Diversity and decay ability of basidiomycetes isolated from lodgepole pines killed by the mountain pine beetle

E. Son, J.-J. Kim, Y.W. Lim, T.T. Au-Yeung, C.Y.H. Yang, and C. Breuil

Abstract: When lodgepole pines (*Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson) that are killed by the mountain pine beetle (*Dendroctonus ponderosae*) and its fungal associates are not harvested, fungal decay can affect wood and fibre properties. Ophiostomatoids stain sapwood but do not affect the structural properties of wood. In contrast, white or brown decay basidiomycetes degrade wood. We isolated both staining and decay fungi from 300 lodgepole pine trees killed by mountain pine beetle at green, red, and grey stages at 10 sites across British Columbia. We retained 224 basidiomycete isolates that we classified into 34 species using morphological and physiological characteristics and rDNA large subunit sequences. The number of basidiomycete species varied from 4 to 14 species per site. We assessed the ability of these fungi to degrade both pine sapwood and heartwood using the soil jar decay test. The highest wood mass losses for both sapwood and heartwood were measured for the brown rot species *Fomitopsis pinicola* and the white rot *Metulodontia* and *Ganoderma* species. The sap rot species *Trichaptum abietinum* was more damaging for sapwood than for heartwood. A number of species caused more than 50% wood mass losses after 12 weeks at room temperature, suggesting that beetle-killed trees can rapidly lose market value due to degradation of wood structural components.

Key words: mountain pine beetle, lodgepole pine, fungal diversity, basidiomycete, white rot, brown rot, decay test, mass losses.

Résumé : Lorsque le pin tordu latifolié (*Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson) tué par le dendroctone du pin ponderosa (*Dendroctonus ponderosae*) et ses champignons associés n'est pas récolté, les propriétés du bois et de la fibre peuvent être affectées par la pourriture fongique. Les ophiostomatoïdes tachent l'aubier mais n'affectent pas les propriétés structurelles du bois. Par contre, les basidiomycètes responsables de la carie blanche ou brune dégradent le bois. Nous avons isolé des champignons qui tachent ou qui dégradent le bois à partir de 300 pins de Murray tués par le dendroctone du pin ponderosa aux stades vert, rouge et gris, répartis sur dix sites dans toute la Colombie-Britannique. Nous avons retenu 224 isolats de basidiomycètes que nous avons classés en 34 espèces selon leurs caractéristiques morphologiques et physiologiques, ainsi que selon la séquence de l'ADNr de la grande sous-unité. Le nombre d'espèces de basidiomycètes variait de 4 à 14 par site. Nous avons évalué la capacité de ces champignons à dégrader l'aubier et le bois de cœur du pin à l'aide d'un test de décomposition in vitro. Les pertes en masses de bois les plus élevées, tant pour l'aubier que pour le bois de cœur, ont été mesurées avec l'agent de la pourriture brune *Fomitopsis pinicola* et les espèces de pourriture blanche *Metulodontia* et *Ganoderma*. L'agent responsable de la pourriture de l'aubier *Trichaptum abietinum* était plus dommageable pour l'aubier que pour le bois de cœur. De nombreuses espèces causaient plus de 50 % de perte en masses de bois après 12 semaines à la température de la pièce, ce qui suggère que les arbres tués par le dendroctone peuvent perdre rapidement leur valeur marchande à cause de la dégradation des composantes structurelles du bois.

Mots-clés : dendroctone du pin ponderosa, pin de Murray, diversité fongique, basidiomycètes, pourriture blanche, pourriture brune, test de décomposition, perte de masses.

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Introduction

The mountain pine beetle (*Dendroctonus ponderosae*; MPB) has infested and killed lodgepole pine (*Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson) in over 16 million ha of forest in British Columbia (http://www.for.gov.bc.ca/hfp/mountain_pine_beetle). A major concern arising from this epidemic is the loss of yield and value for wood fibre over large geographic areas. It is estimated

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that British Columbia will process up to 900 million m^3 of MPB-killed wood by 2010.

After a successful MPB mass attack, a tree undergoes 3 phases, indicated by changes in the tree's foliage colour. With some variation due to weather and the tree's physiological condition, "green", "red", and "grey" phases occur 1, 2–3, and 3–5 years following the initial MPB attack, respectively (Whitney 1982; Kim et al. 2005). The primary forest management needs are to contain the spread of the epidemic and minimize the loss of timber and fibre. Trees in the green phase tend to be harvested, leaving trees from red and grey phases. However, for logistic reasons, many green phase trees are left in the forest, where they will eventually turn red and then grey. While MPB-killed trees are still usable, yield and fibre quality are less than that of sound trees (Pousette and Hawkins 2006). Thus, the secondary forest management needs are to manage the infested trees and to decide which trees can be left standing and for how long and which should be harvested and processed rapidly.

