Effects of leaching on fungal growth and decay of western redcedar

Russell J. Chedgy, Young Woon Lim, and Colette Breuil

Abstract: We tested the effect of leaching on the concentration of western redcedar (WRC; *Thuja plicata* Donn ex D. Don) heartwood extractives that are known to exhibit antimicrobial activity and correlated this with fungal growth and decay. We assessed the extractive tolerance of the following fungal species: *Acanthophysium lividocaeruleum*, *Coniophora puteana*, *Heterobasidion annosum*, *Pachnocybe ferruginea*, *Phellinus sulphurascens*, and *Phellinus weirii* by measuring their growth rate (mm/day) on media with or without WRC leachate. These data were correlated with the ability of the fungal species to grow on and decay leached versus nonleached WRC. We used an ergosterol assay to estimate growth and a standard soil-block test to assess decay. We estimated that leaching reduced the concentration of 5 major extractives: (–)-plicatic acid, β-thujaplicin, γ-thujaplicin, β-thujaplicinol, and thujic acid by ~80%. *Phellinus sulphurascens* exhibited the lowest extractive-tolerance in vitro, grew poorly on and caused minimal decay in nonleached WRC, but it grew well on and decayed pine and leached WRC. *Coniophora puteana*, *H. annosum*, and *P. weirii* displayed moderate to high tolerance to leachate, grew on and caused decay in nonleached as well as leached WRC, but their growth and decay were always greatest on leached WRC and pine, suggesting that leaching enhances decay by these fungi. *Acanthophysium lividocaeruleum* and *Pachnocybe ferruginea* exhibited high extractive-tolerance. Whereas *A. lividocaeruleum* clearly caused decay on all types of wood, no decay was observed with *Pachnocybe ferruginea*, which grew very slowly in the different wood species, and it may or may not be able to decay wood.

Key words: decay, ergosterol, extractive-tolerance, high performance liquid chromatography, western redcedar (*Thuja plicata* Donn ex D. Don).

Résumé : Nous avons testé les effets du lessivage sur la concentration des produits d'extraction du duramen de thuja géant (*Thuja plicata* Donn ex D. Don) qui sont connus pour leur activité antimicrobienne, et nous avons corrélé ces effets avec la croissance des champignons et la pourriture du bois. Nous avons évalué la tolérance aux produits d'extraction des espèces fongiques suivantes : *Acanthophysium lividocaeruleum*, *Coniophora puteana*, *Heterobasidion annosum*, *Pachnocybe ferruginea*, *Phellinus sulphurascens* et *Phellinnus weirii* en mesurant leur taux de croissance (mm/jour) dans du milieu de culture contenant ou non du lixiviat de thuja géant. Ces résultats ont été corrélés avec la capacité des espèces fongiques à croître et faire pourrir le thuja géant lessivé et non lessivé. Nous avons utilisé un essai mesurant l'ergostérol pour estimer la croissance, et un essai sur sol standard pour estimer la pourriture. Nous avons déterminé que le lessivage réduisait la concentration de 5 produits d'extraction : l'acide β-naphtoïque, la β-thujaplicine, la γ-thujaplicine, le β-thujaplicinol et l'acide thujique d'environ 80 %. *Phellinus sulphurescens* était le moins tolérant aux produits d'extraction in vitro, il croissait faiblement et causait une pourriture minimale des TGC non lessivés, mais il croissait bien et il causait la pourriture du Pin et le TGC lessivé. *Coniophora puteana, H. annosum* et *P. weirii* étaient de modérément à fortement tolérants au lixiviat, ils croissaient bien et causaient la pourriture du thuja géant lessivé et non lessivé, mais la croissance et la pourriture étaient toujours supérieurs sur le thuja géant lessivé et sur le pin, suggérant que le lessivage augmente la pourriture causée par ces champignons. *Acanthophysium lividocaeruleum* et *Pachnocybe ferruginea* étaient les plus tolérants aux produits d'extraction. Alors que *A. lividocaeruleum* causait clairement la pourriture de tous les types de bois, aucune pourriture n'était observée avec *Pachnocybe ferruginea* qui croissait très lentement sur les différentes espèces de bois et pouvait ou non pourrir le bois.

Mots-clés : pourriture, ergostérol, tolérance aux produits d'extraction, chromatographie liquide à haute performance, thuya géant (*Thuja plicata* Donn ex D. Don).

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Introduction

Western redcedar (WRC, *Thuja plicata* Donn ex D. Don) wood products are highly valued for their durability, which is conferred by naturally occurring extractive compounds (Barton and MacDonald 1971). These products have extensive residential applications and represent a significant portion of Canada's forest products industry (Gonzalez 2004). The extractives of WRC comprise a mixture of lignans and tropolone compounds. Several of the tropolones, namely the thujaplicins (α-, β-, and γ-thujaplicin) have been shown to have strong antimicrobial activity against a range of wood-inhabiting and plant-pathogenic fungi in vitro (Inamori et al. 2000; Morita et al. 2004*a*, 2004*b*). These compounds are believed to act as a natural chemical defense shield against pathogen invasion in standing trees (Van der Kamp 1986; DeBell et al. 1997) and to confer durability in cedar wood products (Rennerfelt 1948; Rudman 1962; Barton and MacDonald 1971).

