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Relationship between attenuated total reflectance Fourier transform infrared spectroscopy of western juniper and natural resistance to fungal and termite attack

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Abstract: Wood extractives are considered the major factor determining the natural durability of wood. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was assessed for rapid determination of western juniper (*Juniperus occidentalis* Hook. var. *occidentalis*) durability based on extractives in heartwood, sapwood-heartwood and sapwood regions. Durability was assessed by exposing samples to brown-rot decay fungi [*Gloeophyllum trabeum* (Pers.) Murrill and *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel] or eastern subterranean termite (*Reticulitermes flavipes* Kollar). Durability classifications were compared to their extractive contents, along with ATR-FTIR spectra of extracted and unextracted blocks to establish relationships using hierarchical cluster analysis (HCA) and principal component analysis (PCA). Western juniper durability varied with test organisms, but the majority of samples had high fungal and termite resistance. Moderate to weak connections were observed between durability and extractive content, but HCA and PCA analysis were unable to classify durability with accuracy. The absence of non-resistant samples may have influenced the ability of the chemometric methods to accurately categorize durability.

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Keywords: ATR-FTIR, decay fungi, extractives, *Juniperus occidentalis*, natural durability, termite, western juniper

Introduction

Durability is a highly desirable quality that creates a premium for some wood species. Durable woods have broad applications in exterior applications such as poles, fences, flooring, cladding, shingles and marine pilings. Restrictions on applications for certain wood preservatives, such as chromated copper arsenate (Schultz and Nicholas 2000) and increasing public concerns about chemical effects on health and the environment have encouraged the use of naturally durable woods. Compounds in these woods may also serve as models for synthesizing naturally protective compounds (Binbuga et al. 2008).

Resistance to wood decay fungi and insects in many durable wood species has been linked to their extractives content (Rudman and Da Costa 1958). Extractives are minor, non-structural components, mostly ranging from 1 to 5% of total mass, but increasing up to 30% for some tropical species (Hillis 2011). Extractives consist of a broad group of compounds that are soluble with organic or inorganic solvents, or water (Scheffer and Cowling 1966). Non-polar solvents (e.g. hexane, dichloromethane and diethyl ether) remove lipophilic extractives, while hydrophilic extracts are obtained using water or other polar solvents (e.g. acetone, methanol). Extractives may act as toxicants, repellents or antioxidants (Rudman and Da Costa 1958; Woodward and Pearce 1988; Ajuong and Breese 1997; Dizhbite 2004; Hassan et al. 2017), although the exact protective mechanisms of many extractives remain poorly understood (Schultz and Nicholas 2000; Onuorah 2002; Taylor et al. 2006).

Extractive gradients within individual trees and among trees further complicate durability studies (Gierlinger and Wimmer 2004; Guilley et al. 2004). Extractives production is genetically controlled; while cambial age, soil and environmental factors can cause differences in expression among individual trees (Taylor et al. 2002). Furthermore, extractives production is controlled

by many genes, making breeding for durability a major challenge (Harju et al. 2001; Bush et al. 2011; Paques and Charpentier 2015). Extensive lists of durability classifications are available for wood of many species (Scheffer and Morrell 1998; Clausen 2010; Standards Australia 2016), and a given species may vary from durable to non-durable depending on the timber source and conditions under which it was tested. For example, teak (*Tectona grandis* Linn. F.), has a reputation for high durability, but this is reduced for plantation sourced wood (Haupt et al. 2003; Lukmandaru and Takahashi 2009).

The genus *Juniperus* spp. (Cupressaceae) contains a number of highly durable species including western juniper (*Juniperus occidentalis* Hook.), a native of the western United States (Gedney et al. 1999). The heartwood of this species contains cedrol, widdrol and sesquiterpene alcohol compounds that show strong termiticidal and antifungal properties (Orejuela 1995; Craig et al. 2004; Liu 2009; Mun and Prewitt 2011). Changing land utilization practices have resulted in western juniper becoming increasingly abundant on Oregon's eastern rangeland. The presence of this species can reduce the value of rangeland and increase the risk of wildfire, but its utilization is hampered by a lack of viable markets (Leavengood 2008). Interest exists in using western juniper in exterior applications where durability has value, but data is lacking on variation among individual trees.

Durability testing generally exposes wood in the field or uses smaller scale laboratory studies that expose wood blocks to a specific wood decay fungus or insect species (CEN 1994; AWPA 2017a). The process is laborious, time-consuming and limited to a small number of samples that may not be representative of the overall durability of a species. There is a need for more rapid techniques that can properly classify the durability of large numbers of samples rather than a presumed durability based on limited testing.

One possible solution for assessing durability is infrared spectroscopy, a surface-based analytical method that examines the vibration of molecules when a material is exposed to infrared light to generate chemical "fingerprints" unique to the analyzed material (Siesler et al. 2008). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy coupled with chemometric analysis has markedly increased the use of this technique for chemical analysis and predictions of wood constituents such as cellulose, hemicellulose and lignin (Pandey 1999; Stark and Matuana 2007; Zhou et al. 2015a). Extractives-related studies on Scots pine (Holmgren et al. 1999; Nuopponen et al. 2003), eucalyptus (Popescu et al. 2007) and rosewood (Wang et al. 2015), have also produced promising results for the detection of extractives important to durability. Near infrared (NIR) spectroscopy has received considerable attention for estimating wood properties, while relatively few studies have used ATR-FTIR spectroscopy for this purpose. However, the latter technique covers the molecular vibration region making it potentially more useful for identifying important functional groups related to extractives as opposed to the NIR region which is dominated by overlapping overtone and combination bands of mid-IR vibrations.

The aim of this study was to explore the relationships between western juniper extractives content and variations in resistance to fungal and termite attack using ATR-FTIR. Spectral data were assessed using principal component analysis and hierarchical cluster analysis to determine if these techniques could be used to non-destructively establish durability classes for this wood species. It was hypothesized that variability in extractives, fungal and termite resistance from pith-to-bark (from heartwood to heartwood-sapwood to sapwood region), contributes to wood durability and could be detected using ATR-FTIR.

Materials and methods

Samples and sample preparation: Wood disks (150 mm thick) were cut at breast height from five freshly felled western juniper trees in northern California. Disk diameter, sapwood (SW) and heartwood (HW) width (easily observed owing to reddish brown heartwood), and number of growth rings were recorded for each disk (Table 1). The disks were air-dried outdoors, under cover with regular air flow for approximately 3 months. Radial strips (50 mm wide) were then cut from each disk, with strip lengths varying from 100 to 150 mm (the wood within 5 mm of the pith was discarded). The remainder of each strip was cut into 15 mm squares moving from the HW to the HW-SW boundary and finally to the SW (Figure 1). Blocks with defects were discarded and the remainder labeled and separated for extractives analysis, infrared spectroscopy, wood decay tests, and termite tests. Prior to testing, each block was oven-dried at 50 ± 2°C for 24 h to determine density and moisture content according to ASTM Standard D4442-16 (ASTM 2017), except that 50°C was used instead of 103°C to minimize extractives degradation, as certain extractives are known to be highly volatile and sensitive to elevated temperatures (Scheffer 1973).

