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Fungal diversity from western redcedar fences and their resistance to β-thujaplicin

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Abstract

The work reported here investigated the fungal community inhabiting western redcedar fence material with a focus on species colonizing wood below the surface, of which little is known. From seven pieces of fence material, twenty-three different fungal species were isolated and characterized using both traditional morphology and molecular identification methods. The species identified included thirteen ascomycetous and ten basidiomycetous fungi. Isolates were tested for their resistance to β-thujaplicin – one of the principle fungicidal agents of western redcedar heartwood extractives. Generally, ascomycetous fungi exhibited greater resistance to β-thujaplicin than basidiomycetous fungi. Interestingly, three ascomycetous and two basidiomycetous species frequently isolated had high tolerance to this compound. These species could be candidate 'pioneer' species that invade and detoxify western redcedar extractives, paving the way for colonization by decay fungi.

Introduction

Canada's value-added forest product industries depend in part on the unique qualities of some of this country's native wood species. Western redcedar (WRC) (*Thuja plicata* Don) is a well-known and commercially important coniferous tree species common in the Pacific northwest. Its heartwood is valued for the natural durability conferred by fungicidal agents in its extractives (Wethern 1959); in particular, by a group of tropolone compounds known as 'thujaplicins' (Rennerfelt 1948). Of the several classes of thujaplicins characterized, β-thujaplicin (2-hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one) appears to be the most prevalent and effective against decay fungi (Arima et al. 2003; Erdtman and Gripenberg 1948; Inamori et al. 1999, 2000; Trust and Coombs 1973). Despite such extractives, decay fungi are still a major factor in product failure in service. Furthermore, products manufactured from second growth WRC may have lower extractive contents than the best of the old growth (Nault 1986). Optimizing the service life and value of products that rely on such natural protection requires an understanding of how extractives and fungal communities interact and evolve in service.

In WRC trees 'pioneer' fungal species can detoxify fungicidal extractives, clearing the way for less specialized fungi to colonize and decay wood freely (Jin 1987; Van der Kamp 1975). However, to our knowledge, the fungal succession in biodeterioration has not been documented for WRC products and little is known about the microbial communities that these products harbor. While research groups have reported a limited number of decay fungi from WRC utility poles (Eslyn and Highlery 1976; Morrell et al. 2001; Scheffer et al. 1984) and shingles/shakes (Smith and Swan 1975), these studies relied on species identification by morphology only. This approach has two major limitations. Firstly, fungi in artificial cultures