MPB vector fungi through the tree bark to modify the wood environment (like moisture content) so that the beetles can reproduce (green attack). Trees weakened or killed by MPB and its fungal associates can be further colonized by secondary beetles that vector different fungi. The most common fungi spread by MPB are ophiostomatoids and basidiomycetes. The ophiostomatoid fungi stain the tree sapwood — while they cause a cosmetic defect, they do not affect the structural properties of wood. The basidiomycetes include decay fungi that degrade structural wood components like cellulose and lignin, and other fungi, like *Entomocorticium* species, that do not. The sap-staining fungi that are associated with MPB are well characterized. They belong to the genera *Grosmannia*, *Ophiostoma*, and *Ceratocystiopsis*. Some of the species are pathogenic and kill trees (e.g., *Grosmannia clavigera*), while others are less pathogenic (e.g., *Ophiostoma montium*) or nonpathogenic (Solheim and Krokene 1998; Plattner et al. 2008). In contrast, little information is available on the decay fungi that are carried by MPB or other beetles.

While the identity of the decay fungi affecting lodgepole pine forests after MPB outbreaks is still largely unknown, a reasonable amount of information is available about the susceptibility of pine to different types of decay (Venäläinen et al. 2004). Lodgepole pine covers large areas of even-aged stands or is mixed with other species (e.g., spruce) in the northern and southern interior of British Columbia and western Alberta. In contrast to some other conifer species, pine is not a durable species. Pine species are susceptible to white and brown rot fungi. White rots are capable of degrading carbohydrates (cellulose and hemicelluloses) and lignin, the 3 major components of the wood cell wall, while brown rots degrade mainly the wood carbohydrates and not the lignin (Blanchette et al. 1990; Schwarze 2007). Both types of decay gradually decrease the structural properties of wood, and so storage for long periods may be not desirable. The most commonly reported decay fungi affecting pine in British Columbia and Alberta are (1) root rots *Armillaria ostoyae*, *Armillaria sinapina*, and *Heterobasidium annosum*; (2) sap rots *Gleophyllum separium* and *Trichaptum abietinum*; and (3) heart rots *Phellinus pini* and *Fomitopsis pinicola* (see Canadian Forest Service Web site, http://cfs.nrcan.gc.ca; Allen et al. 1996; Dettman and van der Kamp 2001). These fungal species are not specific to pine and have been reported to affect other coniferous trees (Blanchette 1984; Hsiang and Edmonds 1989; Bader et al. 1995).

The work presented extends previous studies on fungi sampled from lodgepole pines with green, red, and grey crowns across British Columbia that reported staining fungi (Kim et al. 2005). Here, we report the identity and diversity of the basidiomycetes isolated from these trees and then assess the ability of some of these species to degrade both sapwood and heartwood using the soil jar decay test.

Materials and methods

Sampling and fungal isolations

Fungal isolates were collected from 10 different sites across British Columbia, Canada, from June 2003 to September 2004. Ten trees of each MPB-attack phase — green, red, and grey — for a total of 30 trees were sampled from each site. The sites were Manning Park (site 1), Riske Creek (site 2), Radium (site 3), Cranbrook (site 4), Little Fort (site 5), Robson Park (site 6), Monte Lake (site 7), Burns Lake (site 8), Prince George (site 9), and Quesnel (site 10). Two logs (~0.6 m long) from each tree, top and bottom, were collected to characterize the fungal isolates. The location, age, and moisture content (MC) of each tree are shown in Table 1. A disk from the bottom of the middle bolt of the tree was used for determining MC of sapwood and heartwood (Kim et al. 2005). The MC of the trees was measured by the oven-dry method (standards D 2016) following the American Society for Testing Materials (ASTM 2000). Then significant differences of tree MC with tree types were examined using a Duncan's multiple range test (SAS Institute 2001). Logs were debarked and inspected for signs of stain and decay. MPB galleries were always observed on the surface of the sapwood. Then disks of ~2–3 cm thickness were further cut for fungal isolation following the method of Kim et al. (2005). Wood chips from either the sapwood or heartwood were placed on 2% OMEA (33 g Oxoid malt extract agar, 10 g Oxoid agar, and 1000 mL distilled water) and 2% OMEA with benomyl (Clubbe 1978) and ampicillin to preferentially isolate the basidiomycetes (Kim et al. 2005). The fungi were allowed to grow for 7–14 days before being transferred onto new media (Kim et al. 2005).

Morphological and molecular identification of fungal isolates

We identified the fungal isolates to either genus or species by morphological and molecular techniques. Isolates were regrown onto 2% OMEA plates, and the isolate's morphological characteristics were compared with those of reference cultures (von Arx and Hennebert 1965). The isolates were grouped by colony morphology, colour, and growth rate (slow, medium, and fast). DNA from representative isolates of each group was further extracted and characterized. For fungal DNA extraction, isolates were grown on 2% MEA overlaid with cellophane. The fungal mycelium was collected and processed following the method of Kim et al. (2005). The 28S ribosomal DNA large subunit (LSU)

Table 1. Characteristics of the harvested green-, red-, and grey-phase lodgepole pine trees after mountain pine beetle attack.

