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VARIATIONS IN DECAY RESISTANCE OF *Cryptomeria fortunei*

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ABSTRACT

Cryptomeria fortunei has been widely planted in many cities in southern China. Eventually some of this material may be utilized for timber, but there are relatively few studies of durability of this resource. There is also some question as to whether *Cryptomeria fortunei* is a synonym for *Cryptomeria japonica* or Japanese cedar (Sugi). Evaluating the durability of the Chinese resource will help ensure that the decay resistance of this urban plantation resource is properly categorized. The decay resistance of *Cryptomeria fortunei* wood was assessed in soil block and agar block tests against *Trametes versicolor*, *Gloeophyllum trabeum* and *Rhodonia placenta*. Hot water and ethanol extractive contents of the heartwood were determined on sections from various distances above ground and then FTIR spectroscopy was used to characterize the wood before and after fungal exposure. Weight losses in sapwood were consistent with the minimal decay resistance of this portion of the wood. Inner and outer heartwood weight losses were more variable suggesting that the heartwood of this species would be considered to be only moderately durable. Extractives were weakly correlated with decay resistance. FTIR results were more variable, although they suggested heavier attack of lignin components by the brown rot fungi. The results suggest that *Cryptomeria fortunei* would need to be protected from the weather unless supplemental preservative treatments were applied.

Keywords: Brown rot, *Cryptomeria fortunei*, decay resistance, extractives, heartwood, *Robinia pseudoacacia*, white rot.

1. INTRODUCTION

Chinese cedar (*Cryptomeria fortunei*) is native to southern China and has long had a reputation for producing a heartwood that is resistant to fungal and insect attack. The wood of this species is used in a variety of applications including construction, coffin making, and furniture. The species is either synonymous with, or closely related to, Japanese cedar or Sugi (*Cryptomeria japonica*) which has been widely planted globally (Tsumura 2011). Several reports suggest that Sugi and *C. fortunei* are synonymous although the exact source of the tree remains cryptic because of continued trade between Japan and mainland China extending back several thousand years (Tsumura 2011).

Sugi has been extensively studied and has been planted in several other countries. Japanese tests suggest that sugi heartwood will provide 4 to 6 years of service life in soil contact (Matsuoka *et al.* 1970, Usta *et al.* 2006, Yamamoto *et al.* 2004). Limited laboratory tests of materials from other areas where the species has been grown in plantations have produced conflicting results, with one study suggesting that the heartwood was highly resistant to decay and another indicating that it was highly susceptible to attack by white rot fungi (Cappellazzi and Morrell 2016, Freitag and Morrell 2006, Morita *et al.* 1997, Okitani *et al.* 1999). These materials likely originated from different seed sources and illustrate the difficulty in classifying natural durability when a species is used in plantation strategies. One worldwide review of natural durability categorized sugi durability as ranging from low to highly decay resistant, illustrating the difficulty in categorizing the heartwood of this species (Scheffer and Morrell 1998). *Cryptomeria* has also been widely planted within China, where it has become an important shade tree in many southern cities. There are an estimated 366100 ha of plantation Chinese cedar with a volume approaching 30 million cubic meters of wood. While these materials are currently too small to be utilized for timber, the time is fast approaching when mature trees will need to be removed and may constitute a local source for potentially durable timbers. The lack of reliable data on the durability of this species will make it difficult to effectively utilize this material.

The purpose of this study was to examine the decay resistance of *C. fortunei* logs and compare these with the durability of black locust *(Robinia pseudoacacia*). Black locust was chosen for comparison because it too is now widely planted globally (including in Kunming) and appears to be highly durable in these disparate locations (Latorraca *et al.* 2011, Pollet *et al.* 2003).

2. MATERIALS AND METHODS

2.1 Materials Tested

Six *Cryptomeria fortunei* Hooibrend logs (30-40 cm in diameter 1,3 meters above ground) were obtained from a forest in Kunming City, Yunnan Province and eight black locust logs (10-15 cm in diameter 1,3 m above ground) were obtained from a forest near Changzhi City, Shanxi Province. The logs were used to prepare 19 mm cubes from the sapwood approximately 1,3 m above ground, and the inner and outer heartwood 1,3 m, 3,0 m, 6,0 m, 9,0 m and 12,0 m above the ground. Outer heartwood samples were obtained from the zone with the first colored wood (indicative of heartwood formation), while the inner zone was taken adjacent to the pith. Only one sapwood location was used because sapwood generally has little natural resistance to decay, regardless of tree position (Pollet *et al.* 2003). The inner and outer heartwood locations were chosen because heartwood durability is known to vary both radially and vertically in a tree. A minimum of 12 blocks were prepared for each location on each of the test trees. The cubes were oven dried (60 °C) and weighed prior to use.

