Ophiostomatoid and basidiomycetous fungi associated with green, red, and grey lodgepole pines after mountain pine beetle (*Dendroctonus ponderosae*) infestation

Jae-Jin Kim, Eric A. Allen, Leland M. Humble, and Colette Breuil

Abstract: The mountain pine beetle (MPB) is a major concern for lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) forests in British Columbia, Canada. MPB and the ophiostomatoid staining fungi for which they serve as vector have a close, mutualistic relationship. In this work, we determined which fungi colonized MPB-killed standing trees with green, red, and grey crowns and quantified how rapidly the fungi stained and reduced the moisture content of sapwood. Green trees were mainly colonized by *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, *Ophiostoma montium* (Rumbold) von Arx, *Ophiostoma nigrocarpum* (Davidson) De Hoog, *Ophiostoma minutum* (Olchow. & Reid) Hausner, and unknown *Leptographium* species. In red and grey pines (2 and 3 years after the original MPB attack, respectively), the frequency of the original fungal colonizers decreased, and other sapstaining fungal species were encountered. Among basidiomycetous fungi, decay fungi were rarely present in green trees but were isolated more frequently in red and grey trees. The frequency and the type of decay fungi isolated varied between harvesting sites.

Résumé : Le dendroctone du pin est une préoccupation majeure dans les forêts de pin lodgepole (*Pinus contorta* var. *latifolia* Engelm.) en Colombie-Britannique, au Canada. Le dendroctone et les champignons agents du bleuissement du genre *Ophiostoma* qu'il transporte ont une relation mutualiste étroite. Dans cette étude, nous avons déterminé quels champignons colonisent les arbres encore debout qui ont été tués par le dendroctone du pin selon que leur cime était verte, rouge ou grise et quantifié avec quelle rapidité les champignons ont causé le bleuissement et réduit la teneur en humidité du bois d'aubier. Les arbres dont la cime était encore verte étaient surtout colonisés par *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, *Ophiostoma montium* (Rumbold) von Arx, *Ophiostoma nigrocarpum* (Davidson) De Hoog, *Ophiostoma minutum* (Olchow. & Reid) Hausner et une espèce inconnue de *Leptographium*. Chez les pins dont la cime était rouge ou grise (respectivement 2 et 3 ans après l'attaque initiale par le dendroctone du pin), la fréquence des premiers champignons à coloniser le bois avait diminué et d'autres espèces de champignons responsables du bleuissement étaient présents. Parmi les champignons basidiomycètes, les champignons de carie étaient rarement présents dans les arbres dont la cime était verte mais ils ont été plus fréquemment isolés chez les arbres dont la cime était rouge ou grise. La fréquence et le type de champignons de carie qui ont été isolés variaient selon le site.

[Traduit par la Rédaction]

Introduction

Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) accounts for 50% of the total growing stock and 25% of the total volume of timber harvested in interior British Columbia, Canada (http://www.mountainpinebeetle.com/beetle_biology.html). Although the mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) and its fungal associates are natural components of lodgepole pine ecosystems, they threaten wood and fiber supplies under epidemic conditions. Recent moderate winters have caused lower larval mortality (http://www.mountainpinebeetle.com/epidemic_factors.html), resulting in an epidemic that is currently spreading across British Columbia and causing extensive economic and environmental damage to lodgepole pine forests. As of fall 2003, lodgepole pine infested by MPB was 203.5 million m^3 spread over 10.1 million ha, which is by far the largest beetle epidemic in Canada's history (http://www.mountainpinebeetle.com/epidemic_facts.html).

The association between the MPB and its fungal associates is mutualistic. MPB carry a diversity of fungi on their body surface and in invaginations of their exoskeleton and in mycangia (Six 2003; Whitney and Farris 1970; Whitney 1971). Within a tree, the beetles remain in the region just under the bark, mining the phloem and reproducing, while the fungi, mainly staining fungi, propagate in beetle galleries in the phloem and into the underlying sapwood. The beetles benefit because (1) the staining fungi lower the wood moisture content and may produce an environment more favor-

Received 5 July 2004. Accepted 23 September 2004. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 9 March 2005.