Total extractives analysis: A total of 102 blocks (15 mm cubes) from the heartwood, sapwood-heartwood boundary and sapwood of each disk were ground to pass a 60 mesh screen using a Wiley mill (Arthur H. Thomas Co., Swedesboro, NJ, USA) and the resulting ground wood sealed individually inside air-tight plastic bags that were stored in the dark at 5°C until used. Total extractives analysis was conducted using a soaking technique allowing extraction of many samples simultaneously (Taylor et al. 2006). This method was chosen instead of conventional Soxhlet extraction owing to the large number of

Table 1: Characteristics of the five western juniper disks used to evaluate extractives content and durability.

Disk	Diameter (mm)	Heartwood diameter (mm)	Sapwood diameter (mm)	Heartwood: sapwood ratio	Growth rings
1	344	175	169	1.04	92
2	425	260	165	1.58	103
3	400	195	205	0.95	102
4	458	310	148	2.09	107
5	520	356	164	2.17	77

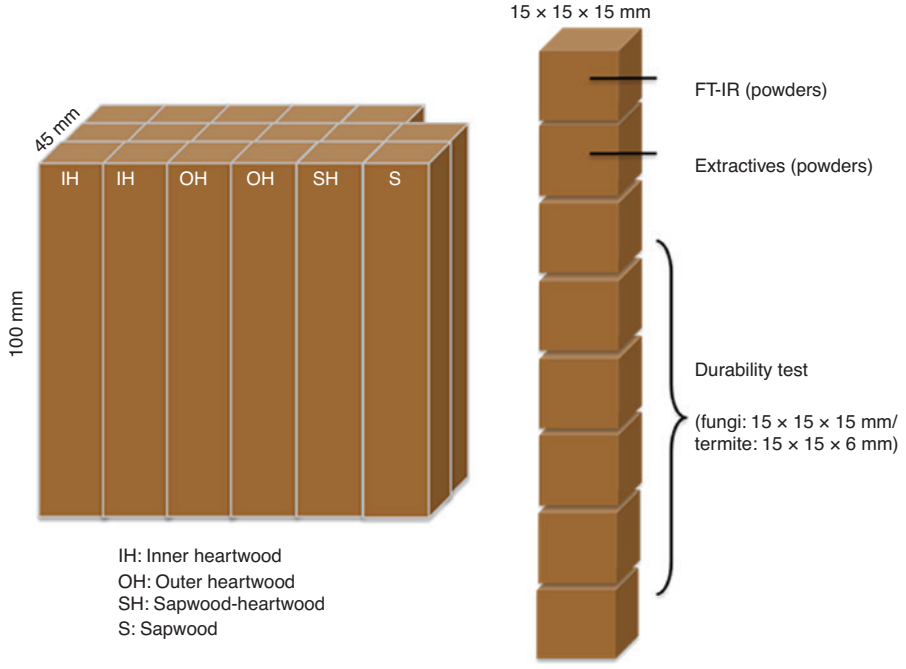

Figure 1: Illustration of the western juniper sample cutting pattern and test blocks prepared for each test in this study.

extractions required. However, complete extraction may not occur, and results can only be compared between tested wood samples, and not as an absolute measure of extractives content.

The ground wood powder was weighed (0.5 ± 0.05 g) and placed inside an individual fabric filter bag (Nuiby unbleached tea filter bags, 6.1 × 8.1 cm), labeled and weighed again to obtain an initial wet weight with bag. Three individual tea bags (replicates) were prepared for each wood powder from each radius location from the five disks. The bags were then oven-dried at 50 ± 2°C for 24 h and an initial oven-dried weight was recorded. The samples were then sequentially extracted using hexane (Fisher Scientific, 99.9%), methanol (Fisher Scientific, 99.9%) and hot water. Extracted samples were dried in a glass desiccator prior to weighing. The solvent selection was based on previous literature that showed effective removal of extractives from western juniper using these solvents (Mun and Prewitt 2011; Tumen et al. 2013a). We recognize that this process might not recover all extractives, but it provided a rapid reproducible method for processing many samples.

A 10 l Erlenmeyer flask was filled with 9 l of hexane along with the filter bags and a stir bar. The flask was then heated at 60°C with continuous stirring for 24 h. The bags were removed, rinsed with hexane and oven-dried at 50 ± 2°C for 24 h. Mass loss after extraction was recorded as the extractives content of each sample. These steps were then repeated using 95% methanol. For hot water extraction, the bags were placed in a 6 l Erlenmeyer flask filled with distilled water and boiled in a water bath for 6 h. The bags were removed from flasks and rinsed with distilled water, then air-dried overnight before they were oven-dried at 50 ± 2°C for 48 h and weighed. Weight loss after all three extractions were recorded as the total extractives content.

Durability study

Wood decay testing

Decay resistance was assessed following procedures described in AWPA Standard E10 (AWPA 2017b). A total of 204 western juniper blocks (15 mm cubes) were conditioned at 20°C and 65% relative humidity to constant weight. Blocks were then oven-dried at 50 ± 2°C, weighed (nearest 0.01 g) and sterilized by exposure to 2.5 mrad of ionizing radiation from a ^{60}Co source. Blocks were kept in a sealed sterile container for no more than 2 weeks until testing.

Decay chambers were 454 ml French square glass bottles half-filled with soil and a western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] wood feeder strip (25 × 20 × 3 mm) was placed on the soil surface. The bottles were sterilized by autoclaving at 121°C for 45 min. A small plug (10 mm diameter) cut from the edge of an active culture of *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel (isolate Madison #698, USDA Forest Products Laboratory, Madison, WI, USA) or *Gloeophyllum trabeum* (Pers.) Murrill (isolate Madison #617) grown in 1.5% potato-dextrose agar (PDA) was placed on one edge of the wood feeder. These fungi cause brown-rot decay and are among the principle degraders of wooden structures, especially in temperate regions (Green and Highley 1997).

The jars were incubated at 28°C for 1 week for *G. trabeum*, or 2 weeks for *Rh. placenta*, to allow fungal mycelium to cover the feeder strips. A sterilized western juniper block from each disk and radius location was then placed, cross-section down, on the feeder strip and the jar was incubated at 28°C for 12 weeks. Three replicate blocks from each radius location of each disk were prepared for each fungus. Additionally, ten blocks of non-durable ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) sapwood were used as a control for each fungus. Two pine blocks were removed, oven-dried and weighed each week starting from week 8 to monitor whether weight loss had reached 50%. The test was terminated once weight loss was greater than 50%.

