NEW PERSPECTIVES ON THE ROLE OF EXTRACTIVES IN THE DURABILITY OF WESTERN REDCEDAR

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Summary

Western redcedar (WRC) heartwood is well known for its durability. It contains several groups of extractives, including thujaplicins, terpenes, and lignans. The thujaplicins are the most acutely toxic to decay fungi, and as such have often been assumed to be critical for durability in service. Research over the last thirty years that investigated the depletion of thujaplicins from wood in service or attempted to correlate thujaplicin content to decay resistance suggests that the thujaplicins are not the primary contributors to the natural durability of this wood in service. Recent work suggests that plicatic acid (a lignan), and potentially other compounds, play a greater role in durability in service than previously thought.

In the present work western redcedar blocks were exposed to controlled above-ground and soil contact pre-conditioning with very low leaching hazards for up to one year. The thujaplicins and terpenes were substantially depleted, while the lignans remained relatively abundant. Subsequent decay testing showed that both above-ground and soil contact pre-conditioning resulted in increased susceptibility to decay by a brown rot fungus. These data provide further evidence that due to rapid depletion the thujaplicins may not be associated with the durability of WRC wood in service, and suggest that detoxification is a likely depletion pathway. This means that durability predictions based on thujaplicin content or on soil block tests without pre-exposure to detoxification may not be representative of field performance. Further elucidation of the compounds responsible for natural durability and the mechanisms through which they operate is essential to ensure that the western redcedar resource is properly utilized, and may also lead to new approaches to wood preservation.

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1. Introduction

Canada's three primary naturally durable wood species all go by the name of "cedar" yet none are true cedars (genus *Cedrus*). Eastern (northern) white-cedar (*Thuja occidentalis L.*) grows from Manitoba to Nova Scotia and as far south as the Appalachians in North Carolina. Yellow-cedar (*Callitropsis nootkatensis* D. Spach) and western redcedar (*Thuja plicata* Donn) grow on the west coast in British Columbia, Alaska and the Pacific Northwest states. The decay resistance

of these species is variable within and between trees, and also varies depending on test and classification methodology. In European Standard EN350-2, yellow-cedar is rated 2-3 (durable to moderately durable) and western redcedar (North American grown) is rated 2 (durable). Australian Standard AS 5604 classifies yellow-cedar as Class 1 (very durable) in both above-ground and in-ground exposures and western redcedar as Class 2 (durable) above-ground and Class 3 (moderately durable) in-ground. Eastern white-cedar, yellow-cedar and western redcedar heartwood are all classified as "resistant to decay" in the USDA FPL wood handbook (Clausen 2010). In 2010 the value of western redcedar lumber exported from Canada was $465 million (Poon 2011). This dwarfs the $59 million value of yellow-cedar lumber, and the even smaller market for eastern white-cedar lumber. As a result the major focus of our Durability by Nature research program, and of the present paper, is on western redcedar.

The heartwood of western redcedar (*Thuja plicata* Donn) is widely recognized as durable (Cartwright 1941; Scheffer 1957; Freitag and Morrell 2001; Laks et al. 2008). This durability is associated with extractable compounds (Sowder 1929; Cartwright 1941) that include thujaplicins, lignans and terpenes. Several thujaplicins have been isolated from WRC heartwood and identified. These include alpha-thujaplicin (Gripenberg 1948), beta-thujaplicin (Anderson and Gripenberg 1948), gamma-thujaplicin (Erdtman and Gripenberg 1948), beta-thujaplicinol (Gardner et al. 1957) and beta-dolabrin (Gardner and Barton 1958). Beta- and gamma-thujaplicin are generally most abundant (Barton and MacDonald 1971; Daniels and Russell 2007), though recent work from our lab has shown alpha-thujaplicin to be more abundant than previously thought (C.R. Daniels, unpublished).