J.-J. Kim and C. Breuil.[1] Department of Wood Science, The University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.
E.A. Allen and L.M. Humble. Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, BC V8Z 1M5, Canada.

[1]Corresponding author (e-mail: breuil@interchg.ubc.ca).

 doi: 10.1139/X04-178 © 2005 NRC Canada

able for the beetle brood and (2) some of the fungal associates are potential nutrient sources for the next beetle generation (Caird 1935; Craighead 1928; Hsiau and Harrington 2003; Nelson 1934; Nelson and Beal 1929; Paine 1984; Paine et al. 1997; Reid et al. 1967; Wagner et al. 1979; Whitney and Cobb 1972). Usually, the life cycle of the MPB is 1 year. Within the tree, the fungi rapidly colonize a fresh, moist, and nutrient-rich environment that is free of competing microflora. Some of the fungi produce a pigment, melanin, which causes a blue to black discoloration of the sapwood (Zink and Fengel 1988; Zabel and Morrell 1992).

Controversy still exists over MPB attacks and the relative role of beetles and fungi in tree death (Paine et al. 1997). However, two issues seem clear. First, the vectored staining fungi help the beetle reproduce, accelerating their spread (Reid 1961; Six and Paine 1998; Solheim 1995). Second, after tree defense mechanisms have been overwhelmed by a mass MPB attack, trees become vulnerable to later attack by other bark beetles (e.g., *Ips* and ambrosia species) that carry other types of microflora (Paine et al. 1997; Solheim 1995). Such a secondary attack usually occurs within a few weeks of the primary attack.

Foliage color changes after a successful primary attack. A tree's foliage fades and changes from green to yellow in the year between the initial MPB attack and the emergence of new adults. In the second and third years following attack, the foliage changes to red, then to grey. The timing of color changes can vary greatly depending on the weather and the physiological condition of a tree. In trees that have sustained high levels of attack by MPB, the sapwood is stained by fungi within a few weeks. Given this, trees that are harvested commercially after MPB attack will typically yield discolored wood. Stain is a cosmetic defect. As there is no appreciable loss in most strength properties, only a small decrease in toughness, stained wood can be used in most applications where appearance is not an important factor (Scheffer 1973). However, MPB-killed trees that are left uncut for extended periods are susceptible to fungal decay that will further reduce their value in markets that are otherwise open to stained wood (Eaton and Hale 1993).

Because fungi typically decrease wood value and marketability following MPB attack, wood products companies operating in regions with beetle–fungus epidemics can better manage harvesting by understanding the dynamics of stain and decay. Part of this understanding involves knowing how fungal diversity changes with time. Currently, however, little is known about fungal diversity in green trees (Solheim 1995; Robinson 1962), and nothing is documented about diversity in red or grey trees.

Most fungi associated with the MPB bark beetles are ascomycetes in the genera *Ophiostoma* H. & P. Sydow that produce asexual and sexual spores in slimy masses. These spores are well adapted to dispersal by sticking to insects (Harrington 1993; Paine et al. 1997). Most *Ophiostoma* species cause blue to black stain in sapwood. Some species are saprobes, while others are pathogenic (Gibbs 1993; Harrington 1993). For example, among the associates of MPB, *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington causes tree death, while *Ophiostoma montium* (Rumbold) von Arx does not (Solheim 1995; Whitney 1971; Whitney and Farris 1970; Yamaoka et al. 1995). Besides staining fungi, MPB also carry yeasts and basidiomycetes, in particular *Entomocorticium* species (Whitney et al. 1987).

In this work, the staining and decay fungi colonizing the sapwood of standing lodgepole pines were identified in green, red, and grey trees at four sites in British Columbia Reduction in sapwood moisture content caused by the staining fungi introduced by MPB and the diversity of staining and decay fungi in green, red, and grey trees were characterized.