			Moisture content (%)*				
Location	Phase	Age (years)	Sapwood	Heartwood	Tree diameter (cm)	Sap rot†	Heart rot†
Manning Park (site 1)	Green	79.3±16	66.9±30c	32.7±5cd	21.3±3	0	5
	Red	80.1±25	26.9±9hi	27.6±4efgh	19.1±5	0	6
	Grey	88.6±14	18.8±8hij	19.7±5klm	19.0±4	0	4
Riske Creek (site 2)	Green	60.9±5	79.4±19b	33.9±11cd	22.5±4	0	0
	Red	70.3±21	19.9±6hij	24.3±5ghijk	27.0±3	0	0
	Grey	52.6±6	14.2±9ij	15.5±2mn	19.5±4	0	1
Radium (site 3)	Green	59.5±5	42.5±12ef	34.0±2cd	21.7±3	0	1
	Red	70.3±8	19.8±9hij	24.2±4ghijk	26.3±5	0	3
	Grey	67.1±11	10.4±3j	14.6±5n	26.4±5	0	8
Cranbrook (site 4)	Green	75.9±9	55.4±23cd	33.1±1cd	26.2±4	0	0
	Red	81.1±6	19.9±1hij	24.1±2ghijk	21.6±4	0	7
	Grey	69.4±7	15.1±4ij	17.6±6lmn	21.1±3	0	5
Little Fort (site 5)	Green	92.4±8	26.8±2hi	31.1±2cdef	29.4±4	0	4
	Red	100.4±6	22.3±3hij	29.1±7defg	32.2±5	1	7
	Grey	96.8±5	21.7±5hij	21.8±5jkl	30.9±4	3	5
Robson Park (site 6)	Green	78.2±9	64.4±30c	33.9±5cd	27.0±2	0	1
	Red	73.5±13	27.5±8hij	27.3±4efghi	26.2±3	0	1
	Grey	72.0±11	22.2±4hij	26.5±6fghij	29.6±3	1	1
Monte Lake (site 7)	Green	95.8±3	100.6±26a	34.4±4cd	23.6±3	0	0
	Red	92.8±6	28.2±7ghi	29.0±3defg	25.2±3	0	3
	Grey	85.1±9	17.7±4hij	20.3±5klm	24.4±3	0	1
Burns Lake (site 8)	Green	61.3±1	41.6±20efg	33.1±3cd	21.8±4	0	3
	Red	57.1±5	31.6±6fgh	31.8±4cde	23.9±4	9	4
	Grey	59.8±2	22.6±5hij	22.6±5hijkl	23.4±2	7	1
Prince George (site 9)	Green	138.2±7	58.3±25cd	43.0±7a	23.8±2	0	7
	Red	137.0±21	32.0±9fgh	35.7±4bc	27.5±3	2	8
	Grey	130.9±16	31.2±9fgh	35.8±7bc	27.2±2	2	11
Quesnel (site 10)	Green	86.2±4	50.8±24de	40.0±9ab	23.7±2	0	1
	Red	88.1±5	21.7±2hij	22.2±2ijkl	24.7±3	6	6
	Grey	93.6±10	20.0±2hij	22.8±6hijk	24.4±5	3	6

*Values are mean of 10 middle bolts per tree, including measurements from 4 pieces of wood per bolt. Numbers followed by the same letter in a column are not significantly different ($\alpha = 0.05$) according to Duncan's method.

†Number of bolts or trees with visible heart rot (97 bolts) or sap rot (34 bolts).

genes were PCR amplified using general primers (White et al. 1990; Gardes and Bruns 1993; Adair et al. 2002). PCR products were purified using the Qiaquick Purification kit and were sequenced at Macrogen (Seoul, Korea). Sequences for each isolate were compared against sequences retrieved from GenBank database. Isolates were identified to the species level when the LSU sequence similarity to GenBank data was greater than 99% and to the genus level when the similarity was less than 99%. Some fungal isolates like *F. pinicola* and *Trichaptum abietinum* were easily identified to the species level because of their close sequence match with that of related taxa from GenBank.

We also differentiated white rot from brown rot fungi by growing the isolates on 2% MEA containing tannic acid. A red reaction zone is formed in this medium, when a white rot fungus releases lignolytic enzymes (Nobles 1965).

Statistical analysis of diversity index

Fungal diversity throughout the sites was compared using the Simpson diversity index (Simpson 1949). The index value ranges between 0 and 1; a value close to 0 indicates the presence of dominant species in a population, and a value close to 1 suggests equilibrium of species.

Determination of wood decay rates

For the decay tests we followed the standard procedure for laboratory soil block culture (Standard E10-01) described by the American Wood Protection Association (AWPA 2002). Small sapwood and heartwood blocks (2 cm × 2 cm × 2 cm) were obtained from sapwood and heartwood boards processed from a sound 118-year-old tree harvested at Williams Lake (British Columbia). First, wood blocks were oven-dried at 105 °C for 24 h to determine the wood dry mass. Then, wood blocks were soaked for 2–3 h in sterile distilled water and autoclaved. Plugs of fungi grown on 2% MEA were inoculated on a wood strip that rested on the soil in a jar. Once the mycelium completely covered the feeder strip, 2 wood blocks per jar were then placed on the feeder strip. In total, 58 isolates belonging to different genera or species were tested on sapwood and heartwood blocks with 2 jars for the sapwood (4 wood blocks) and 2 for the heartwood (4 wood blocks) for a total of 2 replicates each. When possible, several strains of the same species were tested. The decay tests were run for approximately 12 weeks at ~22 °C, after which the wood blocks were removed and oven-dried for measuring wood mass losses.

