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Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi

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Abstract The effect of selective removal of extractives on termite or decay resistance was assessed with matched samples of Thuja plicata Donn ex D. Don and Chamaecyparis nootkatensis (D.Don) Spach heartwood. Samples were extracted using a variety of solvents and then exposed to the subterranean termite Coptotermes formosanus Shiraki in a no-choice feeding test or to the brown-rot fungus Postia placenta (Fr.) M. Larsen & Lombard in a soil bottle test. At the same time, the effect of naturally occurring variations in heartwood extractives on termite or decay resistance was evaluated by testing samples from the inner and outer heartwood of five trees of each species against C. formosanus and P. placenta and analyzing matched wood samples for their extractive content. The results suggest that the methanol-soluble extractives in T. plicata and C. nootkatensis play an important role in heartwood resistance to attack by C. formosanus and P. placenta. Total methanol-soluble extractive content of the heartwood was positively correlated with both termite and decay resistance; however, there was much unexplained variation and levels of individual extractive components were only weakly correlated with one another. Further studies are under way to develop a better understanding of the relationships between individual extractive levels and performance.

Key words Western red cedar \cdot Alaska cedar \cdot Coptotermes formosanus \cdot Postia placenta

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Introduction

Concerns over the safety and environmental impacts of chemically treated wood have increased demand for naturally durable wood products. At the same time that demand is increasing, the nature of the forest resource that will be used to meet this demand is changing from older, larger trees, which contain wood with a proven history of durable performance, to younger trees whose wood properties are not as well understood. Changes in the use of wood products in housing applications, and expansion of the areas threatened by termites are putting additional demands on the performance criteria for wood products. Research to better understand the effects of changes in wood properties on resistance to termites and decay fungi could lead to more rational utilization of naturally durable timber.

The heartwood of some tree species is naturally resistant to termite and fungal attack, largely due to the presence of nonstructural chemical "extractives." For example, the heartwoods of *Chamaecyparis nootkatensis* (D.Don) Spach (Alaska cedar or yellow cedar) and *Thuja plicata* Donn ex D. Don (western red cedar) have significant resistance to *Coptotermes formosanus* Shiraki and other termite species and against decay fungi. Unfortunately for the users of naturally durable wood products, termite and decay resistance varies significantly among individual pieces of wood and this variability can be difficult to predict. 10-12

Heartwood extractive chemistry is complex and varies significantly within and between trees and tree species (reviewed by Taylor et al.¹²). Lower amounts of extractives have been observed in the heartwood near the pith of a number of species, including *T. plicata*.¹¹ Lower extractive content has been correlated with reduced termite and fungal resistance.^{1,13} Grace and Yamamoto⁴ observed significant variation in termite resistance properties of *C. nootkatensis* wood and suggested that this was a function of heartwood extractive variability.

Heartwood extractives within a piece of wood can range from low molecular weight volatile compounds to large polymers, 14 and it appears that not all of these components

Table 1. Mass loss due to decay and termite feeding on blocks from natural variability test

Species	Tree number	Disk age (years)	Disk diameter	Average growth increment (mm/year)	Average deca (%) ^a	у	Average termite feeding (%) ^a		
			(cm)		Inner heartwood	Outer heartwood	Inner heartwood	Outer heartwood	
Thuja plicata	1	101	13	1.28	10.3(8.3)	-0.1(2.0)	2.3(1.0)	2.1(0.9)	
	2	40	21	5.34	12.7(20.7)	5.5(7.3)	3.3(1.8)	2.1(1.0)	
	3	103	18	1.78	17.6(18.2)	2.1(2.2)	2.9(0.3)	2.0(0.6)	
	4	51	33	3.26	17.1(4.7)	2.7(2.4)	5.1(0.6)	2.9(0.2)	
	5	78	29	1.86	21.2(13.4)	3.1(5.9)	11.4(4.3)	3.2(0.6)	
	All trees				15.8(12.8)	2.7(4.3)	5.0(3.9)	2.5(0.8)	
Chamaecyparis	1	157	26	1.63	7.7(5.8)	3.3(0.5)	3.6(0.3)	3.0(0.1)	
nootkatensis	2	166	27	1.65	1.6(1.3)	1.4(0.5)	2.7(0.2)	2.4(0.5)	
	3	310	26	0.82	0.9(0.8)	2.0(0.7)	2.3(0.2)	2.0(0.1)	
	4	403	51	0.63	1.7(3.2)	0.7(1.1)	3.4(0.4)	2.3(0.3)	
	5	283	51	0.91	3.6(3.1)	4.3(6.3)	2.7(0.3)	2.4(0.3)	
	All trees				3.1(3.8)	2.3(2.8)	2.9(0.6)	2.4(0.4)	