The thujaplicins possess strong antimicrobial activity. They have been shown to inhibit decay and blue stain fungi on agar at an order of magnitude lower concentration than pinosylvin and its monomethyl ether, and have similar toxicity to pentachlorophenol (Rennerfelt 1948; Rennerfelt and Nacht 1955). In an agar-based test, beta-thujaplicinol had strong activity against brown-rot fungi, but was less active against white-rot fungi (Roff and Whittaker 1959). Beta-thujaplicin, gamma-thujaplicin and beta-thujaplicinol have also been found to be toxic to decay fungi when added to extractive-free wood (Rudman 1962). Thujaplicins also have activity against gram-positive and gram-negative bacteria (Saleh et al. 1988). More recent theories on the causes of natural durability suggest that extractives with antioxidant and metal chelating activity may be in large part responsible for natural durability (Schultz and Nicholas 2000, 2002). All of the thujaplicins are strong metal chelators (MacLean and Gardner 1956; Gardner et al. 1959) and prevent reduction of Fe^{3+}, potentially preventing the Fenton reaction from degrading lignocellulose (Gérardin et al. 2002). Beta-thujaplicinol has potent radical scavenging activity (Stirling et al. 2007).

WRC heartwood also contains substantial amounts of other extractives including plicatic acid, several related lignans, thujic acid and its methyl ether. Thujaplicin-free extracts that would have been primarily plicatic acid have been reported to have mild antifungal activity (Roff and Atkinson 1954). Plicatic acid is much more abundant than the thujaplicins. It is also an antioxidant and a metal chelator (Gardner et al. 1959), and therefore may have some indirect fungal toxicity. Thujic acid and its methyl ether are not known to have any activity against fungi.

To date no one has demonstrated that any one extractive causes WRC heartwood to be durable. The substantial amount of data on thujaplicin toxicity has led some researchers to infer that thujaplicins are primarily responsible for the natural durability of WRC heartwood (Nault 1988). However, there is a significant amount of evidence that suggests that thujaplicins are not even closely associated with the natural durability of wood in service. Thujaplicins rapidly lose antibacterial efficacy after light exposure (Coombs and Trust 1973). They also rapidly deplete from WRC in service (Johnson and Cserjesi 1980) yet the wood remains durable (Ingram and Morris 2006). Depletion occurs primarily via leaching and biodegradation (Chedgy et al. 2005). In trees, they are broken down by fungi (Jin et al. 1988), and they have been found to be sensitive to peroxidase (Gérardin et al. 2002). In laboratory decay tests, correlations with thujaplicin concentration have been weak (DeBell et al. 1997). In field stake tests poor correlations were found between thujaplicin concentration and decay resistance (Morris and Stirling 2011).

There is also evidence that plicatic acid, and potentially other lignans or unknown compounds, play a greater role in natural durability than previously thought. Though plicatic acid is also leachable (Chedgy et al. 2009), it was the only extractive found in significant quantities in WRC shakes and shingles after decades in a field test (Stirling 2010). The presence of plicatic acid has been associated with the low equilibrium moisture content of WRC (Stirling and Morris 2006). This may contribute to durability above ground where wood is subject to periodic wetting and drying. Fixative agents that reduced leaching of plicatic acid improved the resistance of WRC to decay by a brown-rot fungus (Stirling and Morris 2010). Dark coloured heartwood has been associated with poorer decay resistance (Eades and Alexander 1934). This colouration is believed to be caused by the polymerisation of plicatic acid (Kai and Swan 1990; Johannson et al. 2000). Finally, in field stake tests plicatic acid and an unknown compound were moderately correlated with decay resistance in ground contact (Morris and Stirling 2011).

Exposure to staining fungi has been shown to reduce thujaplicin concentration and make heartwood more susceptible to decay in living trees (Van der Kamp 1986; Jin et al. 1988). While it is important to distinguish between natural durability in service and natural durability of wood in a living tree, these data suggest that microorganisms might be associated with thujaplicin depletion.

Work within our lab has focussed on elucidating the extractives responsible for durability in service and understanding the mechanisms through which they protect the wood. In this paper we report on the depletion of extractives and loss of decay resistance associated with colonisation by microorganisms, and relate these data to the broader question – what causes natural durability in WRC?

2. Methodology

Western redcedar was obtained from the University of British Columbia's Malcolm Knapp Research Forest and confirmed to be free of fungal infection (Chedgy et al. 2007). Outer heartwood was cut into 19 x 19 x 19 mm cubes. Ten WRC blocks were randomly selected to provide an extractives baseline using the methods of Daniels and Russell (2007). An additional set of ten blocks was stored in the lab under dry, dark conditions and analysed for extractives

after one year. Sixty blocks were left unexposed to serve as controls in soil block decay testing. Thirty WRC blocks were Soxhlet extracted with a series of solvents (hexane, dichloromethane, ethyl acetate, methanol, ethanol and water), soaked in distilled water, and oven-dried at 105°C to provide a set of samples with minimal extractives as a control in the soil block decay tests. All samples were conditioned at 40°C for 48 hours and weighed before pre-conditioning. Ninety blocks were pre-conditioned in soil contact and ninety were pre-conditioned above ground.