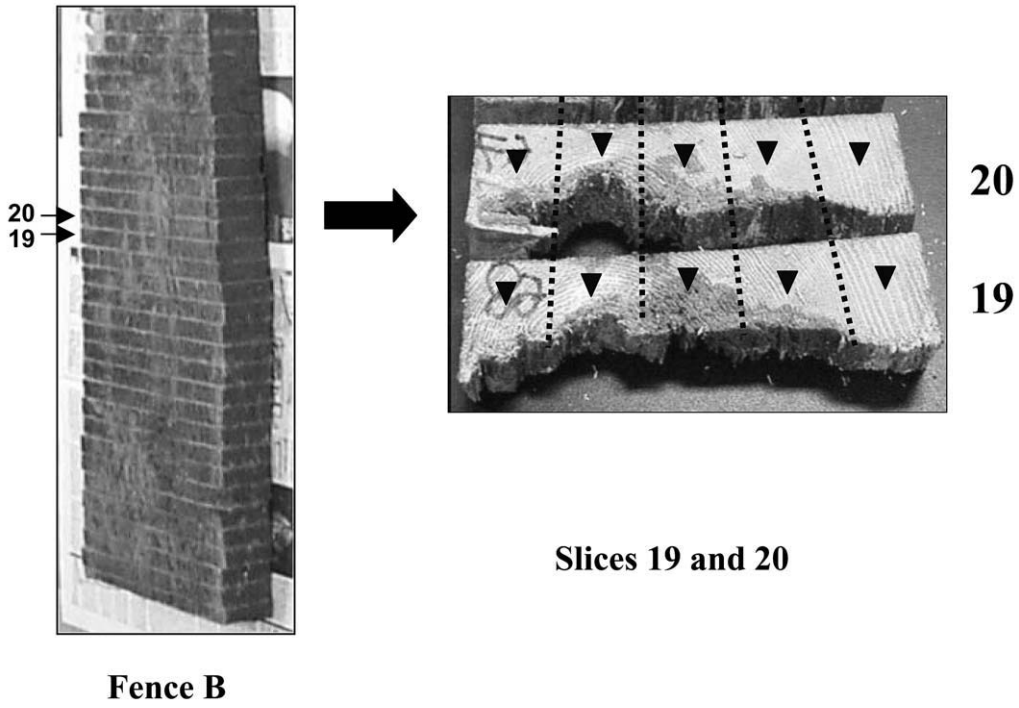

Figure 1. Schematic diagram for fungal isolation from WRC fences. In the example shown, fence material (Fence B) was cut horizontally into 2 cm slices and then divided further into five lateral sections. Wood flecks (shown by bold arrows) from the central regions were aseptically detached and inoculated onto plates.

often exhibit fewer morphological features than in their natural environments. This impedes identification, especially for fungi that lack asexual spores e.g., Homobasidiomycetes. Secondly, a fungal species' characteristics can vary when it is grown on different media or under different culture conditions. The wealth of sequence information that has been compiled in databases means that it is now possible to identify fungi at a far higher resolution using molecular techniques than can be achieved using morphological methods. However, databases contain sequences from only a fraction of all known species and consequently morphological methods are still the method of choice to identify fungi (Allen at al. 2003; Wirsel et al. 2001).

In the work reported here we characterized a) the fungal communities in WRC fences that had been taken out of service due to decay after thirty and forty years of service in British Columbia (BC), Canada, and b) the tolerance of the isolated fungi to β-thujaplicin. In order to identify fungi accurately, classical methods were complemented with molecular techniques.

Materials and methods

Collection sites and fungal isolations

WRC fence material was collected in Vancouver, BC, Canada. Five pieces (125 × 19 × 4.5 cm) were from a fence in service between 1960 to 2001 and two (166.5 × 23 × 8 cm) from a fence in service between 1970 to 2001. Each piece was sliced horizontally into 2 cm blocks. Each block was further divided into five lateral sections. Wood flecks taken from within and near decay pockets were aseptically detached, briefly flamed to remove contaminating surface microflora and plated (Figure 1). Eight decay areas from seven fence pieces were labeled fence A – G. A 1% malt extract agar (MEA) was used for isolating the general microflora and 1% MEA with benomyl (BMEA) was used for the basidiomycetous fungi (Clubbe and Levy 1977). The plates were incubated at room temperature for several weeks with fungi routinely subcultured from mycelial margins to new plates in order to obtain pure cultures. Species identification via classical methodology was achieved by macro- and micro-morphological analyses using taxonomic

guides and standard procedures (Arx 1981; Barnett and Hunter 1987; Carmichael et al. 1980; Cole and Kendrick 1973; Ellis 1971; Nobles 1965; Schol-Schwarz 1970; Stalpers 1978; Wang and Zabel 1990). This was complemented by molecular techniques for species identification.

DNA extraction, PCR and sequencing

DNA was extracted from mycelium scraped from the fungal colonies and placed into micro-centrifuge tubes with 300 μl of extraction buffer [100 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 100 mM NaCl and 2% SDS]. The mixture was vortexed for 10 s, incubated at 75 °C for 30 min. 4/5 volumes of glass beads were then added into the tube and vortexed for a further 10 min. DNA was purified via a two step phenol-chloroform extraction and precipitated with one volume of iso-propanol then centrifuged immediately at 12,000 rpm at room temperature for 10 min. After removing the supernatant, the pellet was washed with 70% ethanol, allowed to air dry and resuspended in 40 μl of distilled water. The extracted DNA was stored at − 20 °C until further use.

To achieve PCR amplification of the internal transcribed spacer (ITS) regions, fungal universal primers (ITS5 and ITS4) and the basidiomycetous specific reverse primer (ITS4B) with ITS5 primer (Gardes and Bruns 1993; White et al. 1990) were used. Amplification was performed as described by Lee et al. (2000). Usually 3 μl of each PCR product was used for the electrophoresis on 0.5% agarose gel containing EtBr in Tris-acetate EDTA (TAE) buffer. The PCR product sizes were determined by comparison to a 1 kb DNA marker (GIBCO BRL, U.S.A.). The PCR products were purified using a Qiaquick PCR Purification Kit (Qiagen Inc.). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer Inc. USA) at the DNA synthesis and Sequencing Facility, MACROGEN (Seoul, Korea). All of the nucleotide sequences determined in this work have been deposited in the GenBank, their accession numbers are shown in Table 1. The ITS region sequences were analyzed using BLAST in order to find the most similar available database sequences. The closest matched sequence for each species is shown in Table 1.