For each tree, n = 3

are equally important in determining natural durability. There is evidence that the volatile fraction is particularly effective in reducing termite attack. 10,15-18 In his review of the literature, Scheffrahn² observed that the most commonly identified antitermitic compounds in various wood species were terpenoids. Lower molecular weight terpenoids include the thujaplicins in T. plicata and nootkatin in C. nootkatensis.14 The thujaplicins are fungitoxic and so have also been a focus of research in durability to fungal attack; 11,19-21 however, there are many other compounds in T. plicata and C. nootkatensis heartwood that make up the extractive mixture in addition to the low molecular weight terpenoids.^{22,23} Many of these compounds have not been identified or examined for their toxicity; furthermore, it is not known how these compounds vary within and between trees. Thus, much remains to be discovered about the relationship between extractives and natural durability in these two species.

Recent work has demonstrated that changes in the tree's environment during growth can alter heartwood extractive content in some species.²⁴ If such variations in heartwood extractives affect the natural resistance of the wood to termites and fungi, then this finding suggests that silvicultural treatments and changes in how forest resources are managed may impact on the natural durability of future wood products.

The objectives of this study were (1) to determine which extractive components in *T. plicata* and *C. nootkatensis* most affect attack by the termite *C. formosanus* and the brown-rot fungus *Postia placenta* (Fr.) M. Larsen & Lombard; and (2) to assess the relationship between naturally occurring variations in heartwood extractive content and resistance to biodeterioration.

Materials and methods

This study consisted of two parts, each comprising multiple tests. In the extractive fraction test, matched heartwood

samples were first subjected to extraction treatments and then tested for termite resistance and decay resistance. For the natural variability test, untreated heartwood samples were assessed for their termite resistance, decay resistance, and extractive content.

Wood materials

Fresh, debarked basal cross sections (approximately 10cm in the longitudinal direction) from five trees of *Thuja plicata* and *Chamaecyparis nootkatensis* were provided by a utility pole supplier (Cascade Pole, Tacoma, WA, USA). The trees varied in growth rate, age, and diameter (Table 1). For each disk, the width of each growth ring (nearest 0.001 mm) from the pith to the cambium was measured along one radius on a dissecting microscope mounted over a moving stage connected to a linear variable differential transformer (LVDT) displacement transducer (Acu-Rite, Jamestown, NY, USA).

Sample preparation

For the natural variability test, blocks ($30R \times 20T \times 40 \text{mmL}$) were cut from the inner and outer heartwood of each of the five trees of each species. Heartwood was differentiated from sapwood by color. These blocks were cut into three samples 10 mm thick (radial) with a band saw. From each sample, 10 mm (longitudinal) was cut from one end for inclusion in the decay test and 10 mm was cut from the other end for use in the extractive analysis. The remaining 20 mm were used for the termite test. After cutting, the samples were stored at room temperature (20° – 23° C) in the dark in sealed plastic bags.

For the extractive fraction test, blocks $(40 \, \text{R} \times 40 \, \text{T} \times 30 \, \text{mm L})$ were cut from the outer heartwood of each of the five trees of each species. These blocks were cut into $(40 \times 40 \times 2 \, \text{mm})$ thick longitudinal) subsamples with a band saw. Subsamples were subjected to the following treatments: (1)

^a Values given in parentheses are standard deviations

vacuum drying at $50^{\circ} \pm 2^{\circ} C$ and $13 \, kPa$ for $48 \, h$ (to eliminate volatile fractions), (2) vacuum drying followed by soxhlet extraction with hexane (to remove mostly nonpolar extractives), or (3) vacuum drying, followed by extraction with hexane, followed by soxhlet extraction with methanol (to removing a broad spectrum of extractive components). Additional samples ("fresh sawn") were stored at room temperature (20° – $23^{\circ} C$) in the dark in sealed plastic bags directly after cutting to serve as nonextracted controls.