In a second trial, increment cores (~8 mm in diameter) were taken 1,3 m above the ground from 52 *Cryptomeria fortunei* trees at the Kunming site. These cores were divided into sapwood, outer heartwood and inner heartwood before being oven dried and weighed. This trial was performed to gain a boarder sense of decay resistance without having to cut the trees down.

2.2 Decay Tests

The 19 mm blocks were briefly soaked in water and then sterilized by autoclaving for 20 minutes at 100 °C. The authors recognize that exposure to excess heat can reduce heartwood durability, but alternative methods were not available (Scheffer and Cowling 1966; Taylor *et al.* 2002). The heating times were limited as much as possible to minimize this risk. Decay chambers were prepared by half-filling 450 mL French squares with moist forest loam and placing a pine sapwood (*Pinus* sp) (for brown rot fungi) or *Paulownia fortunei* (for white rot fungi) feeder strip on the soil surface. The bottles were loosely capped and autoclaved for 45 minutes at 121 °C. After cooling, the bottles were inoculated with 3 mm diameter malt agar disks cut from the actively growing edges of cultures of *Gloeophyllum trabeum* (Pers.ex. Fr.) Murr. (Isolate # Madison 617), *Rhodonia placenta* Niemelä, Loss & Schigel (Isolate # Mad 698), and *Trametes versicolor* (L. ex Fr.) Pilát (Isolate # R-105). The former two fungi produce brown rot, while the latter produces white rot. Sterile test blocks were placed on the surfaces of the feeder strips once the test

fungus had grown, and the bottles were incubated at 28 °C for 12 weeks for the brown rot fungi or 16 weeks for *T. versicolor*. Twelve blocks were evaluated from each tree and position for each decay fungus. Non-fungal exposed controls were included to provide a measure of the weight losses occurring from block handling.

At the end of the incubation period, the blocks were removed, scraped clean of adhering soil and mycelium and weighed. The difference between initial oven dry weight and wet weight were used to calculate moisture content to ensure moisture levels were in a range suitable for fungal attack (>30 % moisture content). The blocks were then oven-dried at 60 °C and weighed. The resulting difference between initial and final oven dry weight was used to calculate fungal associated weight loss.

The increment core segments were also sterilized by autoclaving but the decay tests were performed using a modified agar plate assay. Malt extract agar in plastic Petri dishes was inoculated with agar plugs cut from actively growing cultures of the same test fungi. The plates were incubated until mycelium covered the agar surface, then a sterile glass rod was placed on top of the mycelium and increment core segments were added. The plates were sealed with wax film to retard drying and then incubated at 28 °C for 12 or 16 weeks for the brown or white rot fungi, respectively. At the end of the test, the segments were carefully removed, and oven-dried at 60 °C before being weighed. As with the block tests, differences in weight before and after fungal exposure served as the measure of decay resistance. Additional sapwood core segments were exposed in petri dishes with no fungus to serve as controls.

2.3 Chemical Analysis

Control and fungal exposed sapwood, outer heartwood and inner heartwood collected 1,3 m above the ground that had been subjected to the oven-drying and autoclaving conditions were ground to pass a 20-mesh screen and the resulting material was thoroughly mixed. A small sample of the resulting powder was mixed with KBr, pressed into a pellet and analyzed on a Nicolet i50 FTIR Analyzer (Thermo Scientific, Waltham, MA, USA). The resulting spectra were baseline corrected and then analyzed for differences in spectra between the various locations in the cross section as well as the between different fungal exposures.

2.4 Extractives Content

Three g of the ground material that had not been exposed to a decay fungus was weighed and immersed in hot water (80 °C) for 6 hours or 100 % ethanol for 6 hours at room temperature. The ground wood was recovered by filtration and the liquid extract was discarded. The material was dried at 60 °C

before being weighed. Differences between the initial and final weight were used to calculate total extractives content.