Table 2. The number of basidiomycetes isolated and identified at each study site.

Location	Total no. of isolates	Isolates for DNA analysis per site	No. of species per site	Most frequent species per site*
Manning Park (site 1)	33	15	14	5
Riske Creek (site 2)	10	4	4	3
Radium (site 3)	35	9	12	4
Cranbrook (site 4)	35	12	13	4
Little Fort (site 5)	17	11	9	4
Robson Park (site 6)	10	5	5	1
Monte Lake (site 7)	10	3	5	2
Burns Lake (site 8)	28	18	11	7
Prince George (site 9)	24	14	12	9
Quesnel (site 10)	22	14	10	6
Total	224	105	na	na

Note: na, not applicable.

*Most frequent species is defined as the number of species found at more than one site with more than 1 isolate.

Results

Tree characteristics from the 10 British Columbia sites

The oldest trees were from Prince George (mean ages: 130.9–138.2 years), while the youngest trees were from Riske Creek (mean ages: 52.6–70.3 years). Lodgepole pine trees are considered mature after 60–80 years. MPB preferentially inhabits mature pine; however, in British Columbia's current epidemic, young 20- to 30-year-old lodgepole pines have been also attacked by MPB (British Columbia Ministry of Forests 1995; Maclauchlan 2006). Sapwood MC for all green trees was over 40%, except for those harvested at Little Fort (Table 1). At Monte Lake, sapwood MC in green trees was higher than at any other site. When comparing green and red trees, significant higher values in sapwood MC can be seen in green trees regardless of the site locations. However, when the sapwood MC is compared between red and grey trees, the difference was significant in some locations and not in others (Table 1). We were unable to isolate fungi from red and grey trees with MC up to 20%. Heartwood MC in the green, red, and grey trees from all the sites ranged from 31.1% to 43.0%, 22.2% to 35.7%, and 14.6% to 35.8%, respectively. Heart rot was observed at all the sites (Table 1). Riske Creek trees showed the lowest number of bolts (only 1) with visible heart rot damage, while Prince George had the highest numbers (26). Sap rot was only observed at 5 of the 10 sites.

Isolation and identification using morphological grouping and DNA sequencing

We isolated the fungi from the sapwood and heartwood of the bottom and top billets of 300 green, red, and grey trees. From the 1200 wood chips plated, ~900 isolates were recovered on MEA or MEA–benomyl, and of those only 224 were potential basidiomycetes. Most of the other isolates were staining fungi, molds, yeasts, or undefined filamentous fungi that have been described in previous work (Kim et al. 2005; Lee et al. 2006). Here, we report mainly the work on the basidiomycetes obtained from MEA supplemented with benomyl. However, *Trichaptum abietinum* did not grow in the presence of benomyl, and it was the only species recovered from MEA without antibiotic. Sequence analyses of the rDNA LSU (Lim et al. 2005; Kim et al. 2005) were completed for 105 isolates from 10 sites (Table 2). Based on the LSU data, 29 genera and 34 potential species were tentatively identified. The isolated genera or species are shown in Table 3 and listed in the text below.

Basidiomycete diversity at the different sites

Overall, some basidiomycetes were present at only 1 or 2 sites, while others were present at 6, 8, or 10 sites. For example, *Entomocorticium* species more commonly found in MPB beetle galleries (Whitney et al. 1987; Hsiau and Harrington 2003; Kim et al. 2005) were also frequently isolated from sapwood that contacted these galleries. Species of this genus were the most frequently isolated, occurring at the 10 sites, with 84 isolates in total. *Peniophora* sp., *Sistotrema brinkmannii*, and *Trichaptum abietinum* were also isolated frequently, being found at 9, 8, or 7 of the 10 sites, respectively. The highest basidiomycete diversities were observed at Manning Park (14 species), Cranbrook (13 species), Prince George and Radium (12 species), Burns Lake (11 species), and Quesnel (10 species), while the lowest diversities were found at Robson Park and Monte Lake (5 species) and Riske Creek (4 species).

Seventeen species were only isolated once or twice at a single site: *Acanthophysellum lividocoeruleum*, *Athelia epiphylla*, *Clavulina cristata*, *Coprinellus xanthothrix*, *Diplomitoporus crustulinus*, *Gloeocystidiellum clavuligerum*, *Hypholoma fasciculare*, *Lentinellus omphalodes*, *Metulodonia* sp., *Oligoporus placentus*, *Oligoporus rennyi*, *Panus rudis*, *Phanerochate* sp., *Phellinus ferreus*, *Phlebia uda*, *Pleurotus pulmonarius*, and *Sarcomyxa serotina*.