Inhibition of fungal growth by β-thujaplicin

The agar dilution method was used for the antifungal activity tests. Mycelial growth was measured on MEA plates with various concentrations of β-thujaplicin. A 10 mg ml^{-1} stock solution was prepared in 50% ethanol and kept in the dark at 4 °C. Concentrations of 0, 2, 8, and 32 ppm were prepared. Vigorous mixing of the medium prevented the precipitation of the chemical. Ethanol had no effect on the hyphal growth at the low concentrations present in the growth medium. The medium was inoculated with a 5 mm plug of agar taken from the edge of actively growing isolate colonies. The cultures (three replicates used) were maintained in the dark at 20 °C and growth was evaluated after 12 days by measuring two perpendicular diameters of the colony.

Results and discussion

Isolation and morphological grouping of fungi

Regions of wood decay generally occurred in or around the ends of fence panels. A total of 144 fungal isolates were recovered from 303 sampling sites in 8 regions of decay present on 7 WRC fence sources. The highest count of fungal isolates was recorded in the decay area of fences G (39 isolates: 27.1%), while the lowest was recorded in fence A and B (7 isolates each: 4.7%). The fungal diversity was highest in decay area F1 (10 different types) and lowest in decay area A (3 types). The macro- and microscopic characterization of the isolates allowed us to recognize 23 different fungal taxa, including thirteen ascomycetous fungi and ten basidiomycetous fungi. The basidiomycetes were identified using three characteristics: growth on BMEA, presence of clamp connection, and PCR amplification using forward primer ITS5 and basidio-specific reverse primer ITS4B (Gardes and Bruns 1993). However, PCR using ITS5 and ITS4B failed to amplify the ITS regions of two basidiomycetous isolates, *Cerinosterus luteoalba* and WRCF-B2. BMEA permits isolation of basidiomycetous fungi and prevents the growth of most microfungi (Clubbe and Levy 1977). Two ascomycetous fungi, WRCF-A2 and *Phialophora* sp. 1, grew on BMEA while a basidiomycetous fungus, WRCF-B2, did not.

Among the ascomycetous species we recognized *Oidiodendron griseum*, *Phialophora* spp., *Rhinocladiella atrovirens*, *Sporothrix* spp. and three unidenti-

Table 1. List of fungi isolated in this work; source, their characteristics and GenBank accession numbers