2.5 Data Analysis

The fungal weight loss data were subjected to an Analysis of Variance and then the resulting means were examined using Tukey's HSD test (α=0,05). Extractives contents varied widely and there was no consistent pattern with stem location. The potential relationship between extractives content and fungal associated weight was instead assessed by plotting the data and determining linear correlations.

3. RESULTS AND DISCUSSION

3.1 Decay Resistance

Weight losses of blocks exposed in bottles with no decay fungus were generally low, ranging from -0,08 % to 1,39 %, suggesting that the test conditions had little noticeable effect on weight loss (Table 1). Some blocks appeared to gain weight over the exposure, but the levels were low.

Weight losses of *C. fortunei* sapwood bocks exposed to *G. trabeum* or *R. placenta* averaged 44,89 % and 37,15 %, respectively, indicating that the wood had little resistance to fungal attack. Weight losses were extremely low with *T. versicolor,* which is consistent with the tendency of white rot fungi to have less effect on coniferous species. Weight losses for inner and outer heartwood of *C. fortunei* were generally lower than those found with sapwood, but almost all were over 20 % for the brown rot fungi. American Wood Protection Association Standard (AWPA) E30 provides a relative guide to heartwood decay resistance with weight losses between 10 % and 25 % classified as resistant, while those between 25 % and 44 % are moderately resistant to decay (AWPA 2017). The results indicate that *C. fortunei* was resistant to moderately resistant to brown rot attack; however, variations in weight losses between individual blocks were extremely high, with coefficients of variation ranging from 15,6 % to 83,5 %. This suggests that it would be prudent to classify this material as only moderately durable. These results would concur with the wide range in decay resistance suggested for *C. japonica* (Scheffer and Morrell 1998). Previous studies have suggested that more recently formed heartwood as well as heartwood nearer to the base of the tree tend to be more decay resistant to decay (Taylor *et al.* 2002). However, there was no evidence of those differences in the current study, possibly because of the extreme variation in weight losses.

Weight losses for black locust sapwood blocks were 29,88 % and 20,61 % for samples exposed to *G.*

trabeum and *R. placenta*, respectively. While the weight losses were somewhat lower than those for *C. fortunei*, they still showed that sapwood had relatively little resistance to fungal attack. Weight losses in blocks exposed to *T. versicolor* were higher than those found with *C. fortunei* sapwood, but still lower than those found for either brown rot and far below those considered to be representative of a valid decay test for this fungus (AWPA 2017). Weight losses for black locust heartwood blocks were below 6 % for the two brown rot fungi indicating that this material was highly resistant to fungal attack. These results would be consistent with previous reports for this species (Scheffer and Morrell 1998, Tewari 1978).

Comparisons between decay tests of blocks from the primary six *C. fortunei* trees and increment cores removed from 52 other trees suggested that there were few differences in weight losses for sapwood exposed to any of the three test fungi; however, weight losses tended to be much lower for heartwood samples exposed to either brown rot fungus in the agar block test (Table 2). The high variation in weight losses between samples limited the conclusions that could be reached from these data, but they suggest that the agar block test presented a slightly lower decay hazard. This reduced decay potential may reflect the greater degree of separation between the wood and the media compared to the soil block test where the blocks are directly on a wooden feeder strip that is in direct contact with the soil. The results illustrate the need to be careful in assigning decay resistance to materials where different assessment methodologies have been employed.

Table 1: Weight losses of *C. fortunei* and *R. pseudoacacia* sapwood and heartwood blocks removed from different heights and exposed to three decay fungi in a soil block test.

Species	**Height** (m)	**Location**	**Average Weight Loss (%)**			
			None	*G. trabeum*	*R. placenta*	*T.versicolor*
C. fortunei	1,3	Sapwood	-0,74 (0,70) B	44,89 (12,74) A	37,15 (8,33) A	3,20 (1,26) A
		Outer HW	0,04 (0,39) A	22,65 (12,67) B	23,69 (52,4) B	2,57 (0,37) B
		Inner HW	-0,08 (0,58) A	29,99 (13,23) B	18,48 (15,43) B	2,62 (0,69) B
	3,0	Outer HW	1,39 (4,41) A	29,75 (13,44) A	26,96 (9,92) A	2,35 (0,65) A
		Inner HW	0,03 (0,38) B	32,37 (5,05) A	27,26 (13,61) A	2,37 (0,48) B
	6,0	Outer HW	0,37 (0,38) A	28,98 (10,34) A	25,27 (15,77) A	2,52 (0,33) A
		Inner HW	0,08 (0,82) B	31,97 (11,41) A	30,14 (14,30) A	2,87 (1,26) B
	9,0[b]	Heartwood	0,49 (0,83) AB	29,19 (7,58) A	21,25 (13,07) A	2,39 (0,53) B
	12,0	Heartwood	0,71 (0,24) A	19,11 (9,42) B	25,03 (10,52) A	3,98 (0,40) A
R. pseudoacacia	1,3	Sapwood	1,23 (1,24) A	29,88 (4,54) A	20,61 (9,34) A	9,09 (1,23) A
		Heartwood	0,50 (0,17) B	5,43 (3,16) B	2,06 (2,43) B	1,24 (0,23) B