Materials and methods

Sampling sites and characteristics of the trees

Lodgepole pine attacked by MPB was harvested in the areas of Manning Park, Riske Creek, Radium, and Cranbrook (British Columbia, Canada). MPB-infested trees were sampled monthly from June to October 2003 for the following phases: early or late green (1 month to 1 year after MPB attack), red (2 years after attack), and grey (3 years after attack). Only four trees from Manning Park were from late green attack (attack occurred in 2002). All other trees from all the sites were confirmed as being in the early green tree. Ten lodgepole pine trees for each infestation phase were selected from each location (Table 1). Two bolts (0.6 m long) per tree were obtained, one taken from the top (3.5 m from ground) and the other taken from the bottom (0.3 m from ground) of the tree. Bolts were kept in plastic bags, transported to the laboratory, and held at 4 °C for 1–3 days before fungal isolation was carried out on the logs from the green trees. Isolation from red and grey trees was done 1 or 2 weeks later.

Fungal isolation and identification

Isolations were carried out in the sapwood and at the boundary between the sapwood and the heartwood in each bolt. Bolts were debarked to investigate the presence of other beetles, signs of decayed areas, and basidiomycetous mycelia. We sectioned the bolts into discs of 1–2 cm thickness. To isolate the fungi, we cracked the discs at the gallery level. Small pieces of wood were removed from inside the cracked sections. We placed the samples on (1) 2% MEA (33 g Oxoid malt extract agar, 10 g Oxoid agar, and 1000 mL distilled water) with 100 ppm ampicillin to isolate the general fungal flora and (2) 2% MEA with 4 ppm benomyl and 100 ppm ampicillin to isolate the basidiomycetes. The ampicillin was incorporated to inhibit bacterial growth in both cases. Isolation plates were incubated at room temperature.

After further subculture, we identified most of the fungi isolated from the specimens to the genus or species level using morphological characteristics with reference cultures (Arx and Hennebert 1965; Barnett and Hunter 1987; Batra 1967; Grylls and Seifert 1993; Jacobs et al. 2003; Jacobs and Wingfield 2001; Nobles 1965; Okada et al. 2000; Stalpers 1978; Upadhyay 1981; Wang and Zabel 1990; Wingfield et al. 1988).

To confirm our morphological results, we also sequenced the ribosomal DNA (rDNA) of representative isolates (Kim et al. 1999). To PCR-amplify the internal transcribed spacer (ITS) regions, we used the fungal universal primers (ITS5 and ITS4) for the ascomycetes and the reverse primer

© 2005 NRC Canada

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

Location (lat., long.)	Phase	No. of trees	Age (years)	Moisture content (%)[a] Sapwood	Heartwood	Diameter (cm)	Heart rot[b] B	T	Date sampled
Manning Park (49°11′35″N, 120°35′05′W)	Green	10	79.3±16	66.9±30a	32.7±5a	21.3±3	3	2	10 June 2003
	Red	10	80.1±25	26.9±9d	27.6±4b	19.1±5	3	3	
	Grey	10	88.6±14	18.8±8de	19.7±5cd	19.0±4	4	0	
Riske Creek (52°01′35″N, 122°31′27″W)	Green	10	60.9±5	79.4±19a	33.9±11a	22.5±4	0	0	12 Aug. 2003
	Red	10	70.3±21	19.9±6de	24.3±5bc	27.0±3	0	0	
	Grey	10	52.6±6	14.2±9de	15.5±2de	19.5±4	0	1	
Radium (50°40′84″N, 115°51′92″W)	Green	10	59.5±5	42.5±12c	34.0±2a	21.7±3	1	0	30 Sept. 2003
	Red	10	70.3±8	19.8±9de	24.2±4bc	26.3±5	2	1	
	Grey	10	67.1±11	10.4±3e	14.6±5e	26.4±5	4	4	
Cranbrook (49°27′08″N, 115°43′45″W)	Green	10	75.9±9	55.4±23b	33.1±1a	26.2±4	0	0	1 Oct. 2003
	Red	10	81.1±6	19.9±1de	24.1±2bc	21.6±4	3	4	
	Grey	10	69.4±7	15.1±4de	17.6±6de	21.1±3	2	3	