Overall, except for site 7, the Simpson index values (ranging from 0.599 to 0.850) were closer to 1 than 0, indicating that the species were evenly distributed at each site. Species richness was high at site 1 (14), and diversity was high at site 4. The data showed that *Entomocorticium* species were the dominant species at all 10 sites.

Some of the species isolated are well-documented white rot and brown rot fungi. Different white rot isolates from the same species showed variations in the intensity of the tannic acid reaction, and a few species were unable to grow on media containing tannic acid and so could not be classified as white or brown rot from this test. Among the white rots isolated, *Ganoderma* species were only found at Ques-

nel, *Trametes versicolor* was isolated at 2 sites (Radium and Prince George), and *H. annosum* was found at 5 sites (Manning Park, Radium, Cranbrook, Burns Lake, and Prince George). *Amylosterum chailletii*, a wood wasp associated fungus, was found at 4 sites (Manning Park, Radium, Cranbrook, Little Fort). *Phellinus pini*, a pine-decaying fungus, was present at 5 sites, including a site with a low frequency of basidiomycete fungi, Robson Park. *Peniophora* sp. and *Trichaptum abietinum* were found at 8 and 7 sites, respectively. Among the brown rots, we found *F. pinicola* at 8 sites, while *Coniophora olivaceae*, *Oligoporus placentus*, and *Oligoporus rennyi* were only present at 1 site. Overall brown rot fungal diversity was low.

Decay tests with lodgepole pine wood from Williams Lake

We assessed the decay potential of 58 isolates belonging to 34 species on pine from Williams Lake. For 11 species, we had only 1 isolate, while for the others we tested up to 4 isolates. The results are reported in Table 3, as well as in the text below. Some species decayed the sapwood preferentially, for example, *Amylostereum chailleti*, *Peniophora* sp., most *Phlebia* sp., *Stereum sanguinolentum*, *Phellinus pini*, and *Phellinus ferreus*. In contrast, other species degraded both the sapwood and heartwood, for example, *Ganoderma* sp., *F. pinicola*, *Trichaptum abietinum*, *Oligoporus* sp., and *Metulodontia* sp. We had only 1 isolate for the following species: *Athelia epiphylla*, *Diplomitoporus crustulinus*, *Hypholoma fasciculare* , *Pleurotus pulmonarius*, and *Gloeocystidiellum clavuligerum*. Except for *Athelia epiphylla*, which only degrades the heartwood, all the other species caused sapwood mass losses. A few species caused no wood mass losses; these included the frequently isolated *Entomocorticium* species and *Sistotrema brinkmannii* (Table 3). Some species, including *Acanthophysellum lividocoeruleum*, *Clavulina cristata*, *Coprinellus xanthothrix*, *Cylindrobasidium* sp., *Lentinellus omphalodes*, *Panus rudis*, *Phlebia queletii*, and *Sarcomyxa serotina* decreased wood mass by only 1%–6%.

Discussion

Diversity of wood decay in trees killed by MPB

Except for Riske Creek, where all but one of the sampled trees were sound, visible decay damage was observed in the green, red, and grey trees processed at the other sites. We also noted that damage due to decay was frequently high enough to indicate that it developed before MPB attacks, and more rarely following MPB attacks. While at some sites we found the same numbers of trees affected by decay in the 3 phases (Robson Park), the numbers of decayed red and grey trees at other sites were slightly higher than the number of green ones (Radium, Cranbrook, Prince George, and Quesnel). These results are not surprising, since Lodgepole pine is not a durable tree species and is among the species most susceptible to stain and decay (Chow and Obermajer 2007). Furthermore, the trees processed were often older than 70 years, and such trees have less efficient defense mechanisms against pests than younger trees (Christiansen et al. 1987; Plattner et al. 2008). We also showed that fungal diversity was high at a number of sites. In addition to variation in temperatures, some regions are dryer than others; Prince George, where the fungal diversity was high, receives more constant precipitation throughout the year (http://www.worldweather.org/056/c00619.htm). In addition, fungal diversity may also be affected by parameters such as soil type, vegetation, and landscape topography.

Identifying fungi by morphology is difficult, time consuming, and prone to error. Instead, a preliminary morphological grouping can be followed by additional DNA sequence work (Lim et al. 2005). Researchers use rDNA (i.e., LSU) for primary identification. For nuclear DNA, rDNA is present as many copies in the genome and so is easily amplified using general fungal primers. While we used this approach, our results show that fungal identification using a single gene is not always possible. Thus, further work on either the internal transcribed spacers of rDNA, a region relatively conserved, or other protein-coding genes will be necessary to confirm our preliminary LSU results and to identify isolates that we were able to classify only at the genus level (Mullineux and Hausner 2009).