No-choice termite tests

Replicates of the extractive fraction test samples (two from each of three trees \times four treatments; n = 24) and natural variability test samples (three each from the inner and outer heartwood of five trees; n = 30) were exposed in a 3-week, no-choice feeding test with Coptotermes formosanus as described in a Japan Wood Preserving Association standard.^{25,26} Samples of *Cryptomeria japonica* D. Don (sugi) sapwood and heartwood were also included in the study to provide a basis for comparison. Cryptomeria japonica sapwood is not resistant to attack by C. formosanus, whereas the heartwood is very resistant.²⁷ The termite test containers were examined daily and the dead termites were removed and tallied. The numbers of living termites in each container were counted after 3 weeks of exposure. At the end of the test period the wood samples were washed, dried at $60^{\circ} \pm 2^{\circ}$ C, and weighed, after which mass loss from the samples was calculated.

Decay testing

Replicates of the extractive fraction test samples (two from each of three trees \times four treatments; n=24) and natural variability test samples (three from the inner and outer heartwood of five trees; n=30) were exposed to *Postia placenta* in soil bottle tests according to ASTM D1413-99. Postia placenta is a brown-rot fungus that often attacks wood products in service. Samples of decay-susceptible *Pinus radiata* D. Don sapwood were included in the test as a control. Each block was cut into two pieces to provide a pseudoreplicate. The average mass lost from the two blocks during the test was used as the (single) decay value.

Extractive fraction relative mass

For analysis of the relative mass of each extractive fraction in the extractive fraction test, fresh-cut wood pieces of each species were ground in a Wiley mill (Arthur Thomas, Philadelphia, PA, USA) to pass a 20-mesh screen. Three replicate weighed samples of the wood flour of each species (~2.0g) were enclosed in heat-sealable polyester filter bags (pore size $25\,\mu\text{m}$, Ankom, Macedon, NY, USA). The bags were oven-dried for 12h at $50^{\circ} \pm 2^{\circ}\text{C}$, reweighed, and then subjected to the vacuum-drying, hexane, and methanol extraction steps described above. After each extraction step, the bags were oven-dried and reweighed. The mass of ex-

tractives removed in each step was calculated as the mass lost from the wood samples.

Gas chromatography and extractives quantification

Matched samples from the natural variability test were reduced to powder in a ball-type tissue pulverizer mill (Garcia, Visalia, CA, USA) for 2 min. The concentration of known extractive compounds in the wood powder was analyzed by gas chromatography, using a technique modified from that described by DeBell et al. 11 as follows. Samples of the wood powder (0.11–0.14g) were weighed into 2-ml centrifuge tubes. One milliliter of methanol was added to each of the tubes, which were then capped, shaken, and allowed to stand at room temperature (20°–23°C) for 18 h. The tubes were then centrifuged for 10 min at 5000 rpm, and 0.6 ml of each methanol/wood extract solution was pipetted into an auto sampler vial, which was then capped. The wood extract solutions were analyzed on a Shimadzu GC-2010 gas chromatograph with a flame ionization detector, using He as the carrier gas. Two-microliter injections were made by autosampler onto a capillary column (Rtx-5: 15 m long, 0.25 mm inner diameter, 0.25 \(\mu\)m thick, 5% diphenyl/ 95% dimethyl polysiloxane coating, Restek, Bellfonte, PA, USA) using a 10:1 split ratio and 1.98 ml/min of column flow. The temperature program began at 100°C, increasing at 5°C per minute to 130°C, holding at that temperature for 3 min, increasing by 30°C per minute to 250°C, and remaining at that temperature for 7 min. The injector and detector were maintained at 275°C. Available standards were used to make calibration curves to determine the concentrations of known T. plicata heartwood extractive components³⁰ in each extract, including β -thujaplicin (CAS Number 499-44-5, Aldrich), carvacrol (CAS Number 499-75-2, Aldrich), carvacrol methyl ether (CAS Number 6379-73-3, Aldrich), and γ -thujaplicin, β -thujaplicinol, thujic acid, and methyl thujate (provided by Dr. R. Daniels, Forintek Canada Corporation). Carvacrol (CAS Number: 499-75-2 Aldrich), carvacrol methyl ether (CAS Number: 6379-73-3, Aldrich) and nootkatone (CAS Number: 4674-50-4, Aldrich) were analyzed in the C. nootkatensis extract analysis.²² "Total GC" extractive content for each sample was calculated using the sum of the areas of all peaks (excluding the solvent peak) in the chromatogram and using the calibration curve for β -thujaplicin (for T. plicata) and carvacrol (for C. nootkatensis). All gas chromatography extractive values were corrected for the mass of wood powder sampled.