[a]Values are mean of 10 bottom bolts per tree, including measurements from four pieces of wood per bolt. Means followed by the same letter within a column are not significantly different ($\alpha = 0.05$) according to Duncan's method.
[b]Number of bottom and top bolts with heart rot: B, bottom bolt; T, top bolt.

(ITS4B) with ITS5 primer for the basidiomycetes (Gardes and Bruns 1993; White et al. 1990). We amplified partial large subunits of rDNA with primers LR0R and LR5 (Vilgalys and Hester 1990). For several staining fungi, we amplified the β-tubulin gene using the primer T10 and BT12 (Kim et al. 2003; Lee et al. 2003). Fungal DNA sequences were then compared with data sets from GenBank or from other reference cultures that we sequenced (see Table 4).

Wood moisture content

An additional disk was cut from the bottom of each tree collected near the bolt center to determine the moisture content of the sapwood and heartwood regions. Percent moisture content (MC) was then measured by the oven-dry method following the American Society for Testing Materials (ASTM) (D 2016) standards (ASTM 2000).

Statistical analyses

We used the Simpson diversity index (C) to compare the fungal diversity in the three phases in the different geographic regions. The Simpson index places more importance on abundant species (Simpson 1949). This index is defined as

$$C = 1 - \sum_{i=1}^{i=S} p_i^2$$

where p_i is the probability of sampling a species; i is the frequency of species i / total frequency for all species; and S is species richness, the number of species per sample. C ranges from zero to one and denotes the probability that two randomly selected individuals in a community belong to different species. A value close to zero suggests that dominant species may exist in a population, and conversely, a value close to one indicates species equitability, that is, the species are evenly distributed. We determined dominance or subordinance in fungal communities using the Camargo index ($1/S$) (Camargo 1993), where S represents species richness, the number of competing species in a community. A dominant species is present if $p_i > 1/S$.

We assessed whether data collected from the four sites could be pooled together by testing whether the site had a significant effect on fungal diversity. We used an analysis of variance (ANOVA) based on a randomized complete block design, with fungal species classed as treatments (ophiostomatoid fungi, $k = 10$; basidiomycetes, $k = 8$) and sites as blocks ($n = 4$).

Using a general linear models procedure, as implemented in a Duncan's multiple range test (SAS Institute Inc. 2001), we tested whether wood moisture contents differed significantly with tree type.