That diversity was higher for white than brown rot fungi is not surprising, since only 6% of the wood decay fungi are brown rot (Gilbertson 1980). In the northern hemisphere most brown rot fungi belong to the *Polyporaceae* family; these fungi appear to be associated with and adapted to conifers, which also show a low diversity (Gilbertson 1980; Rayner and Boddy 1988). Among the brown rots, *F. pinicola* is one of the most frequently occurring decay fungi in British Columbia and one of the most damaging in old-growth forests (Allen et al. 1996). The other brown rots, *O. placentus*, *Coniophora olivaceae*, and *O. rennyi* were rarely encountered. In North America, brown rot fungi are found in standing or fallen trees, as well as in processed wood. Softwood and hardwood species with a high amount of parenchyma cells are more resistant to brown rots; however, overall, softwood species are more susceptible to brown rot than to white rot (Schwarze 2007). Among the white rot fungi, the most frequently isolated species were *Peniophora* species, *Trichaptum abietinum*, *Phellinus pini*, and *H. annosum*. None of these fungal species are specific to lodgepole pine; they are broadly distributed on several conifer species throughout British Columbia (Allen et al. 1996). The genus *Peniophora* is highly diversified and includes more than 300 species. Some of these fungal species seem able to degrade phenolic compounds present in the heartwood of pine trees, while other species (e.g., *Peniophora gigantea*) have been used as a biocontrol agent of *H. annosum* on freshly cut stumps of pine (Loman 1970; Parker 1977). *Trichaptum abietinum*, the only species unable to grow on media with benomyl, produces fruiting bodies on dead trees or logs and forms white rot pockets in sapwood (Allen et al. 1996; Kim et al. 2005). This fungal species is found in many trees species in all regions of British Columbia (Allen et al. 1996). *Phellinus pini* is commonly reported as red ring rot because of the red colour appearing in the heartwood at the early stage of decay. At a more advance decay stage the fungus preferentially degrades lignin in latewood tracheids, creating typical white pockets (Blanchette 1984; Allen et al. 1996). The root rot *H. annosum* is a widespread pathogen in northern hemisphere forests, where it causes important economic losses (e.g., up to $1 billion in

Table 3. Decay tests on wood blocks using species with 1 or multiple isolates.