Fungi	GenBank Acc No.	Source (no. of isolates)[1]	Isolation site[2]	Growth onBMEA	Clamp connection	ITS5 / ITS4B	ITS5 / ITS4	Closest match in BLAST	Identity [%][3]
Basidiomycota									
Acanthophysium lividocaeruleum	AY618666	C(2), D(2), G(9)	B	G	P	A	A	Acanthophysium lividocaeruleum [AF506400]	282/289 (97%)
Cerinosterus luteoalba	AY618667	D(1), F1(1)	D	G	NP	NA	A	Sporobolomyces symmetricus [AY364836]	119/123 (96%)
Hyphoderma praetermissum	AY618668	A(2), E(3), F1(4)	D	G	P	A	A	Hyphodontia flavipora [AF455399]	168/173 (97%)
Pachnocybe ferruginea	AY618669	A(2), B(1), D(1), F1(10), F2(3), G(2)	B	G	NP	A	A	Septobasidium sp. [AB043972]	211/229 (92%)
Stereum sanguinolentum	AY618670	G(2)	D	G	P	A	A	Stereum sanguinolentum [AY089730]	443/466 (95%)
WRCF-B2	AY618671	F1(6), G(2)	D	NG	P	NA	A	Rhodotorula nothofagi [AF444641]	151/155 (97%)
WRCF-B4	AY618672	B(2), F1(1)	D	G	NP	A	A	Hyphodontia radula [AF145580]	555/557 (99%)
WRCF-B5	AY618673	C(2), G(5)	D	G	P	A	A	Butlerelfia eustacei [U85800]	386/413 (93%)
WRCF-B7	AY618674	F2(2)	D	G	NP	A	A	Phanerochaete sordida [AY219381]	580/594 (97%)
WRCF-B9	AY618675	F2(2)	D	G	NP	A	A	Phlebia livida [AB084618]	363/391 (92%)
Ascomycota									
Oidiodendron griseum	AY618676	E(1), F1(4), F2(1)	B	NG	NP	NA	A	Oidiodendron griseum [AF062794]	345/345 (100%)
Phialophora lignicola	AY618677	E(3)	O	NG	NP	NA	A	Salal root associated fungus [AF149081]	482/485 (99%)
Phialophora versicola	AY618678	B(1), F1(1)	B	NG	NP	NA	A	Ectomycorrhizal isolate [AJ430410]	464/465 (99%)
Phialophora sp. 1	AY618679	E(4)	D	G	NP	NA	A	Ascomycete sp. [AY354276]	335/350 (95%)
Phialophora sp. 2	AY618680	E(3), F1(2)	B	NG	NP	NA	A	Cadophora fastigiata [AY249073]	497/497 (100%)
Phialophora sp. 3	AY618681	F2(3), G(3)	B	NG	NP	NA	A	Phialophora sp. [AY465463]	469/471 (99%)
Phialophora sp. 4	AY618682	D(2)	D	NG	NP	NA	A	Phialophora sp. [AY465462]	467/500 (93%)
Rhinocladiella atrovirens	AY618683	E(2), F1(6), F2(1), G(9)	B	NG	NP	NA	A	Rhinocladiella atrovirens [AB091215]	563/567 (99%)
Sporothrix sp. 1	AY618684	F1(2), F2(1)	D	NG	NP	NA	A	Sporothrix schenckii [AF484468]	424/440 (96%)
Sporothrix sp. 2	AY618685	C(3)	D	NG	NP	NA	A	Ophiostoma grandicarpum [AJ293884]	171/174 (98%)
WRCF-A1	AY618686	A(3), B(1), C(1), D(1), E(2)	D	NG	NP	NA	A	Leaf litter ascomycete [AF502745]	422/441 (95%)
WRCF-A2	AY618687	C(3), G(4)	O	G	NP	NA	A	Oidiodendron myxotrichoides [AJ635314]	267/288 (92%)
WRCF-A3	AY618688	B(2), F1(2), F2(3), G(3)	B	NG	NP	NA	A	Phialocephala dimorphospora [AF486121]	486/495 (98%)

Note-G, growth; NG, no growth; P, present; NP, not present; A, amplification; and NA, no amplification. [1]Number of sampling point (isolates) collected from each fence: A, 16(7); B, 35(7); C, 33(11); D, 26(7); E, 30(18); F1, 56(39); F2, 25(16); and G, 82(39). [2]D, decay zone; O, outside of decay zone; and B, both regions. [3]Identity [% similarity] was derived from matched nucleotide/compared nucleotide in GenBank.

fied Ascomycota (WRCF-A1, WRCF-A2, and WRCF-A3). *O. griseum, Phialophora* spp., *Sporothrix* spp. and WRCF-A2 were isolated from a limited number of sites, while three species, *R. atrovirens,* WRCF-A1 and WRCF-A3, were isolated from a broad range of decay areas. These ascomycetous fungi were easily identified by their asexual structures. The most commonly isolated species was from the genus *Phialophora* (Table 1). In this genus six species, including *P. lignicola*, *P. versicola* and four unidentified, were recognized. Two *Sporothrix* species were also isolated but their specific identification was not pursued.

Most ascomycetous fungi found in this study were ubiquitous; this is concurrent with other research groups' findings. For example, *O. griseum* has been isolated from soil of cedar and spruce bogs, wood pulp (Barron 1962), and pulp and paper samples (Wang 1965); it causes a superficial discoloration of wood (Käärik 1980). *Phialophora* species are recognized as staining agents of wood products in service and also as important soft rot fungi (Eaton and Hale 1993). *R. atrovirens* originally isolated from material on decayed wood was also found in wood products (Barnett and Hunter 1987). Many *Sporothrix* species are the anamorphs of *Ophiostoma* and *Ceratocystis* (Domsch et al. 1980); some of them are commonly found in creosote-treated wood products (Wang and Zabel 1990).