The blocks were removed, scraped clean of soil and fungal mycelium, and weighed to determine the wet weight. The blocks were then oven-dried overnight at 50°C and weighed again. The difference between the oven-dried weight before and after the test was used to determine wood mass loss.

Decay resistance was classified using the scale described in AWPA Standard E30 (AWPA 2017a) where 0–10% weight loss is highly resistant, 11–24% is resistant, 25–44% is moderately resistant, and >45% is non-resistant to decay.

Termite testing

Western juniper blocks (15 × 15 × 6 mm, R × T × L) from all five disks and radius locations were tested against the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) following a modified no-choice termite test (termite force fed on only one type of wood block) described in AWPA Standard E1 (AWPA 2017c). Test jars were filled with 50 g white sand and 9 ml of distilled water and allowed to stand for 2 h prior to use. Wood blocks were oven-dried for 24 h at 50°C, weighed to the nearest 0.001 g and conditioned to room temperature before the test was initiated. Each test block was placed on top of a piece of aluminum foil on the surface of the damp sand and 3 g of termites (approximately 150 termites including one to three soldiers) were added to each test jar. Three replicates were used for each test. All test jars were incubated for 28 days at 26 ± 2°C and 50 ± 5% relative humidity. At the end of the test, the blocks were cleaned, and mass loss due to termite feeding was calculated based on differences between oven-dried weight before and after exposure.

Collection of spectra

ATR-FTIR spectroscopy

Mid-infrared spectra in the range 4000 and 650 cm^{-1} were recorded using an ATR system with a ZnSe crystal head Smart iTR (Thermo Scientific, USA) mounted on a Nicolet iS50 spectrometer (Thermo Scientific, USA). Spectral analysis of the powdered western juniper, including extracted samples from the extractives analysis (all stored in a desiccator prior to testing), was done using OMNIC software version 9.2 (Thermo Scientific, USA). A background spectrum was obtained every 15 min to exclude signals that were not relevant to the sample. An individual spectrum represented an average of 32 scans at a resolution of 4 cm^{-1}. Samples were analyzed 3 times and averaged to obtain representative spectra.

The resulting spectra were pre-processed by performing a standard baseline correction (for correcting vertically sloped, curved or shifted spectra), smoothing and converted to second derivatives (smoothed and derived using Savitzky-Golay with seven smoothing points filter and a three-order polynomial). The use of second derivatives eliminated the effects of baseline shift and resolved overlapping bands. The raw spectra and the second derivatives were used for further analysis.

Statistical analysis: All analyses were conducted using R Studio version 1.0.136 (RStudio Team 2016). Average fungal and termite feeding weight losses along the radial transect were compared by two-way analysis of variance (ANOVA) tests using Tukey's honest significant difference (Tukey HSD) test procedure for multiple comparisons ($\alpha = 0.05$). Variables tested for weight loss on each fungal and termite species included disk, radial location and extractives content.

Chemometrics analyses using hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed using the R package "Chemospec" (Hanson 2017) on the second derivatives spectral dataset. Data for this analysis were categorized as N = non-resistant (included non-resistant and moderately resistant categories owing to the small number of non-resistant samples), R = resistant, and RR = highly resistant based on the results of durability tests against *G. trabeum*, *Rh. placenta* and *R. flavipes*.

HCA and PCA are unsupervised pattern recognition techniques, with no assumptions made on group membership, and are commonly used to detect groups in a dataset. HCA plots (dendrograms) calculated based on Euclidean distance were used to examine the similarity of samples, while loadings and score plots were used with PCA to visualize data and identify the spectra regions that best explained the observed variations, respectively.

Results and discussion

Extractives analysis

Total extract yields were between 1.5 and 4.5% (dry weight basis) (Figure 2). Methanol extracts gave the highest yields, followed by hot water and hexane. These results were within the wide ranges found in previous studies (Kurth and Ross 1954; Adams et al. 1988; Tumen et al. 2013a,b). In general, extractive contents were highest towards the sapwood-heartwood boundary and lowest in the mid-heartwood and sapwood regions. This follows the general pattern of extractives distribution observed in many species, for example, western red cedar (*Thuja plicata* Donn ex D. Don) (DeBell et al. 1999)

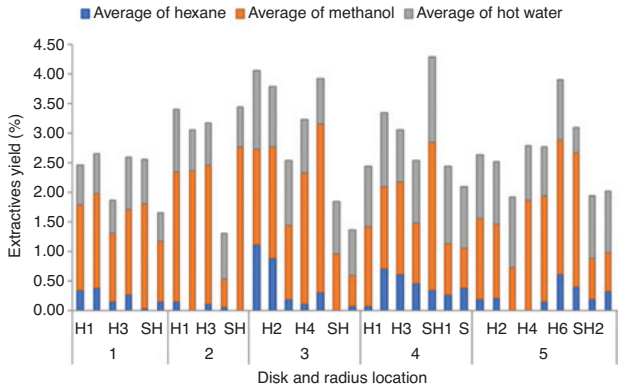

Figure 2: Extractives yield (%) from five disks (1–5) of western juniper radially from the pith (H1) to sapwood (S) after sequential extraction using hexane, methanol and hot water.
Each value consists of the average oven dry weight basis ($n = 3$) for each extraction radially. H, heartwood; SH, sapwood-heartwood; S, sapwood.

and European larch (*Larix decidua* Mill). (Gierlinger and Wimmer 2004).

A related study of western juniper from northern California (Miyamoto et al. 2018) identified the main extractive compounds as cedrol (51%), α-cedrene (5%) and widdrol (3%). Cedrol was also previously reported as the main compound extracted from western juniper heartwood (Adams et al. 1988).

Mass loss by decay fungi

Weight losses after the 12-week exposure to *G. trabeum* and *Rh. placenta* (Table 2) indicated the majority of the samples were classified as either highly resistant (mass loss <10%) or resistant (11–24%), especially against *G. trabeum*. As expected, the inner heartwood (closer to the pith) and outer heartwood (closer to the heartwood-sapwood boundary) showed higher decay resistance, compared to the sapwood regions where the majority of samples were classified as moderately resistant with mass losses ranging from 25 to 44%.

Western juniper is classified as very resistant (Scheffer and Morrell 1998) to resistant to decay fungi (Clausen 2010) which is in agreement with our results. Even under the warm, wet tropical conditions of Hilo, Hawaii, western juniper heartwood displayed high resistance to decay in above-ground field tests (Morrell 2011). However, the sapwood and sapwood-heartwood samples in that study displayed lower resistant to decay. Nevertheless, western juniper fence posts have been observed to provide service lives ranging from 23 to 66 years in Western Oregon (Morrell et al. 1999).