[a]Values represent means of 20 replicates per fungus while figures in parentheses represent one standard deviation. Means followed by the same letter in the same column do not differ significantly for a given fungus (α=0,05).

[b]Small diameters 9 m and 12 m above the groundline limited testing to a single heartwood source instead of inner and outer samples.

Table 2: Comparison between weight losses for *C. fortunei* blocks exposed to decay fungi in a soil block test vs increment cores exposed to the same fungi in an agar block test.

Location	**Average Weight Loss** (%)[a]					
	G. trabeum		*R. placenta*		*T. versicolor*	
	Agar	Soil	Agar	Soil	Agar	Soil
Sapwood	34,1 (11,4) A	44,9 (12,7) A	54,2 (8,1) A	37,2 (8,3) A	5,6 (2,0) A	3,2 (1,3) A
Outer HW	13,1 (14,3) A	22,7 (12,7) B	5,2 (10,6) B	23,7 (5,2) B	3,9 (1,1) B	2,6 (0,4) B
Inner HW	12,3 (15,3) B	30,0 (13,2) B	7,3 (14,0) B	18,5 (15,4) B	3,4 (1,0) B	2,6 (0,7) B

[a]Values represent means of 20 replicates per fungus while figures in parentheses represent one standard deviation. Means followed by the same letter in the same column do not differ significantly for a given fungus (α=0,05).

3.2 Extractives Content

Extractives play critical roles in heartwood durability and, generally, higher extractives levels translate to more durable timbers of a given species (Scheffer and Morrell 1998; Xie *et al.* 2012; 2014). There were no general trends in hot-water extractives levels from *C. fortunei* heartwood with either distance above ground or inner vs outer heartwood (Table 3). Ethanol extractives levels were very low in both inner and outer heartwood 1,3 m and 3,0 m above the ground but were sharply higher in both the inner and outer heartwood 6 m above ground as well as in the 9 m samples. There was little correlation between hot water extractives levels and wood weight loss and only a weak correlation between weight loss and ethanol extractives (Figure 1, 2).

Extractives levels dropped sharply 12 m above the ground, but it is important to note that the diameters at this height were very small as heartwood was just forming. Scheffer and Cowling (1966) suggested that heartwood extractives content should be highest near the base of the tree and decline with height. They also suggested that heartwood extractives should be most active near the heartwood sapwood interface. The results with Chinese cedar do not support either premise.

Table 3: Hot water and ethanol extractives content of the inner and outer zones of *C. fortunei* heartwood.[a]

Height (m)	**Stem Position**	**Hot Water Extractives** (%)	**Ethanol Extractives** (%)	**Total Extractives** (%)
1,3	Outer heartwood	3,87 (0,36)	4,61 (0,46)	8,48 (0,72)
	Inner heartwood	3,71 (0,09)	3,68 (0,88)	7,39 (0,97)
3,0	Outer heartwood	2,66 (0,03)	4,23 (0,14)	6,89 (0,17)
	Inner heartwood	3,16 (0,45)	4,65 (0,76)	7,81 (1,21)
6,0	Outer heartwood	3,21 (0,08)	6,60 (0,39)	9,81 (0,47)
	Inner heartwood	3,26 (0,25)	5,90 (1,75)	9,16 (2,00)
9,0[b]	Heartwood	3,39 (0,25)	6,43 (0,65)	9,82 (0,90)
12,0[b]	Heartwood	2,26 (0,15)	2,97 (0,20)	5,23 (0,35)

[a]Values represent means of six samples per stem position while figures in parentheses represent one standard deviation.
[b]Sections cut 9 m and 12 m above the ground had very little heartwood, resulting in a single sample per tree per height.