Results

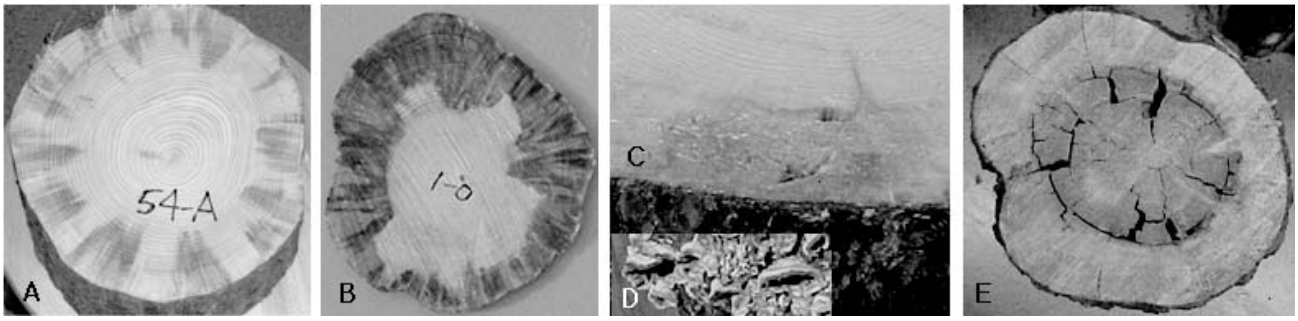
Wood characteristics from sampled trees

Regardless of sample location, the mean diameter of the trees at breast height (DBH) was 23 cm for trees between 40 and 115 years of age (mean: 71 ± 10.3 years; Table 1). Among the 120 trees, 34 were over 80 years old, 32 were between 70 and 79 years old, 25 were between 60 and 69 years old, and 29 were below 60 years old. Of the four sites, on average trees were the oldest at Manning Park and youngest at Radium. The average ages of the green trees at Manning Park and Radium were 79.3 and 59.5 years, respectively.

Sapwood MC for all green trees was over 40% (Table 1). At Riske Creek, sapwood MC was higher (approx. 79%) than at any other site. Sapwood MC was significantly lower in red than in green trees ($\alpha = 0.05$, df = 107, MSE = 185.96; Table 1). Although sapwood MC of grey trees was slightly lower than that of red trees, the difference was not statistically significant ($\alpha = 0.05$; Table 1). For red and grey

© 2005 NRC Canada

Fig. 1. Degradation of lodgepole pine trees attacked by mountain pine beetle: (A) early green tree, (B) fully stained sapwood of red tree, (C) sap rot in grey tree caused by *Trichaptum abietinum*, (D) fruiting bodies of *T. abietinum*, and (E) heart-rotted red tree.



trees at different sites, a number of the sapwood samples had MC below 20%. This should be low enough to prevent fungal growth and shorten fungal survival times (Eaton and Hale 1993; Seifert 1993; Zabel and Morrell 1992). Fungi were absent in 1 of 14 red and 14 of 34 grey trees with MC below 19%. Decay fungi were not isolated from two of the grey trees that had a sapwood MC of 7.8% and 8.3%, respectively.

In the green, red, and grey trees, heartwood MC ranged from 32.7% to 34%, 24.1% to 27.6%, and 14.6% to 19.7%, respectively. Heartwood MC was significantly higher in green than in red or grey trees ($\alpha = 0.05$, df = 107, MSE = 25.12; Table 1). Differences between the MC of the red and grey trees at the same site were also statistically significant ($\alpha = 0.05$; Table 1).

All bottom and top bolts from Manning Park showed evidence of MPB attack. At all sites the bottom bolts were affected by MPB; however, there was some variability in attack densities in the top bolts, for example, at Cranbrook some top bolts showed no sign of MPB attack. At all sites, top and bottom bolts were also often attacked by *Ips* bark beetles. A large number of wood borers (e.g., ambrosia beetles) were observed at both bottom and top bolts in Manning Park trees regardless of the phase. However, no wood borers were found in trees at Cranbrook.

Ophiostomatoid fungi isolated from different phases

A total of 744 fungal isolates were obtained from lodgepole pine trees infested with MPB at the four sites (Table 2). As described below, we were able to identify almost all isolates to species; however, *Ambrosiella* sp., *Graphium* sp., and *Leptographium* sp. could only be identified to genera. These species seemed to be morphologically and genetically different from species reported in the literature (Jacobs et al. 2003; Okada et al. 1998; Okada et al. 2000; Rollins et al. 2001).

We obtained 308 fungal isolates comprising seven species from early or late green trees: *Ambrosiella* sp., *Graphium* sp., *Leptographium* sp., *O. clavigerum*, *Ophiostoma minutum* (Olchow. & Reid) Hausner, Reid & Klassen, *O. montium*, and *Ophiostoma nigrocarpum* (Davidson) de Hoog. These species were identified by their morphological characteristics, and their identities were confirmed by rDNA or β-tubulin sequences (see Table 4). On MEA, *O. clavigerum* was more frequently isolated than *O. montium*. Unexpectedly, on benomyl MEA, a medium that promotes the growth of basidiomycetes, we often isolated the ophiostomatoid fungi *Ambrosiella* sp. and *O. minutum*.