We observed higher variability in weight losses for samples exposed to *G. trabeum* (Table 2). The presence of included sapwood (likely more susceptible to decay and therefore higher weight loss), which is common in western juniper heartwood, may have affected results.

Mass loss by termite attack

Western juniper wood was highly resistant or resistant to *Re. flavipes* attack at all radial positions (Table 3) including sapwood. Excellent termiticidal activity was noted and complete mortality was observed at the end of the 4-week test. Termite mortality in ponderosa pine sapwood (control) ranged from 0 to 20% with mass losses >45%, indicative of healthy and aggressive termites. Resistance of western juniper to termite attack is comparable to (Minore 1983; Gonzalez 2004), or better (Kirker et al. 2013) than western red cedar, a highly valued commercial species frequently used outdoors.

A similar result was observed when western juniper heartwood was exposed to *Coptotermes formosanus* (Shiraki) in an above-ground test while sapwood samples were susceptible (Morrell 2011). The sapwood we used was highly resistant to termite attack, but *Re. flavipes* is less aggressive than *C. formosanus*. While sapwood is generally considered susceptible to decay and termite attack, certain species, including western juniper sapwood, have displayed high termiticidal activity. Similarly, heartwood from western juniper aggressively leached with solvents still exhibits residual termite and antifungal activity (in the 11–24% range = resistant), suggesting that additional underlying mechanisms along with extractives contribute to the durability of this species (Kirker et al. 2013). For example, Adams et al. (1988) reported complete mortality of *Re. flavipes* within the first few days of exposure to western juniper sapwood sawdust. *Coptotermes formosanus* is one of the most aggressive and destructive termites in the United States (Lax and Osbrink 2003), and may have higher resistance to western juniper extractives although these compounds are both antimicrobial and anti-termitic (Adams et al. 1988; Mun and Prewitt 2011).

Sapwood is generally considered as non-durable to biodegradation due to lack of toxic extractives and higher starch content (easily available to decay agents) (Zabel and Morrell 1992). However, the western juniper sapwood we examined displayed excellent resistance to termites, but not to fungal attack; hence, it is possible that the sapwood contained extractives toxic to termites. Essential oils from the sapwood of several Turkish juniper species were reported to contain high concentrations of

Table 2: Weight losses (%) for western juniper after exposure to *G. trabeum*, *Rh.placenta* and *Re. flavipes* by disk and radial position.

			Weight loss (%)					
			G. trabeum		*Rh. placenta*		*Re. flavipes*	
Disk	Label	Radial position	Average	Std dev	Average	Std dev	Average	Std dev
1	H1	IH	3.53 b	3.06	11.75 b	0.69	0.45 c	0.78
	H2	IH	1.98 b	3.42	15.03 b	2.55	3.72 bc	0.90
	H3	OH	6.64 b	7.79	12.04 b	2.11	3.47 bc	0.88
	H4	OH	7.01 b	1.16	12.91 b	0.93	7.75 b	2.04
	SH	SH	3.17 b	1.05	12.83 b	2.31	17.55 a	0.57
	S	S	16.70 a	0.32	33.81 a	1.64	16.31 a	4.21
2	H1	IH	2.82 a	1.43	4.19 b	0.38	0.90 c	0.78
	H2	IH	3.05 a	1.90	13.53 b	2.01	1.04 c	0.90
	H3	OH	1.97 a	1.51	28.19 a	2.70	2.19 bc	0.90
	H4	OH	14.60 a	20.10	29.62 a	7.96	5.33 b	1.79
	SH	SH	10.09 a	8.14	11.01 b	1.13	10.63 a	2.03
3	H1	IH	3.72 cd	0.36	3.42 b	1.89	1.19 bc	0.01
	H2	IH	4.19 cd	0.66	5.96 b	2.04	2.50 b	1.25
	H3	OH	9.82 c	0.56	9.81 b	7.20	1.24 bc	0.03
	H4	OH	4.04 cd	0.43	10.83 b	4.02	0.00 c	0.00
	H5	OH	2.10 d	1.37	4.38 b	1.48	0.00 c	0.00
	SH	SH	36.73 b	0.12	10.36 b	5.34	3.60 b	0.02
	S	S	44.80 a	6.27	29.58 a	1.86	10.74 a	2.05
4	H1	IH	14.98 c	3.59	15.99 bc	2.80	0.69 c	0.59
	H2	IH	5.45 d	1.49	6.02 c	3.31	0.00 c	0.00
	H3	OH	7.32 d	0.79	14.92 bc	1.29	3.26 bc	0.73
	H4	OH	15.13 c	4.67	15.19 bc	3.67	1.58 c	0.67
	SH1	SH	3.18 d	3.11	9.79 bc	1.76	5.64 b	2.49
	SH2	SH	54.29 a	11.36	18.73 ab	8.92	13.23 a	2.25
	S	S	29.37 b	3.42	27.72 a	0.90	13.99 a	0.73
5	H1	IH	6.82 d	1.27	4.13 de	3.08	0.99 a	0.85
	H2	IH	13.44 d	4.80	11.87 cd	5.47	1.51 cd	0.03
	H3	IH	25.35 bc	5.81	10.61 de	3.55	6.63 b	2.29
	H4	OH	17.82 cd	5.02	1.8 e	0.36	3.27 c	1.60
	H5	OH	7.43 d	2.79	4.17 de	2.67	1.32 d	0.01
	H6	OH	4.69 d	2.28	0.92 e	0.83	2.31 cd	0.80
	SH1	SH	9.23 d	2.56	16.80 bc	4.30	0.00 d	0.00
	SH2	SH	48.23 a	3.69	23.47 ab	3.55	11.16 a	1.63
	S	S	38.72 ab	5.18	27.04 a	5.12	11.44 a	0.86

Each value is the average of three specimens per organism and wood location. The same letters in a column indicate that there were no statistical differences between the specimens according to Tukey HSD at $\alpha = 0.05$. IH, Inner heartwood; OH, outer heartwood; SH, sapwood-heartwood; S, sapwood; Std dev, standard deviation.

Table 3: Durability classifications of western juniper samples exposed to *G. trabeum*, *Rh. placenta* or *Re. flavipes* according to radial position in accordance with AWPA Standard E30 (AWPA 2019).