Genus	Species	Foliage colour*	Location of isolate†	ID	% Mass loss‡		Tannic acid test§	Growth rate‖
					Heartwood	Sapwood		
Acanthophysellum	*lividocoeruleum*	Green	Top	111	1.3±0.6	4.2±1.8	S	+
Amylostereum	*chailletii*	Grey	Top	139	2.3±0.5	10.6±1.6	W	+
Amylostereum	*chailletii*	Red	Top	514	1.6±0.8	17.8±1.5	W	+
Amylostereum	*chailletii*	Red	Bottom	428	1.6±0.6	9.3±1.7	S	+
Amylostereum	*chailletii*	Grey	Top	417	0.8±0.2	7.6±2.6	W	+
Athelia	*epiphylla*	Grey	Bottom	119	33.8±2.9	1.2±0.2	S	+
Byssomerulium	*corium*	Red	Top	912	1.4±0.2	27.2±5.6	W	++
Byssomerulium	*corium*	Grey	Bottom	909	1.1±0.1	20.8±3.5	NG	+++
Byssomerulium	*corium*	Red	Bottom	85	2.8±2.7	26.9±6.9	W	+++
Clavulina	*cristata*	Grey	Bottom	926	0.5±0.2	1.3±0.3	W/NG	+
Coniophora	*olivaceae*	Red	Bottom	818	33.2±7.2	34.6±11.9	NR	+
Coniophora	*olivaceae*	Green	Bottom	831	29.3±13.9	10.7±1.4	NR	+++
Diplomitoporus	*crustulinus*	Green	Bottom	316	5.0±2.3	35.2±5.0	S	+++
Coprinellus	*xanthothrix*	Green	Top	509	0.6±0.1	0.6±0.2	NG	++
Cylindrobasidium	sp.	Green	Top	101	0.7±0.2	0.8±0.3	ND	+
Cylindrobasidium	sp.	Grey	Bottom	401	0.9±0.3	0.7±0.3	ND	+
Entomocorticium	sp.	Red	Top	324	0.5±0.2	0.7±0.4	ND	+
Entomocorticium	sp.	Grey	Top	125	1.2±0.4	0.5±0.1	ND	+
Fomitopsis	*pinicola*	Red	Bottom	817	15.7±9.6	56.0±8.1	NR	+
Fomitopsis	*pinicola*	Grey	Bottom	810	23.4±15.5	32.6±18.0	NR	+
Fomitopsis	*pinicola*	Grey	Bottom	530	38.9±12.0	61.5±1.0	NR	+
Fomitopsis	*pinicola*	Grey	Bottom	607	31.6±6.7	61.0±2.2	NR	+
Ganoderma	sp.	Grey	Bottom	1004	24.4±3.8	31.8±12.5	S	++
Ganoderma	sp.	Grey	Bottom	1006	35.6±14.0	49.4±28.6	S	++
Heterobasidion	*annosum*	Green	Bottom	312	0.9±0.3	10.6±3.1	S	+++
Heterobasidium	*annosum*	Green	Top	1017	1.2±0.3	17.3±2.3	S	+++
Lentinellus	*omphalodes*	Grey	Bottom	529	5.8±2.7	5.6±1.2	S	+
Metulodontia	sp.	Grey	Bottom	131	43.6±3.6	67.1±3.4	S	++
Oligoporus	*placenta*	Green	Bottom	816	26.9±4.6	35.6±7.9	NR	++
Oligoporus	*rennyi*	Green	Bottom	153	38.5±4.7	28.9±2.3	NR	+
Panus	*rudis*	Green	Bottom	420	0.4±0.2	1.4±0.2	W	+
Peniophora	sp.	Red	Top	211	3.1±0.9	9.6±0.8	S	+
Peniophora	sp.	Green	Top	616	1.5±0.1	8.9±1.0	S	+
Peniophora	sp.	Red	Top	1010	1.6±0.7	8.7±0.9	S	++
Phanerochaete	sp.	Green	Bottom	149	2.5±1.1	24.8±1.0	W	++
Phellinus	*pini*	Red	Bottom	154	3.4±0.9	9.1±2.7	S	+
Phellinus	*pini*	Grey	Bottom	321	4.6±1.7	18.9±3.2	S	+
Phellinus	*pini*	Red	Bottom	421	7.3±1.2	17.8±1.4	S	+
Phlebia	*radiata*	Green	Top	933	3.9±0.7	23.4±4.6	S	++
Phlebia	*radiata*	Green	Bottom	1022	5.8±1.1	18.9±2.6	S	++
Phlebia	*queletii*	Green	Bottom	422	0.6±0.4	3.3±0.8	W	+
Phlebia	*subserialis*	Grey	Top	802	2.1±2.2	12.0±2.6	NG	+++
Phlebia	*subserialis*	Green	Bottom	1013	2.5±1.5	14.8±2.9	W	++
Phlebia	*tremellosa*	Green	Top	714	1.5±0.4	24.0±2.3	S	+++
Phlebia	*tremellosa*	Red	Top	803	0.5±0.2	18.4±3.4	S	+++
Phlebia	*tremellosa*	Green	Bottom	1011	1.8±1.6	25.8±1.5	W	+++
Sistotrema	*brinkmannii*	ND	ND	LPKR1	ND	0.9±0.1	ND	+
Sistotrema	*brinkmannii*	ND	ND	LPWR	ND	1.5±0.2	ND	+
Stereum	*sanguinolentum*	Grey	Bottom	203	1.1±0.1	1.4±0.6	S	+
Stereum	*sanguinolentum*	Grey	Bottom	524	5.3±1.3	18.1±1.4	S	+
Stereum	*sanguinolentum*	Red	Top	914	4.8±0.9	12.3±0.6	S	++
Stereum	*sanguinolentum*	Green	Bottom	829	1.1±0.2	12.4±2.5	S	++
Trametes	*versicolor*	Green	Bottom	419	3.6±0.7	12.9±1.9	S	++
Trametes	*versicolor*	Green	Top	929	5.9±1.6	12.1±1.9	S	++
Trichaptum	*abietinum*	Grey	Bottom	120B	14.0±1.9	31.6±3.3	S	+

Table 3 (*concluded*).

Genus	Species	Foliage colour*	Location of isolate†	ID	% Mass loss‡ Heartwood	% Mass loss‡ Sapwood	Tannic acid test§	Growth rate‖
Trichaptum	*abietinum*	Red	Bottom	1001	13.8±2.0	36.3±13.0	S	++
Trichaptum	*abietinum*	Grey	Top	927	21.2±4.8	38.7±3.9	S	++
Trichaptum	*abietinum*	Red	Bottom	807	12.3±5.7	27.5±12.9	S	+

*Foliage colour of lodgepole pine indicates the stage of the tree after mountain pine beetle attack.
†Location of where each species was isolated in each log.
‡Mean ± SD (4 replicates).
§Tannic acid test: S, strong; W, weak; NR, no reaction; NG, no growth; ND, no data.
‖Growth rate measured on 1% MEA (colony diameter): +, 0.0–4.9 mm/day; ++, 5.0–9.9 mm/day; +++, >10.0 mm/day.

the United States) in conifer plantations and natural forests (Allen et al. 1996; Garbelotto et al. 1997; Asiegbu et al. 2005). We also found that *Stereum sanguinolentum* causes red heart rot and has been reported to infect different trees species following pruning, logging scars, or after other physical injuries; yet its effect on standing trees is variable or creates minimal damage. This fungus is also reported as a primary colonizer of recently fallen trees, as well as causing sap streak in debarked conifer logs, which seems to result from a dense mycelial formation in the ray tissue (Kleist and Seehann 1997). We also isolated a number of *Amylostereum chailletii*, a well-known mycangial fungus associated with wood wasps of the genus *Sirex* (Talbot 1977; Slippers et al. 2003). Among the other basidiomycetes isolated were *Entomocorticium* species. These species have been reported as a source of nutrients for MPB progeny, especially for the beetle's developing larvae (Whitney et al. 1987; Scott et al. 2008).