Despite their natural durability, however, WRC wood products are susceptible to decay; which may be attributable in part to extractive depletion caused by weathering (Chedgy et al. 2007*a*). Whereas UV radiation from sunlight is known to cause photodegradation of extractive compounds in wood products (Coombs and Trust 1973; Shibata et al. 2003), UV radiation can only penetrate the upper 1 mm of exposed surfaces (Hon 1991) and may have a limited effect on the overall extractive content. In contrast, precipitation can penetrate deeper than radiation within WRC products and may have a significant impact on extractive concentrations by leaching. Leaching can thus result in an increased susceptibility of WRC products to wood-destroying microorganisms and a potentially reduced service life (Chedgy et al. 2007*a*). Johnson and Cserjesi (1980) reported that in WRC shakes the 2 most abundant compounds with antimicrobial activity, β- and γ-thujaplicin, were depleted by 25% after 1 year of exposure to natural weather conditions in a temperate climate and by 90% after 3 years. Biodegradation of extractives by microorganisms may have also contributed to this depletion.

To address the decay of WRC, it is necessary to understand the interactions between WRC extractives, leaching, and the occurrence and activity of fungi in WRC wood products. The aims of this research were to (1) characterize the effect of leaching on the concentration of 5 key extractives ((–)-plicatic acid, β-thujaplicin, γ-thujaplicin, β-thujaplicinol, and thujic acid) known to occur in WRC, (2) examine the resistance to WRC in vitro of 6 fungal species: *Acanthophysium lividocaeruleum*, *Coniophora puteana*, *Heterobasidion annosum*, *Pachnocybe ferruginea*, *Phellinus sulphurascens*, and *Phellinus weirii*, all of which are commonly isolated from WRC products (Lim et al. 2005, 2007), and (3) compare the ability of these fungi to grow on and cause decay in leached and nonleached WRC. The data obtained from this work will provide the forest products industry with information that can be utilized to prolong the service life of WRC products.

Materials and methods

Wood materials

WRC blocks of 19 mm on each dimension were manufactured from the outer heartwood of a 136-year-old tree felled in the summer season at the University of British Columbia Malcolm Knapp Research Forest, Maple Ridge, British Columbia. Prior to the experiment, a sample of blocks were incubated on 1% malt extract agar for 14 days to ensure that they were sound and free of microorganisms. Ponderosa pine (*Pinus ponderosa* P. & C. Laws.) sapwood blocks of the same dimensions were used as controls because this species does not contain any known fungicidal extractives similar to those present in WRC and because sapwood contains a lower resin concentration than heartwood (Smith 1964).

Leaching of WRC blocks and chemical analysis

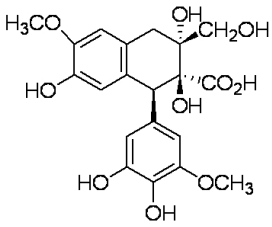
In total, 84 WRC blocks were leached and an additional 84 were not leached. From each set of 84 blocks, 6 were used to test the effect of leaching on the wood chemistry. The remaining 78 were used in the subsequent decay tests. We followed the American Wood-Preservers' Association's (AWPA) standard method of testing wood preservatives by laboratory soil-block cultures (E10-08) (American Wood-Preservers' Association (AWPA) 2008), which includes guidelines for the leaching of wood samples. The 84 WRC blocks were simultaneously immersed in distilled H_2O in a total volume of 4.2 L (50 mL/block), which was collected and replaced daily with fresh H_2O for a period of 14 days. This was conducted at room temperature and shielded from light to prevent photodegradation of the extractives. The extractive content of the leached and nonleached blocks was then compared by estimating the concentration of 5 major extractives: (–)-plicatic acid, β-thujaplicin, γ-thujaplicin, β-thujaplicinol, and thujic acid (Fig. 1). Samples were powdered by first slicing the blocks as thinly as possible. The slices, which were typically 2 mm in width, were then ground to a fine powder (<150 μm) using a freezer mill equipped with a coolant circulation chamber from Bel-art products (Pequannock, New Jersey). A temperature of ~4 °C was maintained during grinding to avoid generation of excess heat caused by friction. Samples were then steeped in methanol and exposed to ultrasonic frequency to extract the compounds of interest. Extractives were then separated using reverse-phase high performance liquid chromatography (RP-HPLC) and quantified using an UV detection system with calibration equations that had been constructed using pure standards against an internal standard. For full method details, see Chedgy et al. (2007*b*). This experiment followed a completely randomized design (CRD) with 2 treatments: no leaching and leaching ($k = 2$). Six replicates of WRC blocks (6 leached and 6 nonleached) were used in the chemical analysis of block extractive content ($n = 6$).

Fungal growth on agar containing WRC leachate

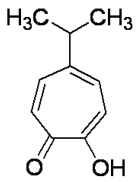
The growth and tolerance of 6 fungal species to WRC leachate was tested in vitro. The fungal species examined were *A. lividocaeruleum*, *C. puteana*, *H. annosum*, *Pachnocybe ferruginea*, *P. sulphurascens*, and *P. weirii*. Two strains of each species were used. The species used were frequently isolated from in-service WRC products (Lim et al. 2005 2007) or dead trees (Larsen et al. 1994).

Following the leaching of WRC blocks, the resulting leachate was retained and stored at 4 °C in the dark. Approximately 25% of the total leachate volume collected over the 14-day period was freeze-dried using an Edwards

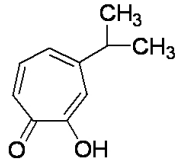
Fig. 1. Chemical structure and nomenclature used for the western redcedar heartwood extractive compounds examined.