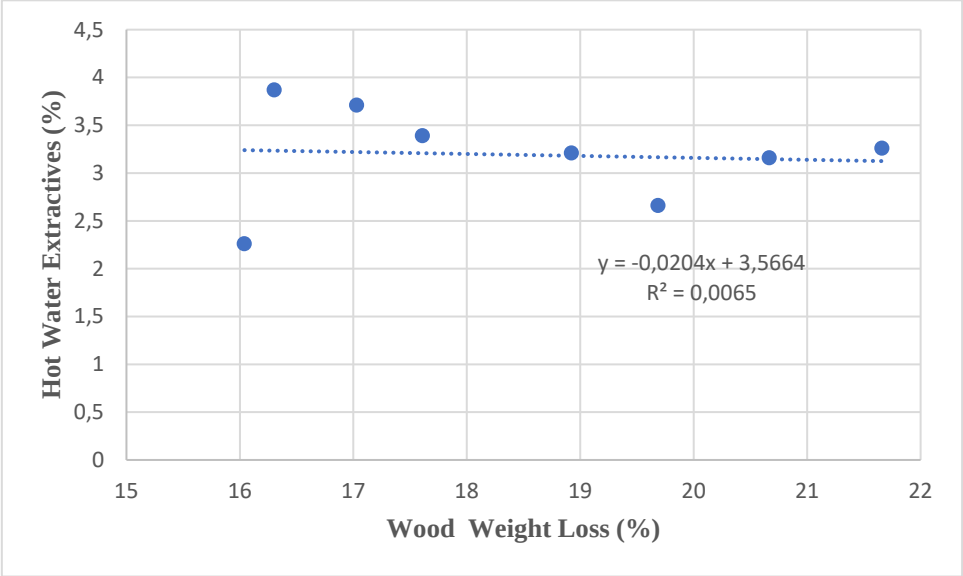

Figure 1: Relationship between hot water extractives content of *C. fortunei* heartwood and average weight loss when exposed to three different decay fungi in a soil block test.

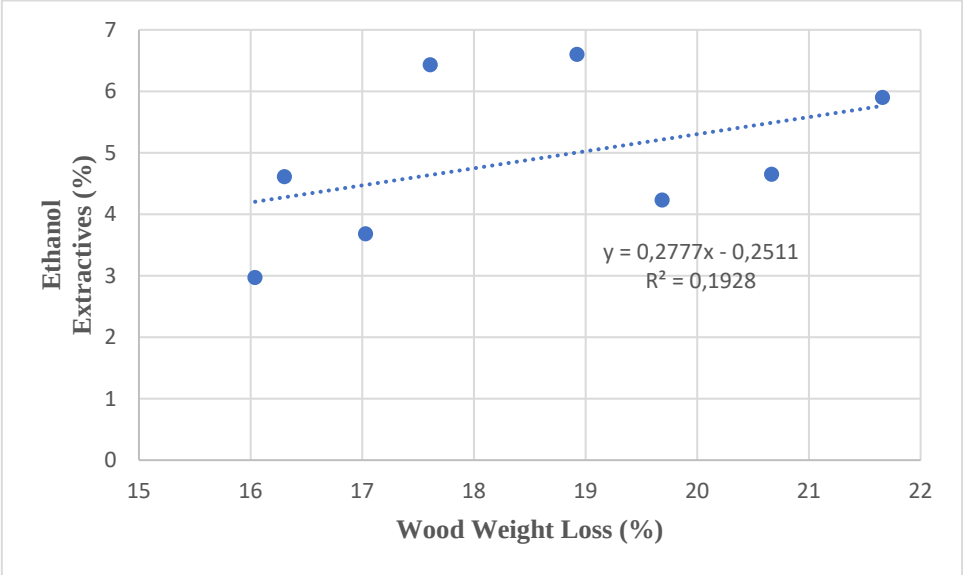

Figure 2: Relationship between ethanol extractives content of *C. fortunei* heartwood and average weight loss when exposed to three different decay fungi in a soil block test.

3.3 FT-IR Spectroscopy

FT-IR spectroscopic analysis is a powerful tool for exploring the effects of fungal activity on various wood components, especially the three main polymers (cellulose, hemicellulose and lignin). Attempts to use this technique to examine extractives content have proven more challenging (Lipeh *et al.* 2019).