The "methanol-soluble" and "ASTM" extractive contents were also determined for the natural variation test samples. Samples of the wood powder (\sim 0.15g) were enclosed in heat-sealable polyester filter bags, oven-dried at $50^{\circ} \pm 2^{\circ}$ C and reweighed, extracted with methanol in a soxhlet apparatus for 6h, redried, and reweighed. The samples were then subjected to the extraction procedure described in ASTM D1105:³¹ successive soxhlet extractions with a 2:1 toluene/ethanol mixture and 95% ethanol, followed by extraction in a hot water bath, redrying, and reweighing. Extractive content from the two extraction

procedures was calculated as the mass lost from the wood powder compared with the mass of the extractive-free wood.

Data analysis

Group values for all parameters in the extraction fraction test were compared by analysis of variance (ANOVA) tests using the Tukey-Kramer procedure for multiple comparisons (S-PLUS 6.1 for Windows; Lucent, Murray Hill, NJ, USA). Correlations among the extractive components (*R* values) were calculated for the samples in the natural variation tests. Simple linear regressions were performed with methanol-soluble extractive content as the explanatory variable and termite feeding values and decay mass lost values as the response variables.

Results and discussion

Extractive fraction test

Effect of extraction on Thuja plicata

The methanol extraction step significantly increased the mass loss from the *Thuja plicata* samples (Fig. 1a). The

patterns of mass loss for the samples indicate that resistance of *T. plicata* wood to *Coptotermes formosanus* attack is similar to that of *Cryptomeria japonica* heartwood, and that the vacuum drying and hexane extractions did not change resistance. Methanol extraction rendered *T. plicata* about as susceptible to attack as the *C. japonica* sapwood.

The pattern of *T. plicata* heartwood resistance to *Postia placenta* was similar to that of the termite test results (Fig. 1b). Although the hexane-extracted wafers had higher average weight loss, only the methanol-extracted wafers showed increased decay levels that were statistically significant compared with the unextracted samples.

Effect of extraction on Chamaecyparis nootkatensis

Chamaecyparis nootkatensis heartwood samples were very resistant to attack by *C. formosanus*. In some cases, all of the termites exposed to fresh-cut *C. nootkatensis* samples died within 48 h. This rapid mortality suggests that volatiles from the wood were toxic to the termites. In most cases, however, many of the termites lived past the end of the test period.

Mass losses due to termite feeding in *C. nootkatensis* samples were low (Fig. 2a). Methanol extraction significantly increased wood susceptibility to feeding by *C. formosanus*, but mass losses never exceeded 10%. The hex-

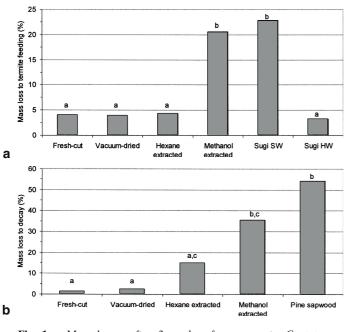


Fig. 1. a Mass losses after 3 weeks of exposure to *Coptotermes formosanus* of *Thuja plicata* blocks that were left untreated or subjected to various extraction procedures. Sugi sapwood (SW) and heartwood (HW) are included for the purpose of comparison. Columns with the same letters are not significantly different at $\alpha = 0.05$. b Mass loss of *Thuja plicata* blocks that were left untreated or subjected to various extraction procedures exposed for 12 weeks to the brown-rot fungus *Postia placenta*. Pine sapwood blocks are included for the purpose of comparison. Columns with the same letters are not significantly different at $\alpha = 0.05$

