 2,4-ditert-butylphenol

TABLE 3 GC-MS analysis results for aqueous extracts from ethylene treated (TRW) and untreated rubber wood (URW).

No.	Compound*	RT**	Area %	
			TRW	URW
1	4-hydroxy-3-methoxy-benzaldehyde (Vanillin)	11.54	***	***
2	2,4-ditert-butylphenol (1,1-dimethylethy)	12.68	***	0.60
3	4-hydroxy-3,5-dimethoxy-benzaldehyde (Syringaldehyde)	14.68	0.85	0.91
4	Tetradecanoic acid (Myristic acid)	15.57	2.39	2.57
5	3,7-dihydro-1,3,7-trimethyl (Caffeine)	16.88	0.82	-
6	Hexadecanoic acid (Palmitic acid)	17.64	1.85	2.41
7	3-Hexadecenenitrile (3E)	18.86	3.02	1.73
8	Octadecanoic acid (Stearic acid)	19.54	1.69	1.81
9	Hexadecanamide (Amide 16)	19.80	6.91	7.54
10	9-Octadecenamide	21.44	76.98	75.97
11	Octadecanamide (Stearamide)	21.58	2.35	2.55

*The identity of these compounds was confirmed and the amount of compound present was determined by analyzing in the same set of experiments standards of compounds in different known concentrations.
** RT: retention time.
*** Detected but not quantitatively

(minor amount found in TRW, but 0.60 area% in URW extracts), 4-hydroxy-3,5-dimethoxy-benzaldehyde (higher in URW than in TRW), and the 3,7-dihydro-1,3,7-trimethyl (Caffeine) that was only found in TRW.

Various phenolic compounds in wood extracts have been reported to inhibit fungi (CHERDCHIM, 2010; MARTÍNEZ-IÑIGO et al., 1999; LEONOWICZ et al., 2001), such as 3-Methoxy-4-hydroxybenzoic acid and 4-Hydroxy cinnamic acid, active against the white-rot *Pleurotuso streatus* and *Trametes versicolor* (CHERDCHIM, 2010). Here, we demonstrated clear negative effects of *H. brasiliensis* extracts on fungal growth (Figures 1 and 2). The phenolic compounds 2,4-ditert-butylphenol and 4-hydroxy-3,5-dimethoxy-benzaldehyde (Syringaldehyde) were found in higher amounts from URW than from TRW.

2,4-ditert-butylphenol has been found effective against an agriculturally important fungus, namely *Fusariumo xysporum*, in inhibiting spore germination and hyphal growth (DHARNI et al., 2014). 2,4-ditert-butylphenol is also active against the growth and aflatoxin production of *Aspergillus flavus* TISTR304 and *Aspergillus parasiticus* TISTR3276 (SANGMANEE; HONGPATTARKERE, 2014). The phenolic compound syringaldehyde enhanced decolorization of malachite green as a highly toxic dyne that inhibits the growth of bacteria and fungi, and reduced growth inhibition has been observed in syringaldehyde-treated samples (MURUGESAN et al., 2009). Syrunggaldehyde also exhibited antifungal activity against *Leucoagaricus gongylophorus* fungus (DE SOUZA et al., 2005). Thus ethylene stimulated *H. brasiliensis* wood lost the antifungal compounds 2,4-ditert-butylphenol and 4-hydroxy-3,5-dimethoxy-benzaldehyde and resistance against some fungi (Figure 1; *G. sepiarium*, *G. striantum*and and *G. lucidum*). Moreover, the antifungal activity of caffeine (3,7-dihydro-1,3,7-trimethyl) has been demonstrated by several authors. According to Miyashira et al. (2011), caffeine inhibits the growth of mutualistic fungus *Atta sexdensrubropilosa*. Ravi et al. (1980) demonstrated activity of caffeine beyond the minimum concentration 1500 ppm. Arora and Ohla (1997) reported that 0.5% caffeine solutions completely inhibit the growth of ten species of wood rotting fungi. Likely caffeine has a wide spectrum antifungal effect, but it can be quickly degraded by fungi in a few days. Nayak et al. (2013) studied the caffeine-degrading abilities of various fungi; *Chrysosporium keratinophilum*, *Gliocladium roseum*, *Fusarium solani*, and *Aspergillus restrictus*. *G. roseum* (followed by *A. restrictus)* showed maximum degradation of caffeine at 0.47 (0.3) mg·ml^{-1}, over 96h in a nitrogen-containing minimal medium. We observed caffeine in extracts from TRW (0.82 area%, Table 3), but not from URW: the ethylene stimulation of rubber wood induced caffeine synthesis. This content of caffeine might inhibit fungal growth, but we incubated wood with fungi for 6 or 12 weeks. The caffeine may have been eliminated during the initial few days of incubation, so its antifungal effects would not show in the results of Figure 1. In conclusion, the aqueous wood extracts certainly contain compounds active against the growth of fungi. However, no single dominant factor that would determine the fungal resistance of rubber wood emerged from this study. It is even likely that some factors contributing to resistance at one point of time promote degradation in other conditions. Furthermore, the potential collaborative functions of various fungi (or their mutual antagonism) would require extensive experimental designs not included in the current study.

CONCLUSIONS

The ethylene stimulation of rubber trees is a common technique to increase the natural rubber latex yields from live trees. However, that stimulation affects rubber wood lumber in various ways, some of which we studied experimentally. The stimulation induced significantly higher lignin and extractives contents in lumber wood (TRW), relative to rubber wood without ethylene treatment (URW). The stimulation also caused a loss in resistance to fungal rotting, and aqueous extraction caused a further loss of resistance. The white rot fungus *G. lucidum* and the brown rot fungus *G. striantum* caused significantly higher mass losses in TRW than in URW, which may be related to the contents of phenolic compounds 2,4-ditert-butylphenol and 4-hydroxy-3,5-dimethoxy-benzaldehyde demonstrated by GC-MS. There may have been chemical effects and physical permeability effects of ethylene stimulation, manifested in the fungal resistances. The extractives have roles in protecting *H. brasiliensis* wood against fungal decay, and *G. lucidum* was the fungus most inhibited by the wood extracts, among those fungi tested. Caffeine was only detected in wood stimulated with ethylene. However, the effects of caffeine were not apparent at 6 or 12 weeks of wood incubation with fungi, which might be due to the rapid degradation of caffeine.

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