			Durability classification (% of samples)			
Organism	**Radial position**	***n***	**Highly resistant**	**Resistant**	**Moderately resistant**	**Non-resistant**
G. trabeum	Inner heartwood	33	75.8	18.2	6.1	0.0
	Outer heartwood	33	72.7	3.0	24.2	0.0
	Sapwood-heartwood	24	54.2	8.3	16.7	20.8
	Sapwood	12	0.0	25.0	66.7	8.3
Rh. placenta	Inner heartwood	33	51.5	42.4	6.1	0.0
	Outer heartwood	33	33.3	51.5	15.2	0.0
	Sapwood-heartwood	24	25.0	66.7	8.3	0.0
	Sapwood	12	0.0	8.3	91.7	0.0
R. flavipes	Inner heartwood	33	100.0	0.0	0.0	0.0
	Outer heartwood	33	100.0	0.0	0.0	0.0
	Sapwood-heartwood	24	54.2	45.8	0.0	0.0
	Sapwood	12	8.3	91.7	0.0	0.0

widdrol (up to 22% of the % total peak area in the GC-MS chromatogram), α-cedrol (up to 19%) and traces of other extractives that may contribute to termite resistance (Uçar and Balaban 2002). Additionally, sapwood resistance to termite attack was also reported on another *Juniperus* species (eastern juniper, *Juniperus virginiana* L.) (Köse and Taylor 2012).

Relationship between mass loss and extractive content

Relationships between extractive contents and mass losses caused by *G. trabeum*, *Rh. placenta* and *Re. flavipes* were assessed using Pearson's correlation coefficient (Figure 3). Negative correlations were exhibited between

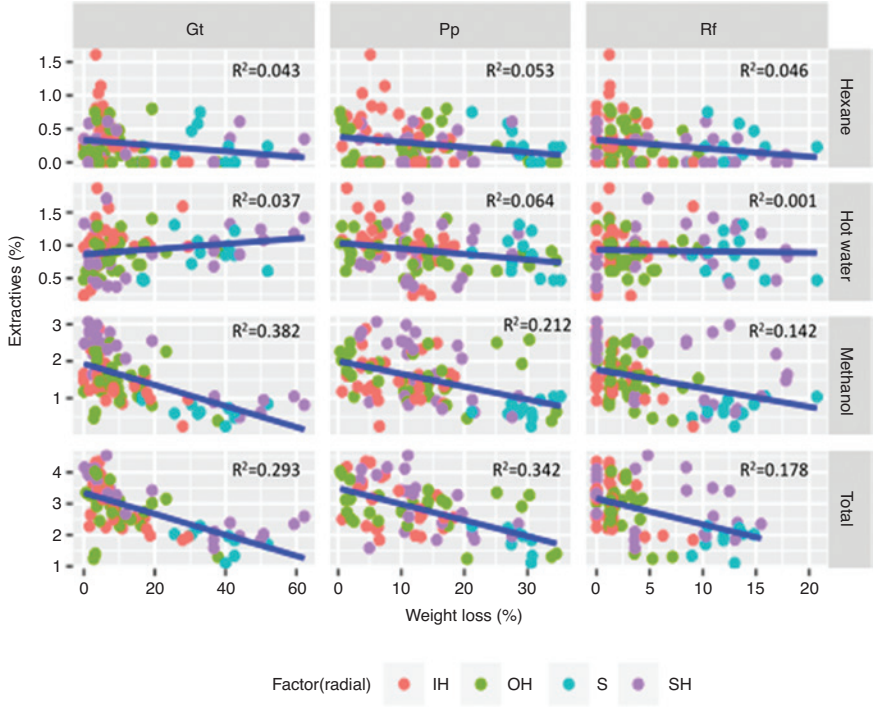

Figure 3: Relationship between weight losses (%) caused by exposure to *G. trabeum* (Gt), *Rh. placenta* (Pp) or *Re. flavipes* (Rf) and extractive contents radially from IH, inner heartwood; OH, outer heartwood; SH, sapwood-heartwood; and S, sapwood.

mass losses and extractives yield, indicating that increasing extractive contents were associated with decreased weight loss, i.e. higher decay resistance. Correlations between mass loss and extractives levels were moderate ($R^2 = 0.3–0.5$) based on Cohen's classification (Cohen 1988) (Figure 3).

Methanol extract levels were most closely correlated with mass losses caused by wood decay fungi, especially *G. trabeum* ($R^2 = 0.38$) (Figure 3). Methanol extracts levels also accounted for 38% and 21% of variability in mass loss to *G. trabeum* and *Rh. placenta*, respectively (Figure 3). Hexane extract levels were poorly correlated ($R^2 = 0.04$) with mass losses to *G. trabeum* and *Rh. placenta* (Figure 3). Previous studies on eastern red cedar indicated strong inhibitory properties of methanol extracts on *G. trabeum* (Mun and Prewitt 2011).

Extractives content and mass losses by termites were poorly correlated, indicating that other factors may contribute to western juniper durability (Kirker et al. 2013) (Figure 3). For example, total extractives accounted for 18% of variability in mass loss to *Re. flavipes* (Figure 3); however, Adams et al. (1988) reported that hexane and methanol extracts of western juniper heartwood were associated with high termiticidal activity against *Re. flavipes*, with complete mortality observed within 1 week of exposure. Other potential reasons for the poor relationships include absence of low durability samples, incomplete extraction, the range of extractive content was relatively small and included sapwood which increased variability in the results.

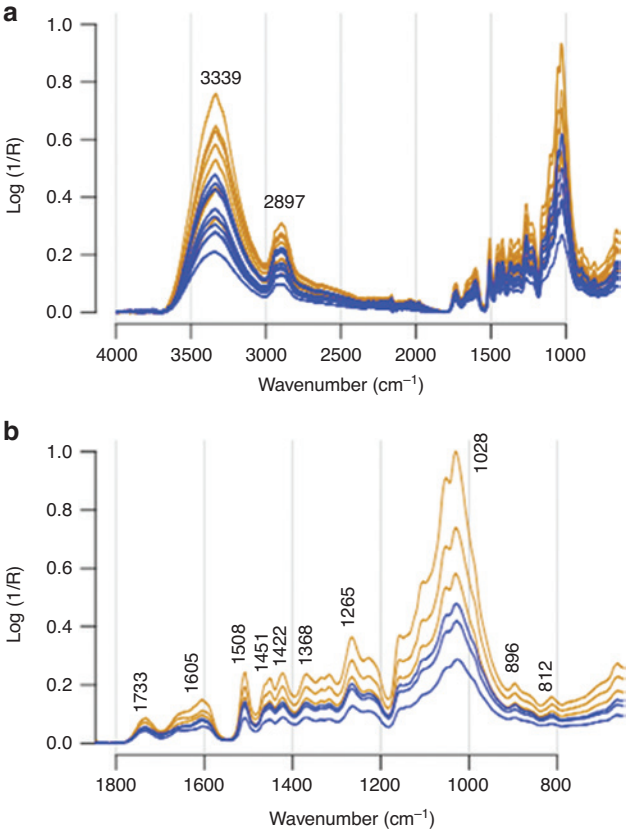

Figure 4: Comparisons between ATR-FTIR spectra of non-extracted (blue) and extracted (yellow) western juniper for bands in the range 4000–650 cm^{-1} (a) and 1800–650 cm^{-1} (b).
Each spectrum is the mean of three spectra from different radial locations.