We identified nine species from 375 isolates obtained from red trees. We also found two fungal species (*Leptographium terebrantis* Barras & Perry and *Ophiostoma minus* (Hedgcock) H. & P. Sydow) not found in the green trees. Isolation frequencies of *O. clavigerum* and *Leptographium* sp. were lower in red than in green trees, but similar for *O. montium*. In contrast, isolation frequencies of three species, *Ambrosiella* sp., *O. minutum*, and *O. nigrocarpum*, were two times higher in the red than in the green trees.

In grey trees, we recovered 91 isolates. In addition to the nine species isolated from the red trees, we found *Ophiostoma olivaceum* Mathiesen at a low frequency and only at Manning Park. The number of fungi in grey trees was lower than in green trees, particularly for *Ambrosiella* sp., *O. clavigerum*, and *O. montium*. The frequency of isolation of *O. clavigerum* was only 3.8% (compared with 61.5% in green trees). A similar decrease was noticed for *O. montium*, while the *Leptographium* sp. was not found at any of the sites. *Ophiostoma clavigerum* and *O. montium* were not isolated from grey trees at Riske Creek. Among the other types of fungi observed, *O. minus* was present at all sites, in low numbers, while *L. terebrantis* and *O. nigrocarpum* were not present in the Cranbrook trees.

The effect of site on fungal diversity was significant for green trees ($F_{[3,27]} = 3.29$, $\alpha = 0.05$) but not for red ($F_{[3,27]} = 2.29$, $\alpha = 0.05$) or grey trees ($F_{[3,27]} = 1.99$, $\alpha = 0.05$) and not for red and grey trees combined ($F_{[3,27]} = 1.81$, $\alpha = 0.05$). Given this, we pooled data from red and grey trees from all sites for further analysis (Table 2). The highest species richness was found in grey trees from Manning Park, followed by red trees from Radium (Table 2). Species richness and diversity were lowest in green trees. *Ophiostoma clavigerum* and *O. montium* were present at all sites. In green trees, the dominant species were *O. clavigerum* and *O. montium*, while in the red and grey trees they were *Ambrosiella* sp., *O. clavigerum*, and *O. montium* (Table 2).

Basidiomycetes isolated from the different phases

In total, we isolated 260 basidiomycetes from the different phases using MEA with benomyl and ampicillin (Table 3). We confirmed the fungal indentities by noting presence or absence of clamp connections and with PCR using basidiomycete-specific primers (Adair et al. 2002). At least eight species of basidiomycetes were identified in trees attacked by MPB: *Amylostereum chailletii* (Pers.) Boidin, *Entomocorticium* sp. type

© 2005 NRC Canada

Table 2. Number of isolates[a] of ophiostomatoid fungi from lodgepole pine trees at each phase attacked by mountain pine beetle.