Rate of decay of some fungal species

Wood decay species require a certain amount of moisture to grow and reproduce; usually wood with moisture content less than 20% is protected from decay (Zabel and Morrell 1992). In MPB-killed pine, decay can precede or follow MPB attack and staining discolouration. Alteration of nutrients and toxic chemicals by staining fungi may provide more suitable environments for wood decay fungi (Hart 1981; Zabel and Morrell 1992). Decay fungi include white rot species that degrade lignin along with cell wall carbohydrates and brown rot species that degrade mainly the carbohydrates and not the lignin (Böttcher et al. 1988; Schwarze 2007). It is also well known that some fungal decay species degrade wood more rapidly than other species. We isolated more white rot than brown rot fungi and noted that the rate of decay on sapwood or heartwood pine wood blocks seemed to depend more on fungal species than on whether the fungus caused brown rot or white rot.

The sapwood of most conifers, especially lodgepole pine, has low amounts of extractives and so is less durable and more prone to decay than the heartwood (Hart and Shrimpton 1979; European Committee for Standardization 1994). Lodgepole pine heartwood can contain 4–5 times more extractives than sapwood and has a markedly different chemical composition (Erdtman and Rennerfelt 1944; Gao et al. 1995). Further, sapwood, the living tissue of the tree stem, contains a high amount of organic nitrogen as proteins and amino acids, high levels of starch and triglycerides, and has a mildly acidic pH, and so is a more favourable environment and source of nutrients for many fungal species (Abraham et al. 1997; Chow and Obermajer 2007). Species like *Entomocorticium* sp. and *Sistotrema brinkmannii* cause small mass losses that are likely due to the removal of nonstructural wood components (i.e., starch, proteins, triglycerides), but do not affect wood structure. While *Sistotrema brinkmannii* has been reported as a wood-rotting basidiomycete (Ullrich 1973), our data and results from the wood industry indicate that this species does not affect wood strength. Similarly, Chee et al. (1998) showed that this species caused little mass loss in a *Pinus radiata* wood block assay.

Brown rot fungi degrade mainly the wood carbohydrates, cellulose, and hemicellulose. They affect both sapwood and heartwood blocks and gave high mean mass losses of 45% and 30%, respectively. The ability of some brown rot species (e.g., *O. rennyi* and *Coniophora olivaceae*) to cause similar mass losses in both sapwood and heartwood is not surprising. For *F. pinicola* we observed variation among isolates and higher mass losses for sapwood than for heartwood. It is possible that the nutrient content of the sapwood stimulated the initial fungal growth, allowing fungi to better colonize the wood blocks. High extractives content in heartwood may slow down the overall degradation process by affecting the growth and enzyme production of different fungal species (Gao et al. 1995). Heartwood extractives degradation has been only reported for a few white rot species (Dorado et al. 2000). Despite the slow growth of *F. pinicola* in MEA, the degradation of the sapwood (i.e., 61% mass loss) by some isolates was among the highest. Only the white rot *Metulodontia* sp. had a similar mass loss; however, we had only 1 isolate and so cannot generalize our findings. Furthermore, wood is not a homogenous substrate, so some of the wood blocks may have contained more extractive compounds than others, and variability between isolates could have resulted from substrate variability rather than from the genetic background of the isolates. Wood mass losses were generally lower for *Trichaptum abietinum*, another white rot species that mainly damages sapwood in coniferous trees (Allen et al. 1996). Slight heartwood degradation with some isolates of this fungal species is not surprising, since autoclaving the wood blocks might have altered the toxicity of the heartwood extractives. *Heterobasidium annosum*, which is reported to attack the heartwood of pine with low pinosylvan alcohol contents (Jorgensen 1961; Prior 1975), grew and degraded only the sapwood and not the heartwood in our decay test. While *Phellinus pini* is

often shown to delignify the heartwood of living conifers, in dead trees it may be able to spread into sapwood (Blanchette 1980). In the wood block test this fungus preferentially degraded the sapwood, as did *Trametes versicolor*, *Phlebia tremellosa*, and *Stereum sanguinolentum*; in all these cases the wood mass losses were moderate. *Ganoderma* sp. degraded both sapwood and heartwood, and it is likely that this species is less affected than other decay fungi by the extractive contents present in the heartwood (Schmidt and Moreth 2003). Finally, *Amylostereum chailletii* caused low mass losses of sapwood, with a mean loss of ~11%. This species is affected by polyphenols, tree defense chemicals that are produced against fungal attack (Hillis and Inoue 1968).

In conclusion, the information generated in this work highlights the potential of some decay fungi to rapidly reduce fibre quality and yield. Thus, it is important to identify which fungi are present in MPB-killed trees to understand how fast wood and fibres may be degraded. We showed that many white and brown rot fungi can decrease wood mass by 50% within 12 weeks. Applying this approach to a larger number of sites should result in more effective ways of prioritizing sites for harvesting MPB-killed trees.

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