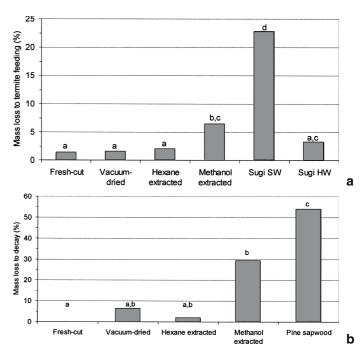
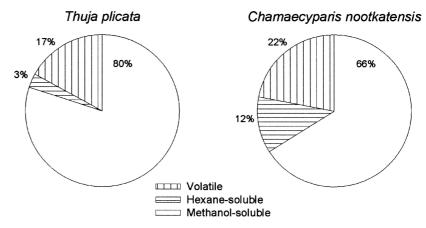


Fig. 2. a Mass losses after 3 weeks of exposure to *Coptotermes formosanus* of *Chamaecyparis nootkatensis* blocks that were left untreated or subjected to various extraction procedures. Sugi sapwood and heartwood are included for the purpose of comparison. Columns with the same letters are not significantly different at $\alpha = 0.05$. b Mass loss of *Chamaecyparis nootkatensis* blocks that were left untreated or subjected to various extraction procedures and exposed for 12 weeks to the brown-rot fungus *Postia placenta*. Pine sapwood blocks are included for the purpose of comparison. Columns with the same letters are not significantly different at $\alpha = 0.05$

Fig. 3. Relative abundance of various heartwood extractive fractions by mass



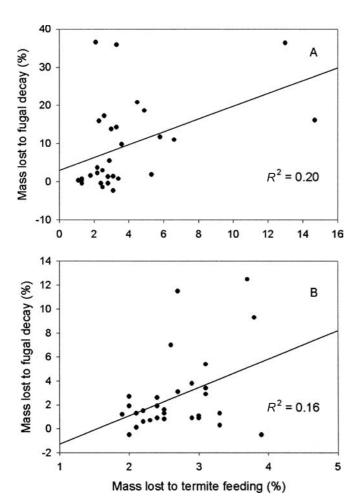


Fig. 4A,B. Mass loss due to feeding by *Coptotermes formosanus* versus mass loss due to decay by *Postia placenta* of matched heartwood samples of *Thuja plicata* (**A**) and *Chamaecyparis nootkatensis* (**B**)

ane-extracted samples had slightly more mass loss than the vacuum-dried samples, but the differences were not statistically significant.

The pattern of *C. nootkatensis* heartwood resistance to *P. placenta* was similar to that of the termite test results (Fig. 2b); only the methanol-extracted wafers showed statis-

tically significant increased decay levels compared with the unextracted samples. These data suggest that the more volatile components of the wood may be less important for termite and fungal decay resistance of *T. plicata* and *C. nootkatensis* than the higher molecular weight or more polar compounds that are soluble in hexane and methanol. This finding differs from previous work with other species that found the volatile fraction to be especially important. ^{10,15,16,18} Whereas some volatile extractives may have remained in the wood after vacuum drying and hexane extraction, one would expect some differences after those treatments if the volatile fraction were critically important.

The relative importance of the methanol-soluble extracts may be a function of their inherent toxicity and/or their relative abundance. Most of the extractives in the heartwood of the two wood species are methanol soluble (Fig. 3), and it was the removal of these extractives that most reduced the durability of the wood pieces in these tests.

Natural variability test

In the natural variability samples of both wood species, *C. formosanus* feeding and *P. placenta* decay losses were generally low, with high variability among the samples (Table 1). The outer heartwood was more resistant than the inner heartwood to termite attack and decay in many cases. Such high variability in the termite and decay resistance of heartwood within and between trees of the same species is consistent with previous observations of these and other wood species. ^{1,11,15,32}

The range of termite feeding values was small in this test, and the susceptibility to decay of a matched sample was positively, but only weakly, correlated to its susceptibility to termite attack over the limited range of values (Fig. 4). Methanol-soluble extractive concentrations were negatively related to decay mass loss and termite feeding values for the two wood species, but this relationship explained relatively little of the variation (Table 2). None of the other extractive measures was a better predictor of the susceptibility of the wood to attack by *C. formosanus* or *P. placenta*. Concentrations of individual components of the heartwood extractives in samples of each wood species were only

Table 2. Results of regression of termite feeding and decay mass loss values on the methanol-soluble extractive content of *Thuja plicata* and *Chamaecyparis nootkatensis*