2,3,6-Trihydroxy-7-methoxy-2-hydroxymethyl-4-(3',4'-dihydroxy-5'-methoxyphenyl)-tetralin-3-carboxylic acid
(–)-Plicatic Acid



2-Hydroxy-5-isopropyl-2,4,6-cycloheptatrien-1-one
γ-**Thujaplicin**



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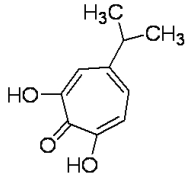
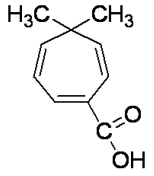
β-**Thujaplicin**



2,7-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one
β-**Thujaplicinol**



7,7-Dimethylcycloheptatriene-3-carboxylic acid
Thujic Acid

Modulyo freeze dryer (BOC Edwards Pharmaceutical Systems, Wilmington, Massachusetts) equipped with a Thermo-Savant VLP 200 vacuum pump (Thermo Electron Corp., Waltham, Massachusetts). This was accomplished by filling multiple 50 mL conical tubes with 35 mL of leachate. The tubes were then frozen at –80 °C in a horizontal position. Once frozen, a small hole was pieced in the cap of each tube to allow air flow. The tubes were then placed into a precooled freeze dryer (–45 °C) under a vacuum of 1 mbar (1 bar = 100 kPa) to freeze-dry for a period of 48 h. The resulting leachate powder was resuspended to a concentration of 50 mg/mL in a 1:1 ratio of H_2O–ethanol, and filter-sterilized. To test the antifungal activity, the agar dilution method was used. Plates were filled with a known volume (25 mL) of molten 1% malt extract agar using a graduated pipette and then allowed to solidify. A solution containing a known concentration of extractives was then prepared and homogeneously spread over the surface. Over time, it will diffuse throughout the agar to give a specified concentration. Concentrations of 0, 16, 32, 64, 128, and 256 ppm of the leachate were used. Control plates contained 50% ethanol at the same volumes used to prepare the various leachate-containing plates. The medium was then inoculated with a 5 mm plug of agar taken from the edge of actively growing isolate colonies. Cultures were maintained in the dark at 20 °C, and the growth was evaluated by measuring 2 perpendicular diameters of the colony every 3 days. The growth rate (mm/day) was then estimated for each isolate during its exponential growth phase. Three replicate plates were prepared for each isolate at each leachate concentration.

This trial followed a CRD with 6 treatments defined as the varying concentrations of *T. plicata* leachate ($k = 6$). Three replicate cultures were established for each species strain at each leachate concentration, and 2 strains were used per species. Statistical analysis was performed on the mean growth rates (mm/day) calculated from both strains together ($n = 6$).

Soil-block decay tests

The ability of the 6 fungal species to cause decay was compared on leached and nonleached WRC blocks, as well as on blocks of ponderosa pine sapwood. The extent of decay was measured by estimating the percentage mass loss (dry mass, DW) of blocks compared with controls after a period of incubation with the various fungal isolates. We followed the standard procedure for laboratory soil-block cultures (E10-08) as outlined by the AWPA (2008). All wood blocks used were free of knots, with 2–4 rings/cm. Blocks showed no visible evidence of infection (e.g., stain) by any other microorganisms, including wood-destroying fungi. Before use, the pine and WRC blocks were placed in a conditioning room at 20 °C with a relative humidity of 65% for 48 h and then sorted by mass into narrow mass-range groups. Pine blocks used in the experiment were 3.3 g ± 0.1 g, and all WRC blocks used in the experiment were 2.1 g ± 0.1 g. The pine blocks were numbered and the DW of each was calculated after oven-drying the blocks at 105 °C for 24 h. The WRC blocks originated from heartwood on a single longitudinal axis parallel with its growth rings. The blocks were sequentially numbered relative to their position. The even-numbered blocks were used in the experiment, placed into a conditioning chamber, and their wet mass was recorded. The odd-numbered blocks were oven-dried at 105 °C for 24 h, and their DW was recorded. Their DW values were used to estimate the DW of the neighboring, even-numbered blocks. This was necessary because several of the extractive compounds of interest are prone to thermal degradation (Johnson and Cserjesi 1980; Shibata et al. 2003) and thus could not be dried at 105 °C, because this would compromise the experiment. In addition, the even-numbered WRC blocks (leached and nonleached) were ion beam sterilized (Iotron Industries Canada Ltd., Port Coquitlam, British Columbia) to limit any alteration of extractives that might occur with autoclaving, which involves temperatures around 120 °C. It is important to note that Curling and Winandy (2008) showed that sterilization by γ radiation can still result in minor modification of structural components, such as lignin and hemicelluloses, while other carbohydrate components remain unchanged in pine species. This represents a deviation from the AWPA protocol that recommends sterilization by autoclaving.

Glass jars containing soil were prepared as described in the AWPA standard protocol. Feeder strips of dimensions 3 mm × 28 mm × 34 mm were manufactured from lodgepole pine (*Pinus contorta* Dougl. ex Loud.) sapwood and placed on the soil surface (1/jar). Jars were autoclaved (with lids) at 103.4 kPa for 30 min on 2 consecutive days and then inoculated with fungal colonies by placing

10 mm × 30 mm agar blocks removed from near the leading edge of the mycelium of actively growing colonies. Agar blocks were placed in contact with 1 edge of the feeder strip and in contact with the soil. The jar lids were loosened by a one-quarter turn from the fully tightened position to allow limited oxygen flow, and the jars were placed in an incubation room (25 °C with a relative humidity of 70%) until the feeder strips were completely covered with mycelium. Test blocks were brought to a moisture content (M_c) of 40% by placing sterilized blocks at 100% + M_c into a sterile fume hood and allowing them to air dry until the appropriate M_c had been reached. Blocks were then placed on the surface of the feeder strips with the tracheids in the vertical orientation to encourage mycelial penetration. All samples were incubated for a period of 16 weeks. Six replicates were used for each fungal isolate and for each wood type: leached WRC, nonleached WRC, and pine sapwood. For each wood type, 72 blocks were used (6 species × 2 strains × 6 replicates). In addition, for each wood type, 6 controls were used, which were not inoculated with fungal cultures to ensure that no contamination occurred, bringing the total number of blocks used for each wood type to 78. To reduce the number of jars used in the experiment, 2 blocks were used per jar.