FT-IR spectra contain a wealth of information on wood structure, primarily focusing on the three primary polymers. For the purposes of discussion, baseline corrected peak heights were used to establish

ratios between specific elements of the spectra (Faix 1992; Lipeh *et al.* 2019; Pandey and Pitman 2003). Spectra were only examined for samples that had been autoclaved prior to exposure in the decay test with or without the test fungus. The peak at 1504 cm^{-1} which is assigned to C=C stretching of lignin was used for comparison with peaks at 895 cm^{-1}, 1155 cm^{-1}, 1367 cm^{-1}, and 1732 cm^{-1}. The first three peaks are assigned to C-H deformation, C-O-C vibration and C-H deformation of cellulose and hemicellulose, respectively, while the peak at 1732 cm^{-1} is related to C=O stretching in xylans and lignin. The selected peaks allowed for comparisons in the ability of the fungi to affect cellulose and hemicellulose in relation to the lignin.

Examples of FTIR spectra from sapwood, outer heartwood and inner heartwood showed substantial differences between non-exposed and fungal exposed samples (Figure 3-5). In general, spectra were similar for wood from non-fungal exposed blocks and those exposed to *T. versicolor*, which reflected the low weight losses associated with exposure to this fungus. However, white rot fungi would be expected to produce relatively few changes until late in the decay process since they tend to utilize decomposition products as they are released (Zabel and Morrell 2020). Thus, there should be relatively little evidence of structural changes in the polymers early in the decay process.

Brown rot fungi tend to produce more dramatic changes in the structural polymers as they rapidly depolymerize the carbohydrate fraction, especially the hemicelluloses at the early stages of attack (Wilcox 1978; Winandy and Morrell 1993). As a result, ratios between the lignin peak at 1504 cm^{-1} and the other selected peaks should increase. Lignin ratios with the xylan peak at 1732 cm^{-1} were slightly lower for samples exposed to either brown rot fungus than those for the non-fungal exposed control suggesting that more xylan was present in the wood (Table 4). This finding contradicts reports that brown rot fungi tend to preferentially utilize hemicelluloses early in the decay process which helps account for their extreme effects on decay (Zabel and Morrell 2020). Ratios at 1367 cm^{-1} and 1155 cm^{-1} for wood exposed to *G. trabeum* both increased, while the ratio at 895 cm^{-1} nearly doubled suggesting preferential carbohydrate utilization. Sapwood samples exposed to *R. placenta* followed similar trends for the ratios at 1732 cm^{-1}, 1367 cm^{-1} and 1155 cm^{-1} but experienced a much smaller increase in the ratio at 895 cm^{-1}.

FTIR analysis of inner and outer heartwood samples tended to be less uniform. For example, 1504/1732 cm^{-1} ratios for outer heartwood samples exposed to *G. trabeum* were slightly higher than those for the control while those for samples exposed to *R. placenta* were slightly lower. Ratios for 1504/1367 cm^{-1} and

1504/1155 cm^{-1} were both higher for samples exposed to one of the brown rot fungi, which would be consistent with selective carbohydrate utilization. Similar trends were noted with inner heartwood. One problem with the FTIR results is the high variability in weight losses among the blocks. Combining materials results in a mixture of materials with a range of degrees of fungal damage. This would have masked larger differences that might exist between blocks.

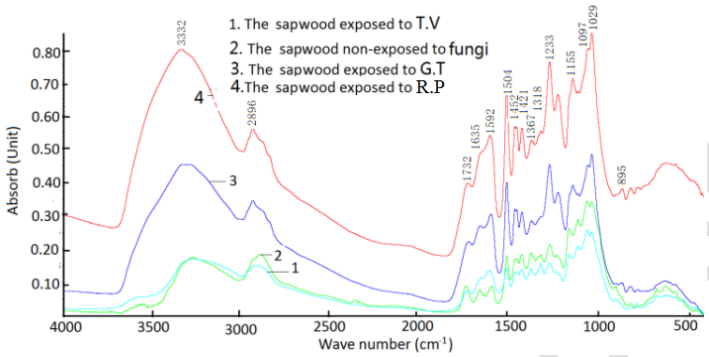

Figure 3: Example of FTIR spectra for *C. fortunei* sapwood blocks analyzed directly or after exposure to *T. versicolor* (TV), *G. trabeum* (GT) or *R. placenta* (RP) in a soil block test.