Among the Basidiomycetes five isolates were identified to the genus or species level by morphological features. The most frequently isolated species was *Pachnocybe ferruginea*; it was present in most of the decay areas except in fences C and E. This species has also been reported on creosote-treated western redcedar poles by Warren and Marshall (1986). Two other species, *Acanthophysium lividocaeruleum,* easily recognized by its scattered clamp connections and gloeocystidia (Nakasone 1990), and *Hyphoderma praetermissum,* with its white mat with subtomentous to short-woolly, nodous septate and spathulate cystidia, were also frequently isolated. One isolate of *Stereum sanguinolentum* was identified by its simple septate, scattered single, double, or multiple clamps, and cystidium-like structures. This species is the only *Stereum* species that occurs primarily on gymnosperms. All of the above basidiomycetous fungi have been associated with white rot of various softwoods and wood products in North America (Eslyn 1970; Gilbertson 1974; Ginns 1986; Lemke 1964; Scheffer et al. 1984; Zabel et al. 1985).

An orange *Sporothrix* colony found in D and F1 fences that we initially identified as *Sporothrix luteoalba* based on its morphology, was renamed using DNA sequence analysis *Cerinosterus luteoalba.* Some *Sporothrix* species are reported as the anamorphs of the basidiomycetous genus *Cerinomyces* (Dacrymycetaceae), and Moore (1987) erected the new genus *Cerinosterus* to accommodate species of *Sporothrix* having dolipores and imperfect parenthesome septa. Finally, some unknown Basidiomycetes WRCF-B2, and WRCF-B5 were isolated with high frequencies but rarely from the decay sites, while WRCF-B7 and WRCF-B9 were isolated only from a decay site (Table 1).

Molecular analysis of fungal isolates

In order to discriminate the isolates to the species level, the ITS regions were amplified. The amplified products ranged from 640 to 770 bp for the Basidiomycota and from 570 to 660 bp for the Ascomycota. However, for two isolates, *Phialophora versicola* and WRCF-A3, the amplified products were larger than the other species reported in this work, at about 1100 bp and 927 bp, respectively. The two fungi have introns of 520 bp for *P. versicola* and 340 bp for WRCF-A3 located near the 3′ end of the 18S rDNA (data not shown). These introns contained four conserved regions, a characteristic of group I introns (Cech 1988; Dujon 1989). Blast searches revealed high similarity between the intron sequences of WRCF-A3 and a *Phialographium*-like fungus (AB038422), while the intron sequence of *P. versicola* matched closely those of *Lachnum sclerotii* (AF505520), *Rhabdocline parkeri* (AF462428), *Cadophora gregata* f. sp. *adzukicola* (AF056487), and *Hymenoscyphus ericae* (AY394907). Although phylogenetically distant, these species are coniferous pathogens or ectomycorrhizal fungi.

Sequences of 18S and LSU rDNA regions have been used for fungal identification in many ecological studies (Hunt et al. 2004; Kernaghan et al. 2003; Tedersoo et al. 2003). In addition to these regions, ITS sequence comparison is regarded as an excellent tool for identifying unknown fungi to broad species groups or genera (Horton and Bruns 2001). The ITS sequence data enabled the linkage of most morphologically unidentifiable fungi to established genera. For example, WRCF-B5 belongs to the genus *Butlerelfia*, WRCF-B7 to *Phanerochaete*, WRCF-B9 to *Phlebia*, WRCF-A2 to *Oidiodendron*, and WRCF-A3

to *Phialocephala* (Table 1). At this stage of analysis, WRCF-B4 was identified as *Hyphodontia radula* since both sequences were 99% similar. *Phialophora* sp. 2 was identified as *Phialophora fastigiata* (telemorph – *Cadophora fastigiata*) with 100% sequence similarity.

Five *Phialophora* species were positioned in five distantly related clades in the ITS phylogenetic tree (data not shown). This result is consistent with previous work that suggested that the genus *Phialophora* is clearly polyphyletic (Gams 2000). Interestingly *P. versicola* and *P. lignicola* were closely related to salal root associated fungi, which were isolated from Vancouver Island (Allen et al. 2003). Some *Phialophora* species are known to form ectendomycorrhizal relationships with *Pinus* and *Larix*, and have also been observed forming ericoid mycorrhizal with *Gaultheria shallon* (Monreal et al. 1999; Yu et al. 2001), though their ecological functions are not well understood. It is important to note that *Phialophora* species have different phylogenetic histories and ecological roles.