Extracted and non-extracted spectra

ATR-FTIR spectra of extracted and non-extracted western juniper were divided into two sections: the functional group region between 4000 and 1800 cm^{-1}, and the fingerprint region of 1800–650 cm^{-1} (Figure 4). Two broad bands were observed at 3339 cm^{-1} (O-H stretching vibrations) and 2897 cm^{-1} (aliphatic C-H) (He et al. 2007; Mattos et al. 2014). The band at 3339 cm^{-1} is characteristic of lignocellulosic materials including bamboo, jute yarn and cotton (He et al. 2007; George et al. 2013; Tomak et al. 2013). Differences in peak intensity between extracted and non-extracted samples were observed (3339 cm^{-1} and 2897 cm^{-1}); however, there was little difference in the actual shape of the peaks (Figure 4a).

The peaks within the region 1800–800 cm^{-1} represent cellulose, hemicellulose and lignin. While specific peak intensities varied, no major changes were observed before and after extraction in the shape of spectral peaks (Figure 4b). This may be a consequence of the low total extractives levels (1.92–3.91%) of the samples (Figure 1). For example, ATR-FTIR spectroscopy could detect carvacrol when added to wood at a high level (5% w/w), but this compound was undetectable at lower levels (1–3% w/w) (Lipeh et al. Unpublished). Previous studies have shown that bands related to wood extractives occur at 1730 cm^{-1}, 1633 cm^{-1}, 1600 cm^{-1}, 1510 cm^{-1} and 1271 cm^{-1} (Nuopponen et al. 2003; Pandey and Pitman 2003; Colom and Carrillo 2005; Schauwecker et al. 2013; Mattos et al. 2014; Zhou et al. 2015b) (Table 4). Peaks were still present in these regions for the extracted spectra, but changes in absorbance at these wavelengths were difficult to detect.

The ATR-FTIR spectral technique used in this study can be affected by surface properties of the analyzed wood (Stuart 2004) hence spectra were converted to the second derivative to enhance apparent resolution (Siesler et al. 2008) and produce clearer separation of features.

Table 4: Possible identities of FT-IR bands found in the western juniper.

Wavenumber (cm^{-1})	Reference wavenumber (cm^{-1})	Band assignment	Potential compounds	Sources
1733	1740–1720	C=O stretching vibrations produced by ester carbonyl	Fat, wax or esterified resin acids	Colom and Carrillo 2005
1605	1610–1590	C=C stretching or aromatic ring deformation	Aromatic compounds, phenolic group	Pandey and Pitman 2003
1508	1515–1505	Olefinic double bond	Aromatic compounds	Schwanninger et al. 2004
1265	1271–1268	Deformation vibration within benzene rings	–	Mohebby 2005
896	930–915	Aromatic C-H out-of-plane deformation, pyrene ring vibration	–	Popescu et al. 2007
812	811	Carbon single bonded oxygen	–	Zhou et al. 2015a,b

Second derivative spectra of samples before and after extraction showed differences at 1800–1750 cm^{-1}, 1600–1500 cm^{-1} and 1200–1000 cm^{-1} (Figure 5). The region at 1800–1750 cm^{-1} is associated with C=O stretching vibrations produced by ester carbonyls (around 1730 cm^{-1}) in lipids, wax or esterified resin acids (Zhou et al. 2015b), while the band at 1750 cm^{-1} is associated with conjugated carboxylic aldehydes of lignin and extractives (Colom and Carrillo 2005; Silverstein et al. 2005). The region at 1600–1500 cm^{-1} is related to lignin structures, specifically at bands 1592 cm^{-1} and 1504 cm^{-1}, and corresponds to the symmetrical stretching of the C=C aromatic benzene rings (Mattos et al. 2014; Zhou et al. 2015b). C=C aromatic rings are also present as functional groups in western juniper extractives that contribute to durability. The bands at 1200–1000 cm^{-1}, specifically 1157 cm^{-1}, correspond to C-O-C asymmetrical stretching in both hemicellulose and cellulose.

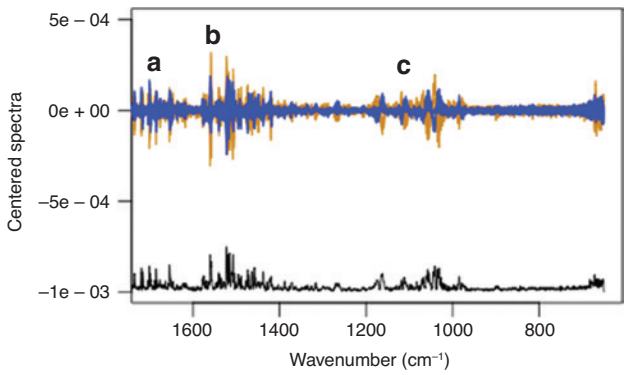

Figure 5: Variation among non-extracted (blue) and extracted (yellow) western juniper ATR-FTIR second derivative spectra from 1800 to 650 cm^{-1}.
(a) 1800–1750 cm^{-1}, (b) 1600–1500 cm^{-1}, and (c) 1200–1000 cm^{-1}. An untreated spectrum is shown below the second derivative spectra.

HCA was performed using second derivative FTIR spectra (Figure 6). The resultant dendrograms from these analyses allowed visualization of extracted and non-extracted western juniper groups based on the complete linkage method with Euclidean distance as the similarity measurement. The full spectrum (4000–650 cm^{-1}) was used to group samples into one block, with the non-extracted spectra, and another with part of the non-extracted and all of the extracted spectra (Figure 6a). This region is not particularly useful for extractives analysis, but it might be useful for classifying the extracted and non-extracted samples. The fingerprint region (1800–650 cm^{-1}) showed no obvious differences between the two groups (Figure 6b). While we used an excess of extraction solvent, it is possible that all extractives were either not removed or deposited or the amount removed did not differ greatly among samples as extractives levels did not have a large range.