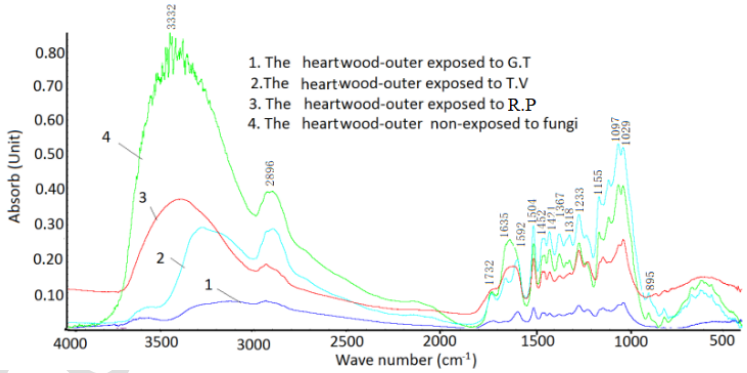

Figure 4: Example of FTIR spectra for *C. fortunei* outer heartwood blocks analyzed directly or after exposure to *T. versicolor* (TV), *G. trabeum* (GT) or *R. placenta* (RP) in a soil block test.

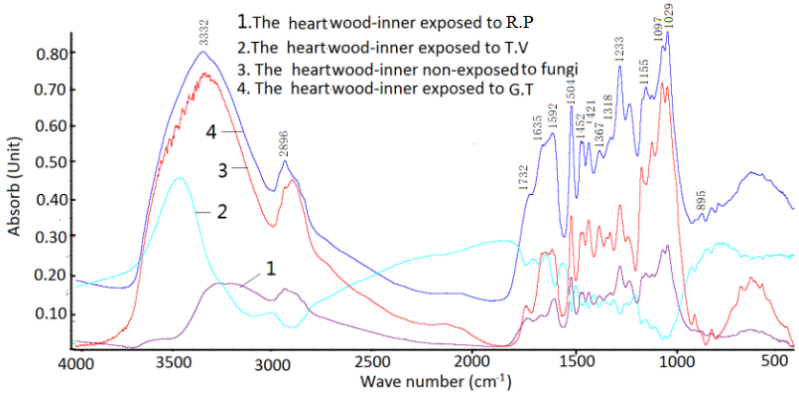

Figure 5: Example of FTIR spectra for *C. fortunei* inner heartwood blocks analyzed directly or after exposure to *T. versicolor* (TV), *G. trabeum* (GT) or *R. placenta* (RP) in a soil block test.

Table 4: Effect of fungal exposure on FT-IR spectra as shown by ratios between the lignin peak (1504 cm^{-1}) and peaks assigned to structures in the carbohydrate fractions.

	Fungus	**Peak Ratios** (cm^{-1})			
		1504/1732	1504/1367	1504/1155	1504/895
Sapwood	*Control*	2,08	0,85	0,99	2,11
	T. versicolor	1,94	0,99	1,05	2,79
	G. trabeum	1,80	1,40	1,12	4,18
	R. placenta	1,70	1,31	1,04	1,85
Outer Heartwood	*Control*	2,38	1,12	0,88	3,28
	T. versicolor	2,79	1,01	0,75	2,75
	G. trabeum	2,76	1,46	1,11	8,75
	R. placenta	1,75	1,32	1,06	2,12
Inner Heartwood	*Control*	3,11	1,07	0,73	3,98
	T. versicolor	1,43	0,89	0,77	1,28
	G. trabeum	1,55	1,20	0,97	1,85
	R. placenta	2,37	1,35	0,98	4,22

4. CONCLUSIONS

Chinese cedar durability varied widely among samples but overall would be rated as moderately durable. Extractives concentrations were weakly correlated with decay resistance, reflecting the high variability in both weight losses and extractives levels among individual samples. FTIR analysis produced conflicting results, again due to the high variability among individual samples. The results suggest that utilization of Chinese cedar would need to be limited to applications that were protected from wetting or that supplemental protection would be required to ensure performance.

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Availability of data and materials: The authors will make the data available upon request

Competing interests: The authors declare no competing interests

Authors' contributions: This research arose out of discussions between Drs. Li and Morrell during a visit to Kunming where the abundance of Chinese cedar as a street tree raised questions about its relative durability. The remaining authors undertook material collection, biological testing and chemical analysis.

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