Estimation of fungal growth in decayed wood

The extent of fungal growth of each species on the wood blocks from the decay test was estimated by measuring ergosterol (ergosta-5,7,22-trien-3β-ol), a sterol present only in fungal cell membranes (Hull and Woolfson 1976). After incubation, the wood blocks were frozen at –80 °C until further use. In total, 234 blocks were analyzed (6 replicate blocks for the 6 fungal species, 2 strains per species plus 6 controls, on 3 different wood types). Blocks were powdered in the same manner as stated previously. Care was taken to ensure that no powder was lost during this process. Samples were steeped in 15 mL methanol and exposed to ultrasonic frequency (40 kHz) for 120 min using a Branson 8510 ultrasonic bath (Danbury, Connecticut) cooled to ~4 °C by the addition of ice. Samples were extracted only once in each case. Extracts were filtered using a 0.2 μm nylon syringe filter to remove any wood particles, and they were stored at 4 °C in the dark. Typically, 12 mL of filtered extract was recovered from each sample. Extracts were separated and quantified by RP-HPLC. The extraction process and HPLC apparatus used were identical to that described by Chedgy et al. (2007*b*). For ergosterol quantification, we followed the experimental conditions outlined by Gao et al. (1993), with 7-dehydrocholesterol as the internal standard, and a column temperature of 25 °C with a mobile phase of methanol–acetonitrile–water (50:45:5, by volume) at a flow rate of 1.2 mL/min. All solvents of the mobile phase were supplemented with 0.1% formic acid (*v*/*v*). The wavelength used was 283 nm. Mixed extracts were injected (50 μL) on to the column and readily separated.

To estimate the total amount of ergosterol (mg/block), calibration equations were constructed relative to the internal standard. This compound possesses a chromatographic retention time (t_R) proximal to ergosterol but does not overlap. Reagents and (or) standards, ergosterol, and 7-dehydrocholesterol were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario). From stock standard solutions of both ergosterol and 7-dehydrocholesterol, a calibration curve was constructed in a similar method as described by Chedgy et al. (2007*b*). The detector responses for each compound were subjected to linear regression analysis, and the response factor was calculated as $y = 1.579x$ with an R^2 value of 0.978, where y is the peak area ratio (ergosterol peak area divided by internal standard peak area) and x is the concentration (μg/mL) ratio ([ergosterol]/[internal standard]). The estimated ergosterol concentration (μg/mL) was then multiplied by the extraction volume (15 mL) to obtain total ergosterol.

This trial followed a CRD with 3 wood treatments defined as pine, leached, and nonleached *T. plicata* ($k = 3$). Six replicates cultures were established for each species strain, for each wood treatment, and 2 strains were used per species. Statistical analysis was performed using the mean total ergosterol (mg) calculated from both strains together ($n = 6$).

Estimating the degree of decay

Following the ergosterol extraction, the powdered wood samples were placed on 1 mm filter paper discs and placed over a vacuum using a Büchner flask. Samples were washed several times with distilled H_2O to remove excess methanol. The samples were then dried in an oven at 105 °C for 24 h, and the DW was recorded. The percentage of mass loss was then calculated. In the case of the pine blocks, the percentage mass loss was simply the percentage difference between the sample DW before and after the decay test. In the case of WRC blocks, the DW after the decay test was compared with the estimated DW of the block, which was calculated by measuring the M_c and DW of its neighboring odd-numbered block from the single longitudinal axis of the heartwood from which it was taken.

This trial followed a CRD with 3 wood treatments: pine, leached and nonleached WRC ($k = 3$). Six replicates cultures were established for each species strain, for each wood treatment, and 2 strains were used per species. Statistical analysis was performed and the mean total percentage mass loss DW was calculated from both strains together ($n = 6$).

Statistical analysis

All experiments followed a CRD with treatments denoted as k and replicates as n. A one-way analysis of variance (ANOVA) ($\alpha = 0.05$) and Tukey's test for comparison of means (Tukey 1949) were performed on the data. Statistical analysis was performed using JMP IN software (version 4.0.3 (academic); SAS Institute Inc., Cary, North Carolina).

Results

Leaching of WRC blocks resulted in significant losses of the 5 key extractives. Leached WRC contained ~80% less extractives than nonleached WRC (Fig. 2). The ability of fungal species *A. lividocaeruleum*, *C. puteana*, *H. annosum*, *Pachnocybe ferruginea*, *P. sulphurascens*, and *P. weirii* to grow (mm/day) on media supplemented with varying concentrations of WRC leachate (ppm) was examined (Table 1). Statistical analysis suggested that the presence of WRC leachate had a significant effect on the mycelial growth of *A. lividocaeruleum*, *C. puteana*, *H. annosum*, *P. sulphuras-*

Fig. 2. Concentration of extractives per block (μg/g dry mass (DW)) in leached and nonleached western redcedar heartwood. *Y* error bars indicate standard deviation. Numbers above the error bars represent the concentration expressed as a percentage of the nonleached western redcedar blocks. ANOVA generated the following critical values for extractives of interest: (–)-plicatic acid, $F_{[1,10]} = 850.3$; γ-thujaplicin, $F_{[1,10]} = 85.1$; β-thujaplicin, $F_{[1,10]} = 76.4$; β-thujaplicinol, $F_{[1,10]} = 36\,613.7$; and thujic acid, $F_{[1,10]} = 63.9$. The treatment effect was considered significant if the *F* value was greater than the tabular value of $F_{[1,10]} = 4.96$.