Above-ground pre-conditioning consisted of placement on stainless steel mesh in an environment chamber at 20°C, with intermittent exposure to outside air, and controlled, fluctuating relative humidity from 80 to 98%. Blocks for soil contact pre-conditioning were first placed in rows in bags made from plastic screen door mesh. These mesh bags were then placed in screened soil at 90% water holding capacity. The soil was a sandy loam with high organic matter content obtained from the FPInnovations field test site at the UBC Malcolm Knapp Research Forest. Both soil contact and above ground pre-conditioning were designed to ensure a minimal leaching hazard.

Blocks were removed in groups of five at regular intervals. Each block was split in half radially; half was used for fungal isolation, and half for extractives analysis as described above. Fungal isolations made during this work are not described in this paper.

Samples were removed from pre-conditioning after six months and one year. Twenty two blocks from each group were put into a soil block test based on the AWPA E10-08 method (AWPA 2008). All blocks were conditioned at 40°C for 48 hours and weighed. In replicates of six each treatment group was exposed to *Coniophora puteana* (Schum. ex Fr.) Karst. 9G, *Postia placenta* (Fr. ex Cke.) M. Lars. et Lomb. 120F, and *Trametes versicolor* (L.: Fr.) Quél. 105C. Four blocks from each treatment group were used as check blocks.

In a follow-up experiment 30 longitudinally matched pairs of WRC heartwood blocks were cut. One set of samples was stored in dry, dark conditions not suitable for fungal growth. The other set was pre-conditioned under controlled conditions conducive to fungal growth (20°C and close to 100% humidity). The first month of pre-conditioning was on inert racks in a growth room with intermittent exposure to outdoor air containing natural airspora brought in by a fan. At that point this chamber had to be shut down for repair. The remaining five months pre-conditioning was in a mould chamber run according to American Wood Protection Association Standard E-24 (AWPA 2010), but without spray inoculation of samples. An additional source of inoculum in this chamber was the fungi sporulating on the surface of soil. After six months both sets of blocks were milled, extracted, and analysed for extractives (Daniels and Russell 2007).

3. Results and Discussion

In WRC blocks pre-conditioned in soil contact, the concentration of thujaplicins and terpenes decreased significantly over the first seven weeks and then remained relatively stable for the duration of the test (Figure 1). After one year, only 10% of the initial thujaplicins remained. The concentration of lignans was highly variable but did not show such a substantial loss. Although WRC extractive concentrations have a naturally high variability, changes in extractive concentrations in this work appear to be influenced by date of sampling. As samples were

analysed in different batches, part of the variability observed may be due to differences in extraction efficiency. Nevertheless major changes in extractive concentrations were observable.

In WRC blocks pre-conditioned above ground, the concentration of thujaplicins decreased significantly over the first two months and then dropped more slowly (Figure 2). After one year only 1% of the initial thujaplicins remained. This loss is consistent with that reported by Johnson and Cserjesi (1980) who found significant reductions from WRC shakes after three years in a field test. The relative permanence of the lignans is consistent with analysis of WRC shakes and shingles after 25 years, which found only plicatic acid in significant quantities (Stirling 2010). The concentrations of lignans and terpenes were highly variable between blocks and over the pre-conditioning period, so no meaningful trends could be determined.

Figure 3 summarizes the concentration of extractives before pre-conditioning and after one year of pre-conditioning. To mitigate potential variations in extraction efficiency the last three data points (Weeks 33, 37 and 52) were averaged to provide an indication of extractives concentration after pre-conditioning. Analysis of the unexposed blocks retained to control for volatilization of extractives showed small statistically significant (p = 0.05) increases in lignans and terpenes, but no significant differences in thujaplicin content. These apparent increases are likely due to the high natural variability, potential differences in extraction efficiency, and relatively small sample size (n = 10). The important point is that no significant losses were observed. Extractive leaching is likely not a factor in extractive loss from samples pre-conditioned above ground as they had no contact with liquid water. Extractive leaching is likely a very minor factor in extractive loss from samples pre-conditioned in ground contact, as there was very little direct contact with the soil, and the soil was maintained below its water holding capacity. Therefore, the losses of thujaplicins and terpenes after one year are likely due to biological activity.