Species	Decay mass	loss		Termite feeding			
	Coefficient	P value	R^2	Coefficient	P value	R^2	
Thuja plicata Chamaecyparis nootkatensis	-1.46 -0.93	0.015 0.117	0.20 0.09	-0.42 -0.26	0.007 0.007	0.23 0.23	

Low P values for the regression coefficients indicate that there is a statistically significant linear relationship between the variables. R^2 values measure the fraction of the variation in the response variable explained by explanatory variable

Table 3. Correlation matrix of extractive measurements of samples of *Thuja plicata* (n = 30)

Extractive components ^a	Degree	of linear as	ssociation	$(R)^{b}$											
	СМ	CA	MT	TA	β -in	γT	β-ol	GC	MS						
Carvacrol methyl ether (CM) [0.018]	1														
Carvacrol (CA) [0.0697]	-0.49	1													
Methyl thujate (MT) [0.0216]	-0.04	-0.06	1												
Thujic acid (TA) [0.571]	-0.61	0.62	0.10	1											
β -Thujaplicin (β -in) [0.092]	-0.30	-0.01	0.49	0.06	1										
γ-Thujaplicin (γ-T) [0.106]	-0.39	0.11	0.42	0.36	0.56	1									
β -Thujaplicinol (β -ol) [0.084]	-0.33	0.09	0.22	0.36	0.53	0.81	1								
Total extractives by gas chromatography (GC) [2.256]	-0.33	0.29	0.41	0.17	0.54	0.67	0.47	1							
Methanol-soluble extractive content (MS) [8.245]	-0.41	0.16	0.27	0.48	0.65	0.82	0.88	0.53	1						
ASTM extractive content [15.572]	-0.40	0.34	0.19	0.66	0.46	0.65	0.66	0.39	0.86						

^a Extractive names are abbreviated in the columns to the right. Values given in square brackets are the average concentrations in wood (% by weight)

Table 4. Correlation matrix of extractive measurements of samples of *Chamaecyparis nootkatensis* (n = 30)

Extractive components ^a		Degree of linear association (R) ^b					
	СМ	CA	NO	GC	MS		
Carvacrol methyl ether (CM) [0.019]	1						
Carvacrol (CA) [0.413]	0.96	1					
Nootkatone (NO) [0.268]	0.50	0.42	1				
Total extractives by gas chromatography (GC) [3.250]		0.39	0.83	1			
Methanol-soluble extractive content (MS) [5.057]	0.41	0.29	0.74	0.69	1		
ASTM extractive content [9.424]	0.48	0.45	0.67	0.52	0.66		

^a Extractive names are abbreviated in the columns to the right. Values given in square brackets are the average concentrations in wood (% by weight)

weakly correlated with one another or with the various measures of total extractive content (Tables 3 and 4). These observations suggest that it is not possible to focus on a single heartwood extractive measurement in order to understand the natural durability of the wood, especially if one is considering the resistance of the wood to multiple biodeterioration agents.

These data support the general belief that heartwood extractives are responsible for the natural resistance of heartwood against termites and fungi. In particular, *C. nootkantensis* and *T. plicata* heartwood is resistant to termites and fungi because of the methanol-soluble extractives, which are present in relatively high concentrations.

However, the high variability within the heartwood extractive mixtures of each species, and the poor correlations of extractive components with fungal and termite resistance of the wood, suggest that the relationship between heartwood extractives and heartwood durability is complex.

Conclusions

Thuja plicata and Chamaecyparis nootkatensis heartwood showed considerable resistance to Coptotermes formosanus and Postia placenta in laboratory tests. Methanol extraction

^bValues range from –1 to 1, where 0 corresponds to no linear association

^bValues range from –1 to 1, where 0 corresponds to no linear association

of the wood samples reduced their resistance, whereas vacuum drying and hexane extraction appeared to have little effect.

Considerable naturally occurring variability was observed in the extractive components of wood samples taken from different locations within trees and from different trees. Variations in extractive components were only weakly correlated with one another, and explained relatively little of the variation in termite and fungal resistance. These results suggest that the methanol-soluble heartwood extractives of *T. plicata* and *C. nootkatensis* are important factors in the complex relationship between the extractive content of heartwood and its natural durability.

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