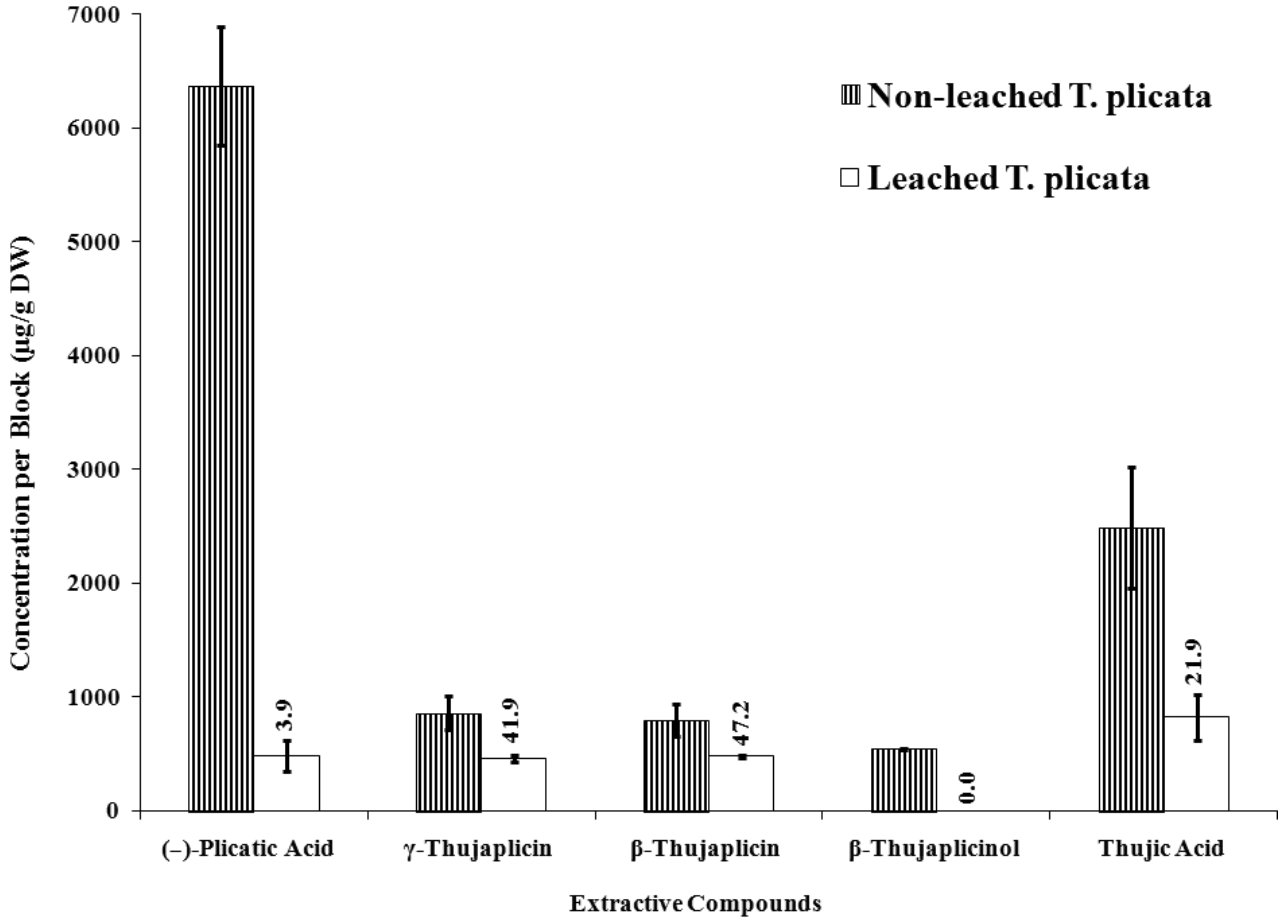


Table 1. Mean fungal growth rate (mm/day) on media containing various concentrations of leachate.