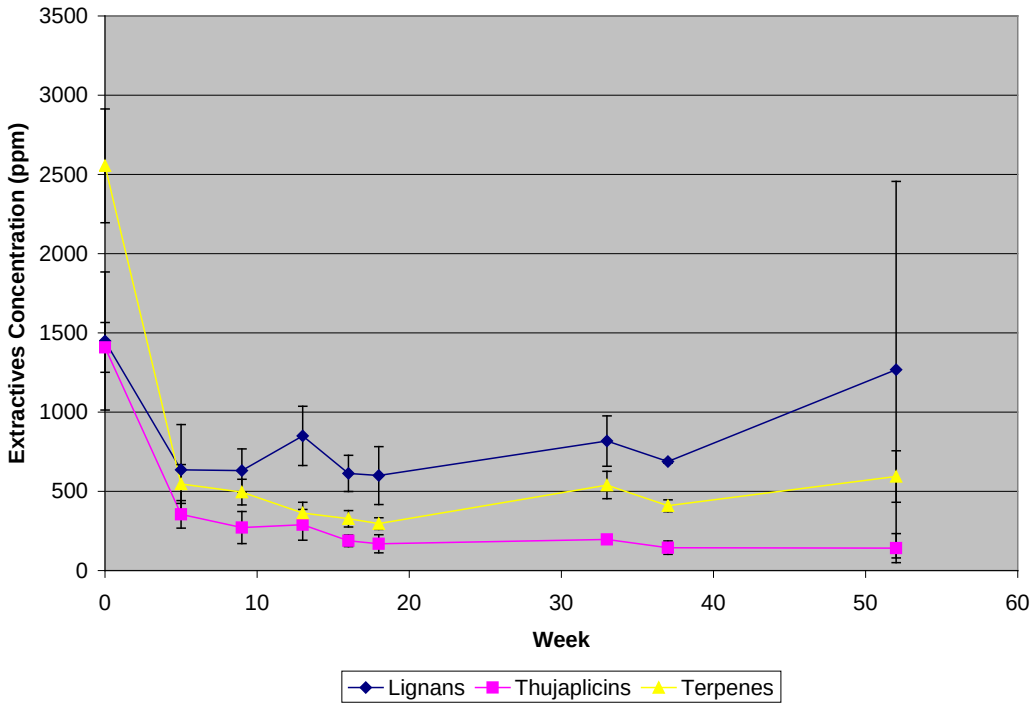

Figure 1: Extractives in Western Redcedar Blocks Pre-conditioned in Soil Contact

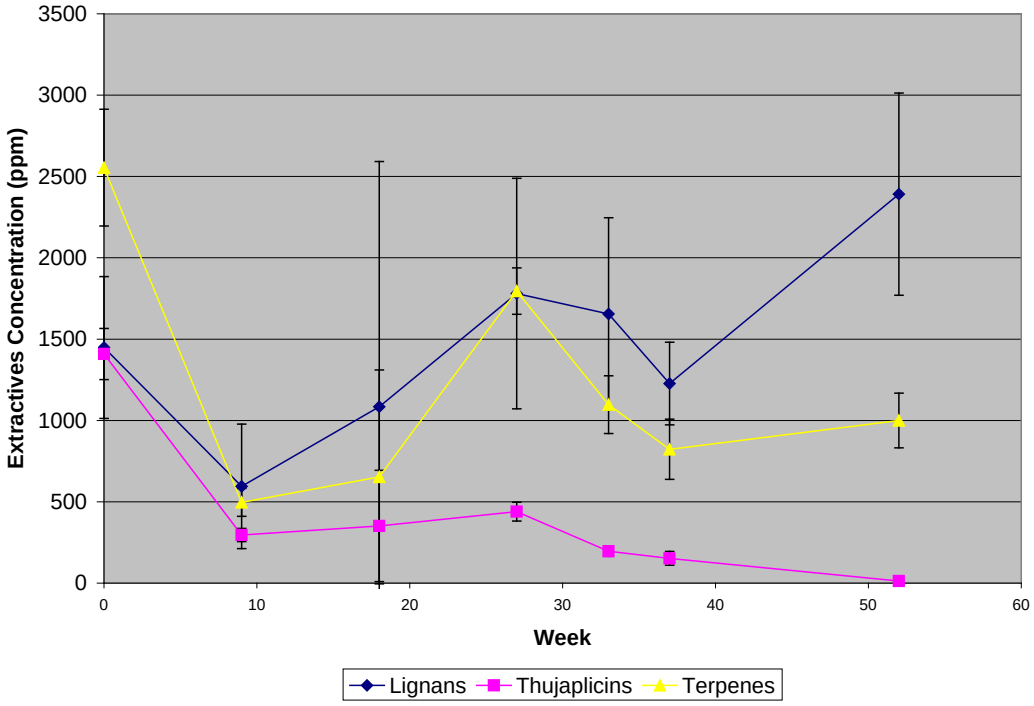

Figure 2: Extractives in Western Redcedar Blocks Pre-conditioned Above Ground

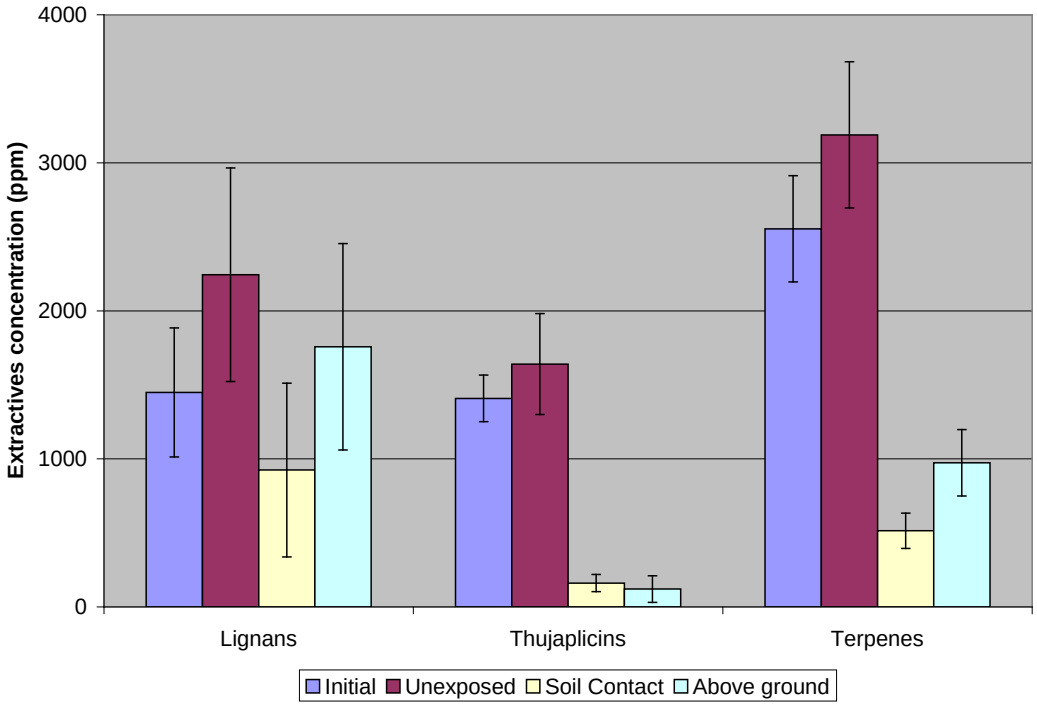

Figure 3: Lignans, Thujaplicins and Terpenes in Western Redcedar Blocks Initially, and After One Year of No Pre-conditioning, Soil Contact, or Above Ground Pre-conditioning

In a follow up experiment to confirm the previous results the concentrations of extractives in matched above-ground pre-conditioned and unexposed samples were determined (Table 1). The concentration of measured lignans was not significantly different in pre-conditioned samples, though thujaplicatin methyl ether showed a slightly higher concentration in pre-conditioned samples. There was a significantly lower concentration of thujaplicins present in pre-conditioned samples. All of the individual thujaplicins were far less abundant in the pre-conditioned samples. Thujic acid was also significantly less abundant in pre-conditioned samples. These data agree with the trends previously observed.

Unknown Compound B was also included in this analysis as recent work suggests it may be an important contributor to durability (Morris and Stirling 2011). There was no significant difference in the amount of Unknown B in pre-conditioned samples.