Pachnocybe ferruginea, Cerinosterus luteoalba and WRCF-B2 were matched to members of a primitive order of Basidiomycota. These results might explain why *C. luteoalba* and WRCF-B2 were not amplified by basidio-specific primers. *P. ferruginea* was closely related to *Septobasidium* sp. which was classified as Urediniomycetes. This is consistent with previous results on 5S rDNA and large subunit rDNA sequence analysis (McLaughlin et al. 1995; Walker 1984). *P. ferruginea*, described first as a Hyphomycetes with reddish brown synnemata by Ellis (1971), was later transferred to Heterobasidiomycetes because of its simple septal pore structure (Kropp and Corden 1986; Oberwinkler and Bandoni 1982). The ITS sequence of *C. luteoalba* confirmed its position within the Basidiomycota instead of Ascomycota, which coincided with Moore's (1987) suggestion.

Overall there was a good agreement between morphological and ITS-sequence based approaches. However, due to the limited ITS sequence data within the database the closest matches of some fungal isolates could not be established. Specifically, ITS sequences of *C. luteoalba, H. praetermissum* and WRCF-B2 had matches to only 5.8S rDNA region sequence of *Sporobolomyces symmetricus* (AY364836), uncultured fungus (AY241671) and *Rhodotorula nothofagi* (AF444641), respectively.

β-thujaplicin resistance and fungal colonization

Although β-thujaplicin concentration varies within WRC trees (MacLean and Gardner 1956), it inhibits many decay fungi at concentrations between 10 to 20 ppm (Inamori et al. 2000; Rennerfelt 1948). In our work, β-thujaplicin showed some antifungal activity against most of the fungi examined (Table 2). Most basidiomycetous fungi tested were inhibited by concentrations between 2 and 8 ppm, while most of the ascomycetous fungi tested were affected at concentrations between 8 and 32 ppm. Our results were consistent with those of Rennerfelt (1948), who also showed that some ascomycetous fungi had higher resistance to thujaplicin than decay fungi. However, two basidiomycetous species, *P. ferruginea* and *A. lividocaeruleum*, and three ascomycetous species, *Oidiodendron* sp. (WRCF-A2), *Phialophora fastigiata* (*Phialophora* sp. 2) and *Phialophora* sp. 3, had high tolerance to this compound (Table 2). That these fungi were isolated both outside and in the center of decay areas suggests that they may be pioneer fungi in WRC fences. Such fungi may tolerate high concentrations of inhibitors and, by detoxifying them, permit other ascomycetous and basidiomycetous fungi to become established.

These suggestions agree with the conclusions of Findlay (1966), Chesters (1950), Meredith (1960) and Shigo (1967) on WRC logs and fallen trees, and of Van der Kamp (1975) on standing trees. They showed that heartwood is invaded by a succession of fungi that allow decay to occur. Later, Jin et al. (1988) demonstrated that a pioneer *Sporothrix* fungus that was consistently isolated from the outer heartwood in WRC tree transformed thujaplicins into thujin, which was nontoxic to decay fungi.

Conclusions

Complementing molecular techniques with traditional morphology based methods greatly increased the accuracy and speed of fungal species identification. Compared to earlier studies on WRC wood products, a variety of different types of wood rotting basidiomycetous fungi were isolated from WRC fence materials. Identified Homobasidiomycetes in the present study were corticioid fungi, which cause white rot, and most of them were not commonly associated with WRC products. The isolates' tolerance to β-thujaplicin, as well as location and frequency of isolation