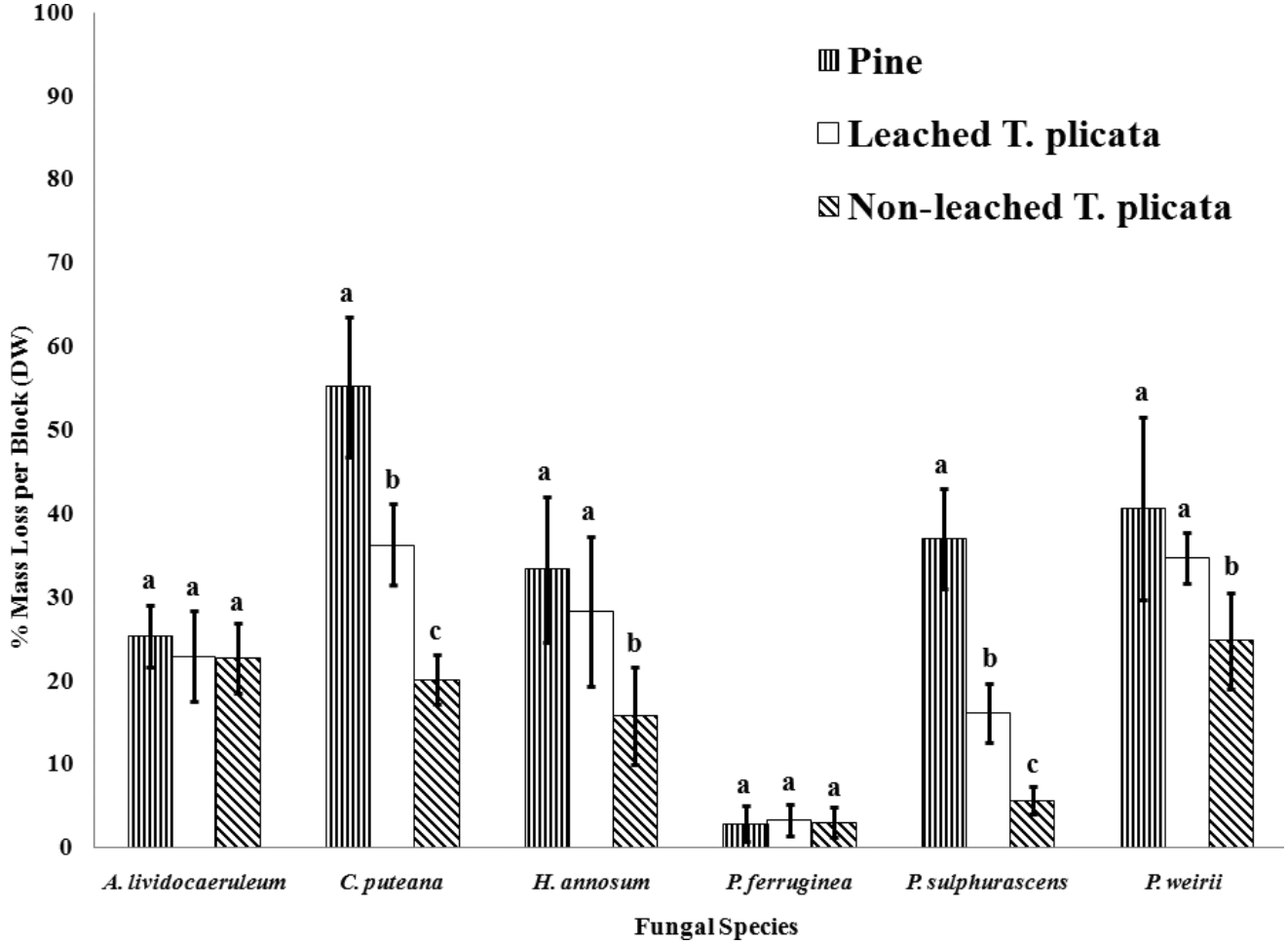
	Leachate concn. ± SD (ppm)					
Species	0	16	32	64	128	256
A. lividocaeruleum	4.1±0.3a	4.5±0.2a	4.6±0.1a	4.2±0.3a	2.3±0.1b	1.3±0.2c
C. puteana	4.0±2.0a	3.1±1.5a	2.4±1.0b	1.9±0.6c	0.0±0.0d	0.0±0.0d
H. annosum	7.8±0.3a	7.4±0.4a	7.6±0.3a	7.1±0.4a	3.7±0.4b	1.7±0.6c
Pachnocybe ferruginea	0.5±0.3a	0.7±0.1a	0.7±0.2a	0.6±0.1a	0.7±0.1b	0.7±0.1b
P. sulphurascens	6.0±0.2a	1.4±0.4b	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c
P. weirii	6.2±0.4a	4.8±0.8b	4.5±0.1b	3.3±0.2c	0.0±0.0d	0.0±0.0d

Note: ANOVA generated the following critical values for the 6 species examined: *Acanthophysium lividocaeruleum*, $F_{[5,30]} = 56.87$; *Coniophora puteana*, $F_{[5,30]} = 13.26$; *Heterobasidion annosum*, $F_{[5,30]} = 21.51$; *Pachnocybe ferruginea*, $F_{[5,30]} = 2.12$; *Phellinus sulphurascens*, $F_{[5,30]} = 509.35$; and *Phellinus weirii*, $F_{[5,30]} = 169.68$. Treatments were considered to have a significant effect if the critical *F* values were greater than the appropriate tabular value $F_{[5,30]} = 2.53$ ($\alpha = 0.05$) for this experiment. Numbers followed by the same letter were not significantly different ($\alpha = 0.05$) according to Tukey's test of multiple comparison of means.

cens, and *P. weirii*. However, the presence of WRC leachate did not significantly affect the growth of *Pachnocybe ferruginea*, even at the highest concentration of 256 ppm. *Acanthophysium lividocaeruleum* and *H. annosum* were also able to grow on plates with 256 ppm leachate but at a slower growth rate than on the control plates.

The results of the soil-block test (Fig. 3) coupled with the quantification of ergosterol (Fig. 4) showed that, in most cases, the 6 fungal species were able to grow and decay pine sapwood to a greater degree than they could decay WRC heartwood. A high degree of variability was observed in the results for wood decay (% mass loss) and ergosterol content (total mg/block). This may be caused by the natural substrate variability and the fungal entities. To assess fungal