Table 1: Extractives Concentrations in Pre-conditioned and Unexposed Matched Samples

Extractive	Unexposed (ppm)	Pre-conditioned (ppm)
Plicatic acid	2937 (2596)	2902 (2263)
Thujaplicatin methyl ether*	869 (371)	1004 (421)
LIGNANS	3806 (2962)	3906 (2646)
Alpha Thujaplicin*	1108 (87)	132 (21)
Beta Thujaplicin*	343 (95)	51 (17)
Gamma Thujaplicin*	1028 (213)	360 (173)
Beta Thujaplicinol*	383 (224)	231 (100)
THUJAPLICINS*	2862 (473)	774 (300)
Thujic acid*	3844 (483)	2360 (236)
Methyl thujate	151 (39)	132 (69)
TERPENES*	3995 (499)	2491 (258)
Unknown Compound B**	1.6 (2.5)	1.7 (2.4)

* Statistically significant difference ($p < 0.05$)

** Expressed as peak area ratio per gram

Significantly higher weight losses from *C. puteana* were found in pre-conditioned blocks (Table 2). Untreated, unexposed WRC heartwood used in this experiment had an average weight loss of 25%, putting this material on the borderline of moderately resistant and resistant according to ASTM D2017 if *C. puteana* were a standard fungus in this test, which it is not. Both above-ground and ground contact pre-conditioning resulted in increased weight loss, putting this material into the category of slightly resistant or non-resistant if *C. puteana* were a standard fungus in this test. This reduced resistance to *C. puteana* corresponds with the loss of extractives reported above. Exposure to UV light prior to exposure did not affect weight loss.

The weight loss of unexposed WRC heartwood samples from *P. placenta* resulted in no weight loss, putting this material into the category of highly resistant according to ASTM D2017. Pre-conditioning above-ground had no effect on decay resistance. Pre-conditioning in soil contact for six months resulted in a weight loss of 11%, dropping this material just into the category of resistant. Exposure to UV light did not result in a consistent change in weight loss.

T. versicolor failed to cause weight loss in the untreated pine controls.

Samples that were extracted with a series of solvents and exposed to *C. puteana* and *P. placenta* had very high weight loss, confirming that the compounds associated with the natural durability of WRC are appropriated dubbed “extractives” (Sowder 1929; Roff and Atkinson 1954).

Table 2: Weight Loss in Soil Block Decay Tests

Species	Pre-treatment	Pre-conditioning	Average corrected weight loss (%)		
			Coniophora puteana	*Postia placenta*	*Trametes versicolor*
WRC	None	No environmental exposure	25 (13)*	-0.36 (0.2)	-0.96 (0.1)
WRC	None	Above ground 6 months	45 (3)	-0.68 (0.1)	-0.86 (0.3)
WRC	None	Above ground 12 months	47 (3)	-1.1 (0.2)	-1.0 (0.1)
WRC	None	Soil contact 6 months	47 (3)	11 (16)	-0.98 (0.3)
WRC	Extracted	No environmental exposure	54 (3)	34 (28)	-1.1 (0.09)
Pine	None	No environmental exposure	61 (1)	63 (1)	1.1 (1)

* Standard deviations are shown in parentheses (n = 6)

The data from above-ground pre-conditioning show that the thujaplicins are rapidly depleted from wood products, even under slow leaching conditions. The detection of microorganisms in this material (not detailed in this paper) suggested biodegradation as the likely pathway. Detoxification of the thujaplicins is the likely cause of the decreased resistance to decay by *C. puteana* and, after soil contact, *P. placenta*. These data provide further evidence that the thujaplicins are not associated with the durability of WRC wood in service. As a result, long-term durability predictions based on soil block data without pre-exposure to fungi associated with extractive depletion may not be representative of field performance. Moreover, the continued presence of plicatic acid suggests that it may play a greater role in the long-term durability of WRC than previously thought. This supports the association of lignans with durability in ground contact (Morris and Stirling 2011).

Note that the decay test was not run according to ASTM D 2017 (ASTM 2005), so the application of durability classifications outlined in this standard based on weight losses would not be appropriate.

Future work will seek to determine whether fungi isolated from WRC samples are the causal agents of extractives loss and reduced decay resistance.

4. Conclusions

Significant losses of thujaplicins and terpenes occurred in both above-ground and soil contact pre-conditioning. Lignan concentrations were highly variable but significant losses were not observed after either pre-conditioning. This was confirmed in a follow up experiment with matched samples. Biological degradation was the most likely cause of extractive depletion in the soil contact and above-ground pre-conditioning in this experiment. Both above-ground and soil

contact pre-conditioning resulted in increased susceptibility to decay by *C. puteana*. Soil contact pre-conditioning resulted in increased susceptibility to decay by *P. placenta*.

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