Table 2. Effect of the various concentrations of β-thujaplicin on fungi isolated

Fungi (identification by ITS sequence)	Fungal growth[1] (mm)			
	0 (Control)	2 ppm (mg/L)	8 ppm (mg/L)	32 ppm (mg/L)
Basidiomycota				
Acanthophysium lividocaeruleum	31.5 (1.3) a[2]	27.0 (1.3) b	23.8 (0.3) c	15.5 (0.9) d
Cerinosterus luteoalba	5.7 (0.6) a	0.0 (0.0) b	0.0 (0.0) b	0.0 (0.0) b
Hyphoderma praetermissum	13.8 (0.8) a	3.8 (0.8) b	0.0 (0.0) c	0.0 (0.0) c
Pachnocybe ferruginea	7.1 (0.5) a	6.9 (0.6) a	7.0 (0.3) a	5.1 (0.3) a
Stereum sanguinolentum	39.0 (1.0) a	14.3 (1.2) b	0.8 (0.3) c	0.0 (0.0) c
WRCF-B2	9.8 (0.3) a	8.1 (0.4) a	1.8 (0.3) b	0.0 (0.0) b
WRCF-B4 (*Hyphodontia radula*)	12.3 (0.6) a	0.0 (0.0) b	0.0 (0.0) b	0.0 (0.0) b
WRCF-B5 (*Butlerelfia* sp.)	15.3 (0.6) a	0.8 (0.3) b	0.0 (0.0) b	0.0 (0.0) b
WRCF-B7 (*Phanerochaete* sp.)	85.3 (0.6) a	23.0 (1.0) b	4.8 (0.3) c	0.0 (0.0) d
WRCF-B9 (*Phlebia* sp.)	35.7 (0.6) a	32.7 (1.2) b	2.8 (0.3) c	0.0 (0.0) d
Ascomycota				
Oidiodendron griseum	3.2 (0.3) a	2.3 (0.3) a	2.0 (0.0) ab	0.0 (0.0) b
Phialophora lignicola	10.7 (1.2) a	9.8 (1.0) ab	7.7 (1.3) b	0.0 (0.0) c
Phialophora versicola	6.7 (0.3) a	5.2 (0.8) a	3.8 (0.3) b	0.0 (0.0) c
Phialophora sp. 1	16.0 (1.0) a	15.7 (1.2) a	7.3 (1.5) b	0.0 (0.0) c
Phialophora sp. 2 (*P. fastigiata*)	17.8 (0.3) a	17.2 (0.3) a	16.5 (0.5) ab	14.3 (0.6) b
Phialophora sp. 3	7.3 (0.3) a	7.0 (0.0) ab	7.0 (0.5) ab	4.8 (0.3) b
Phialophora sp. 4	9.7 (0.6) a	7.2 (0.3) b	0.0 (0.0) c	0.0 (0.0) c
Rhinocladiella atrovirens	3.7 (0.6) a	2.8 (0.3) a	1.4 (0.4) b	0.0 (0.0) b
Sporothrix sp. 1	11.7 (0.6) a	10.8 (0.8) ab	9.7 (0.6) b	0.0 (0.0) c
Sporothrix sp. 2	13.0 (0.5) a	11.8 (0.3) a	9.8 (0.3) b	0.0 (0.0) c
WRCF-A1	8.7 (0.6) a	7.2 (0.3) ab	5.0 (0.5) b	0.0 (0.0) c
WRCF-A2 (*Oidiodendron* sp.)	31.7 (0.6) a	31.2 (0.3) a	26.8 (0.3) b	8.8 (0.3) c
WRCF-A3 (*Phialocephala* sp.)	10.8 (0.8) a	6.8 (0.3) b	1.8 (0.3) c	0.0 (0.0) c

[1]Fungal growth was measured 12 days after exposure on MEA. Values are mean of three replicates and standard error in parenthesis; [2]Numbers followed by the same letter in each row are not significantly different ($\alpha = 0.05$) according to the Duncan's method.

may provide evidence of pioneer species involved in a succession of fungi that ends in decay of WRC products. Two basidiomycetous species: *Pachnocybe ferruginea* and *Acanthophysium lividocaeruleum*; three ascomycetous, soft-rot fungal species: *Oidiodendron* sp. (WRCF-A2), *Phialophora fastigiata* (*Phialophora* sp. 2); and *Phialophora* sp. 3 might be pioneer fungi in WRC fence decay. These findings contrast with previous research that suggested basidiomycetes generally followed ascomycetous, soft-rot fungi (Butcher 1968; Corbett and Levy 1963; Duncan 1960). They might facilitate the entry of other ascomycetous and basidiomycetous (decay fungi) into WRC fence materials. Therefore, these pioneer fungi may play an important role in deterioration of WRC fences. Further research is needed to establish chemical mechanisms of extractive detoxification by these pioneer species.

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