PCA was used for qualitative recognition of different sample groups. The second derivatives (1800–650 cm^{-1}) were subjected to PCA analysis, with the first three principal components (PCs) explaining 82.7% of the variation in the dataset (PC1 = 56%, PC2 = 5.7% and PC3 = 5.7%. The distribution of samples along PC1 (Figure 7a) indicated slight overlap between the extracted and non-extracted groups. The majority of non-extracted spectra

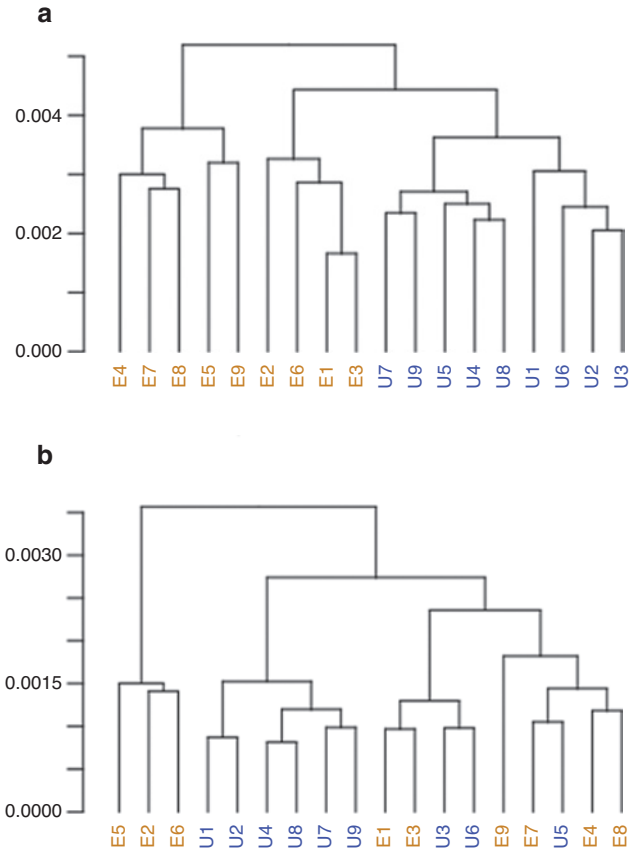

Figure 6: Dendogram of hierarchical clustering (with Euclidean distance) of the non-extracted (U, blue) and extracted (E, yellow) western juniper.
ATR-FTIR spectra using second derivatives at 4500–650 cm^{-1} (a) and 1800–650 cm^{-1} (b).

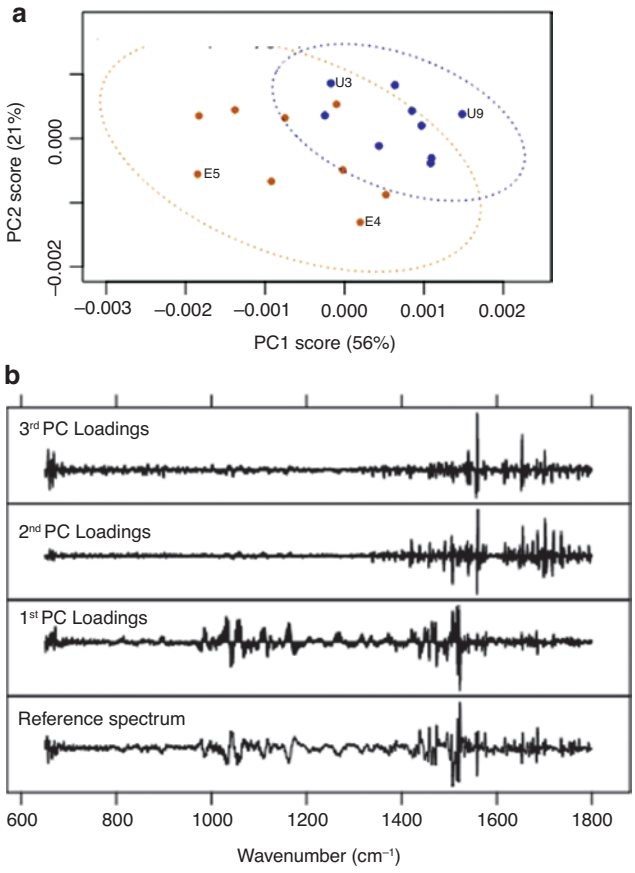

Figure 7: (a) PC1 vs. PC2 scores plotted using classical PCA on the ATR-FTIR spectra of non-extracted (blue) and extracted (yellow) western juniper, and (b) the loadings profile of the first three PCs. Dashed lines on the PC1 vs. PC2 plot indicate the 95% confidence interval for each group.

had positive PC1 scores, while extracted spectra had negative PC1 scores. Based on the loadings profile (Figure 7b), PC1 variation was associated with the regions 1200–1000 cm^{-1}, and 1400–1600 cm^{-1}. These regions are related to C=C stretching or aromatic ring deformation (1610–1590 cm^{-1}) and deformation vibrations within benzene rings (1271–1268 cm^{-1}). Both these regions are associated with wood extractives (Pandey and Pitman 2003; Mohebby 2005). PC2 variation was related to the region between 1800 and 1400 cm^{-1}. C=O stretching vibrations produced by ester carbonyl bonds (1740–1720 cm^{-1}) from fat, wax or esterified resin acids (Colom and Carrillo 2005) are important in this region. While these results suggest that it might be possible to segregate extracted and non-extracted samples there was very little difference among non-extracted samples. The lack of representation of less durable samples made this process more difficult. The inclusion of a broader range of durability categories might help improve separation.

FTIR for wood durability

Averaged ATR-FTIR spectra (triplicate) were used to examine the potential for sorting based upon durability classes established by exposure to *G. trabeum* (Figure 8). Both "moderately durable" and "non-durable" groups (Table 3) were combined as "non-durable" due to lack of representation of both groups. Only two durability classifications were available for termite resistance, "highly resistant" (RR) and "resistant" (R). Comparisons of averaged spectra between the three different durability classes for *G. trabeum* showed no discernable differences between the highly resistant, resistant and non-resistant western juniper (Figure 8). Similar findings were observed for spectra representing different durability classifications for *Rh. placenta* and *R. flavipes*.