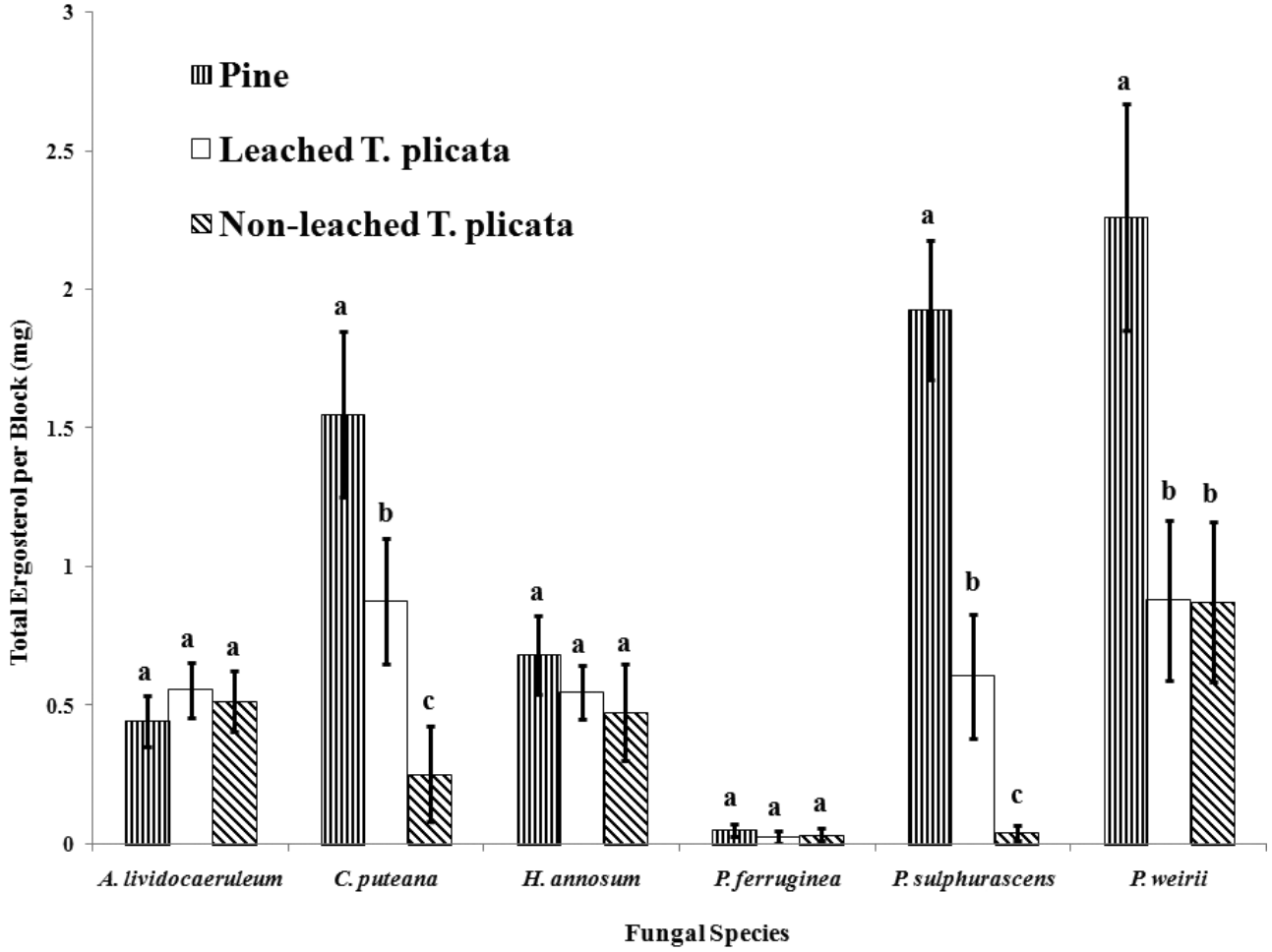
Fig. 3. Estimation of the degree of decay (mean % mass loss) of wood blocks caused by 6 fungal species of interest. *Y* error bars indicate standard deviation. Critical *F* values are as follows for each of the fungal species: *Acanthophysium lividocaeruleum*, $F_{[2,15]} = 2.52$; *Coniophora puteana*, $F_{[2,15]} = 117.49$; *Heterobasidion annosum*, $F_{[2,15]} = 7.35$; *Phellinus weirii*, $F_{[2,15]} = 15.21$; *Pachnocybe ferruginea*, $F_{[2,15]} = 0.09$.; and *Phellinus sulphurascens*, $F_{[2,15]} = 273.82$. The values were considered significant if greater than the tabular value of $F_{[2,15]} = 3.68$ ($\alpha = 0.05$). Bars with the same letter were not significantly different according to Tukey's test for multiple comparison of means ($\alpha = 0.05$).



growth in wood, we used ergosterol, a sterol present only in fungal cell membranes (Hull and Woolfson 1976). However, the amount of ergosterol varies in filamentous fungal cells throughout the 5 distinct phases of growth, namely, the lag phase, the first transition period, the log phase, the second transition period, and the stationary phase (Meletiadis et al. 2001), as well as between fungal species. Therefore, by estimating the total amount of ergosterol per block (mg), it is only possible to compare the relative amounts of fungal growth of a fungal species as a result of the wood treatments; thus, we only made intra-species comparisons for fungal growth. Despite this variability, it was possible to observe statistically significant patterns in the data.

Overall, fungi were able to grow on and decay leached WRC wood more readily than nonleached WRC. For the fungal species *C. puteana*, *H. annosum*, *P. sulphurascens*, and *P. weirii*, the greatest amount of growth and decay occurred on pine, followed by leached WRC, and then nonleached WRC. For example, *C. puteana* produced a mean mass loss of 55.2% ± 8.3% and 1.6 mg ± 0.3 mg ergosterol for pine, 36.2% ± 4.9% and 0.9 mg ± 0.2 mg ergosterol for leached WRC, and 20.1% ± 3.0% and 0.3 mg ± 0.2 mg ergosterol on nonleached WRC. Tukey's test for multiple comparison of means also showed that for *C. puteana*, the degree of decay and fungal growth was significantly different ($\alpha = 0.05$) on the 3 wood types tested. A similar, but less defined, pattern was observed for *H. annosum* and *P. weirii*, with no significant difference in decay between pine and leached WRC wood. However, fungal growth of these 2 species did not follow this pattern exactly. For *H. annosum*, no significant difference was observed between decay of pine (33.3% ± 8.7%) and leached WRC (28.3% ± 9.0%), whereas decay of nonleached WRC was significantly lower at 15.7% ± 5.8%. Growth of *H. annosum* was not significantly influenced by wood treatments: pine, 0.7 mg ± 0.1 mg; leached WRC, 0.6 mg ± 0.1 mg; and nonleached WRC, 0.5 mg ± 0.2 mg. In this case, the fungi were able to grow in the presence of high extractive levels on nonleached WRC but were less effective at causing decay. *Phellinus weirii* exhibited no significant difference in the decay of pine (40.6% ± 10.9%) and leached WRC (34.7% ± 3.0%), but decayed nonleached WRC was significantly less (24.8% ± 5.7%). Fungal growth was significantly greater on pine than on the WRC blocks: pine, 2.3 mg ±