HCA was performed on the second derivative spectra of the fingerprint region (1800–650 cm^{-1}) for different durability classifications established by exposure to

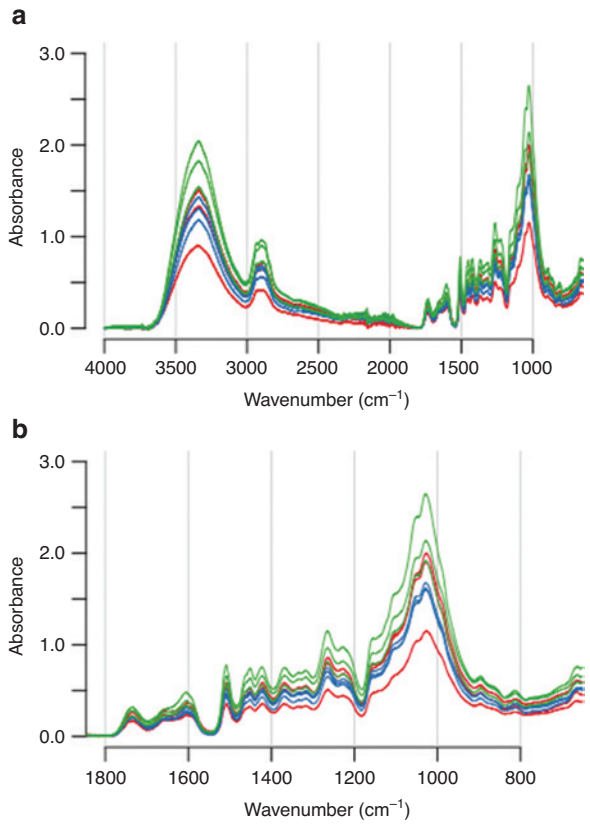

Figure 8: Average ATR-FTIR spectra of western juniper samples according to the durability classification established by exposure to *G. trabeum* at wavenumber (a) 4000–650 cm^{-1}, and (b) 1800–650 cm^{-1}.
Green, highly resistant; blue, resistant; red, non-resistant.

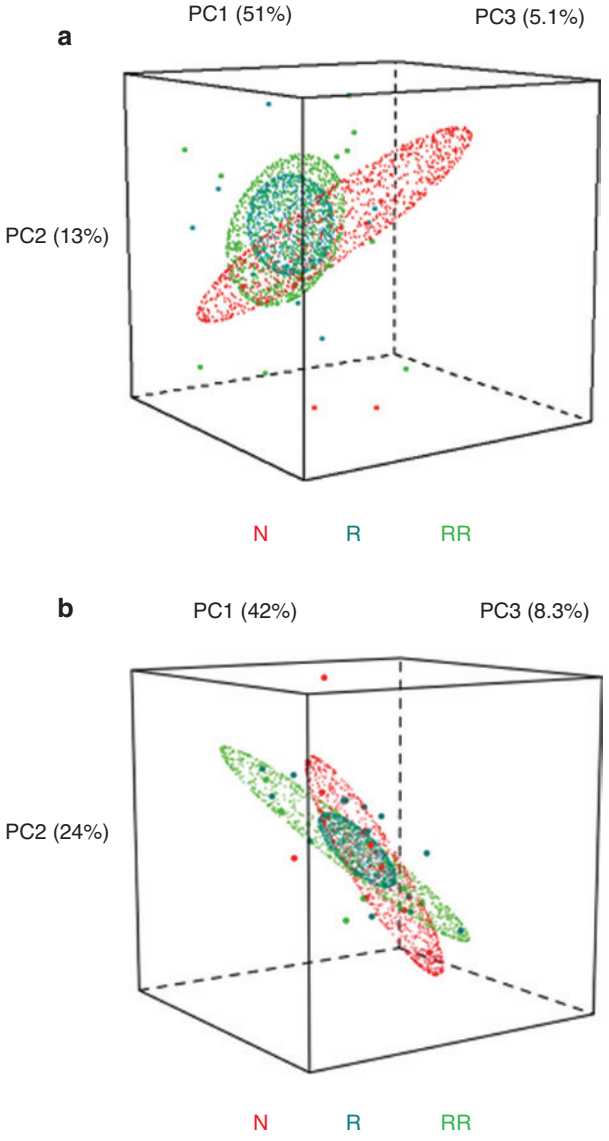

Figure 9: PCA analysis showing 3D representations of the first three principal components (PCs) of spectra using second derivatives for durability classification established by exposure to (a) *G. trabeum*, or (b) *Rh. placenta*.
N, non-resistant; R, resistant; RR, highly resistant.

G. trabeum, *Rh. placenta* and *R. flavipes*, but dendrograms were unable to classify samples based on durability.

Robust PCA (Gharibnezhad et al. 2011) using median absolute deviation (Hanson 2017) was performed on the 1800–650 cm^{-1} region using second derivative spectra to qualitatively discriminate between samples with different durability classifications against *G. trabeum*, *Rh. placenta* and *R. flavipes*. Three PCs (PC selection was based on scree plots) were required to categorize durability of western juniper against *G. trabeum* and accounted for 69.1% of variability in the spectral dataset with PC1 representing 51%, PC2 13% and PC3 5.1% of the variation, respectively. Similarly, the first three PCs were required to categorize resistance against *Rh. placenta* and explained 74.3% of variability (PC1 = 42%, PC2 = 24% and PC3 = 8.3%). *Reticulitermes flavipes* resistance required three PCs for adequate categorization explaining 74.3% of the variability, with PC1 representing 42%, PC2 24% and PC3 8.3% of the variation.

Clustering of data using three-dimensional (3D) PCA plots showed overlap between the three different durability classifications to all the tested organisms (Figures 9 and 10). No obvious groupings were detected using the PCA analysis. Similar results were achieved with the 3D plot using combinations between the first three PCs. These results again illustrate the lack of separation between the durability classes established by exposure to each organism.

Conclusions

Western juniper displayed excellent resistance to fungal and termite attack, but extractives content was poorly correlated to durability classifications. There are a number of

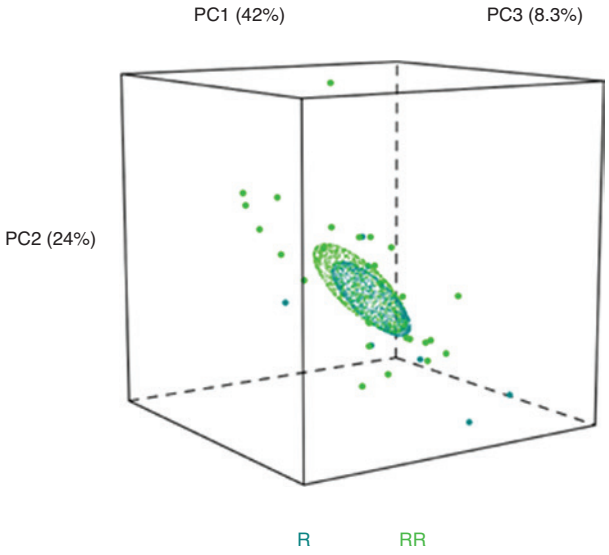

Figure 10: PCA analysis showing 3D representations of the first three principal components (PCs) of spectra using second derivatives for durability classification established by exposure to *Re. flavipes*.
R, resistant; RR, highly resistant.

possible reasons for the separation failure between durability classes. The lack of more decay or termite susceptible samples in the data set likely limited the ability to develop clear delineations between groups. The use of other fungi or termite species might also improve separations.

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