Fig. 4. Estimation of the mean total ergosterol per block (mg) present in wood blocks decayed by 6 fungal species of interest. *Y* error bars indicate standard deviation. Critical *F* values are as follows for each of the fungal species: *Acanthophysium lividocaeruleum*, $F_{[2,15]} = 0.73$; *Coniophora puteana*, $F_{[2,15]} = 38.96$; *Heterobasidion annosum*, $F_{[2,15]} = 3.78$; *Phellinus weirii*, $F_{[2,15]} = 39.54$; *Pachnocybe ferruginea*, $F_{[2,15]} = 1.72$; and *Phellinus sulphurascens*, $F_{[2,15]} = 45.67$. The values were considered significant if greater than the tabular value of $F_{[2,15]} = 3.68$ ($\alpha = 0.05$). Bars with the same letter were not significantly different according to Tukey's test for multiple comparison of means ($\alpha = 0.05$).



0.4 mg; leached WRC, 0.9 mg ± 0.3 mg; and nonleached WRC, 0.9 mg ± 0.3 mg. *Phellinus sulphurascens* caused decay on pine (37.0% ± 6.0% and 1.9 mg ± 0.3 mg) and leached WRC (16.1% ± 3.6%) and 0.61 mg ± 0.2 mg), but could barely grow on nonleached WRC (5.6% ± 1.6% and 0.0 mg ± 0.0 mg).

However, the treatment effects were not significant for *A. lividocaeruleum*, suggesting that loss of extractives is not necessary for growth and decay to occur. This species provided conclusive results because it grew well on and decayed the 3 wood types to a similar extent: pine, 25.3% ± 3.7% and 0.5 mg ± 0.1 mg; leached WRC, 22.9% ± 5.4% and 0.5 mg ± 0.1 mg; and nonleached WRC, 22.6% ± 4.2% and 0.5 mg ± 0.2 mg. *Pachnocybe ferruginea* showed small amounts of growth on all 3 wood types, but failed to cause significant mass loss: pine, 2.9% ± 2.2%, 0.1 mg ± 0.0 mg; leached WRC, 3.3% ± 1.9% and 0.0 mg ± 0.0 mg; and nonleached WRC, 3.0% ± 1.8% and 0.0 mg ± 0.0 mg.

Discussion

Extractive loss generally resulted in a greater degree of decay of WRC. For example, *P. sulphurascens*, a species causing laminated root rot in Douglasfir, decayed pine and leached WRC, but could barely grow on nonleached WRC. This pattern was apparent to a lesser extent with species *C. puteana* (a standard species used in decay tests), *H. annosum*, and *P. weirii*, which were all able to decay nonleached WRC blocks but to a lesser degree than leached WRC and pine blocks. *Heterobasidion annosum* and *P. weirii* cause root and butt rot in softwood species; however, in contrast to *H. annosum*, which has a wide host range, *P. weirii* is associated mainly with WRC in old-growth trees in British Columbia (Allen et al. 1996) and Douglas-fir (Thies 1983). Our data suggest that, for these 3 fungal species, leaching of extractives enhances fungal decay of WRC. However, this was not the case for *A. lividocaeruleum*, which was able to decay pine, leached WRC, and nonleached WRC to a statistically similar degree.

Wood-decaying species *H. annosum* caused less decay than expected. The wood moisture content may have influenced the amount of decay observed with the various fungal species in this work, with each species requiring different moisture levels for optimal growth. In the work reported

here, a M_c of 40% was created, which may be lower than the M_c encountered in natural conditions. For *H. annosum*, which typically causes rot in standing trees, Nielson et al. (1985) reported a typical M_c of 58% in freshly cut WRC.

However, we established a clear correlation between tolerance to WRC leachate in vitro and the ability to decay leached versus nonleached WRC in soil-block decay tests. *Pachnocybe ferruginea* is of particular interest because it exhibited high tolerance to WRC leachate in vitro. This species is commonly isolated from Douglas-fir (Kropp and Corden 1986) and WRC wood products (Lim et al. 2005). However, while it seemed to grow on all wood types, it failed to cause decay. This species is a "'pioneer'" species that has high resistance to β-thujaplicin (Lim et al. 2005). It may detoxify extractives in wood products, paving the way for colonization by less extractive tolerant decay fungi. To our knowledge, there is no evidence in the literature to suggest that this species can cause wood decay by degrading cellulose, hemicellulose, or lignin. The lack of obvious decay may be a function of its slow growth rate, making it difficult to draw any conclusions as to its role in the decay of wood in wood products. We observed that the growth rate of this species marginally increased when grown on media supplemented with WRC leachate compared with controls. To this end, it is possible that it may utilize such extractives as a carbon source. The small mass losses observed in our decay test may be attributable to a loss of simple sugars or extractives. Thus, further work is required to better understand the role of this fungal species in the decay of WRC products.

In this work, we have demonstrated that leaching of WRC causes a significant depletion of several major heartwood extractives. Because many of these have antimicrobial activity, such depletion increases the susceptibility of WRC products to decay by allowing wood-destroying fungi to grow. Further, fungi vary in their resistance to WRC extractives, and a relatively small amount of leaching can result in product decay and reduced service life. Maintaining durability in WRC wood products that are exposed to weathering requires retaining naturally occurring extractives in the products.

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