	Manning Park			Riske Creek			Radium			Cranbrook			Total			
Fungus	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Red + grey
Ambrosiella sp.	6	21[b]	9	14	37[b]	2	2	25[b]	4[b]	—	5	5	22	88[b]	20[b]	108[b]
Graphium sp.	1	2	1	1	—	—	—	2	—	—	—	—	2	4	1	5
Leptographium sp.	5	3	—	5	—	—	—	—	—	—	1	—	10	4	—	4
Leptographium terebrantis	—	—	5	—	2	1	—	2	—	—	—	—	—	4	6	10
Ophiostoma clavigerum	49[b]	28[b]	4	36[b]	43[b]	—	37[b]	28[b]	6[b]	33[b]	31[b]	8[b]	155[b]	130[b]	18[b]	148[b]
Ophiostoma minus	—	—	2	—	—	2	—	3	1	—	4	2	—	7	7	14
Ophiostoma minutum	—	3	2	—	2	—	—	8	3	1	—	—	1	13	5	18
Ophiostoma montium	32[b]	19[b]	6	31[b]	36[b]	—	30[b]	37[b]	6[b]	19[b]	21[b]	7[b]	112[b]	113[b]	19[b]	132[b]
Ophiostoma nigrocarpum	2	6	5	2	2	7[b]	2	3	2	—	1	—	6	12	14	26
Ophiostoma olivaceum	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	1
Total isolates	95	82	35	89	122	12	71	108	22	53	63	22	308	375	91	466
No. of points sampled	80	120	120	60	120	120	64	120	120	48	120	120	252	480	480	960
Percent frequency of total isolates (%)[c]	119	68	29	148	102	10	111	90	18	110	53	18	122	78	19	49
Species richness (*S*)	6	7	9	6	6	4	4	8	6	3	6	4	7	9	9	10
Simpson's index of diversity (*C*)	0.61	0.76	0.84	0.69	0.70	0.60	0.55	0.75	0.79	0.48	0.64	0.71	0.61	0.73	0.83	0.78

[a]Fungi isolated on malt extract agar; fungi were isolated from 20 bolts (4–6 chips from each log) from 10 trees collected at each phase.
[b]Dominant species. Species is considered dominant if $p_i > 1/S$, where p_i is proportion of total sample represented by species i and S is number of competing species present in the community (Camargo 1993).
[c]Percent frequency of total isolates = total isolates / No. of points sampled × 100.

© 2005 NRC Canada

Table 3. Number of isolates[a] of basidiomycetes from lodgepole pine trees at each phase attacked by the mountain pine beetle using malt extract agar (MEA) with benomyl and ampicillin.

	Manning Park			Riske Creek			Radium			Cranbrook			Total			
Fungus	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Green	Red	Grey	Site total
Amylostereum chailletii	—	—	2	—	—	—	—	—	—	—	—	—	—	—	2	2
Entomocorticium sp. type 2	10[b]	12[b]	17[b]	—	25[b]	1	12[b]	24[b]	4[b]	—	16[b]	8[b]	22[b]	77[b]	30[b]	129[b]
Entomocorticium dendroctoni	3	—	—	—	—	—	—	1	—	—	—	—	3	1	—	4
Fomitopsis pinicola	6[b]	12[b]	—	—	—	—	—	—	—	2	—	1	8[b]	12	1	21
Heterobasidion annosum	1	3	—	—	—	—	1	—	—	2	2	5[b]	4	5	5	14
Peniophora sp. D22	—	5	—	—	5	2[b]	2	5	14[b]	—	4	6[b]	2	19	22[b]	43[b]
Sistotrema brinkmannii	2	1	2	—	1	1	—	1	—	—	—	1	2	3	4	9
Trichaptum abietinum	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	1
Unidentified basidiomycetes	—	2	6	—	—	2	—	1	8	—	1	17	—	4	33	37
Total isolates	22	35	27	0	31	6	15	32	26	4	23	39	41	121	98	260
No. of points sampled	80	120	120	60	120	120	64	120	120	48	120	120	252	480	480	1212
Percent frequency of total isolates[c]	28	29	23	0	26	5	23	27	22	8	19	33	16	25	20	22
Species richness (*S*)	5	5	3	0	3	3	3	4	2	2	3	6	6	6	7	8
Simpson's index of diversity (*C*)	0.69	0.7	0.35	0.00	0.32	0.73	0.34	0.37	0.35	0.50	0.43	0.74	0.65	0.53	0.66	0.61

[a]Fungi isolated on MEA; fungi were isolated from 20 bolts (4–6 chips from each log) from 10 trees collected at each phase.

[b]Dominant species. Species is considered dominant if $p_i > 1/S$ where p_i is the proportion of total sample represented by species i and S is the number of competing species present in the community (Camargo 1993). Unidentified basidiomycetes were excluded from the above statistics.

[c]Percent frequency of total isolates = total isolates / No. of points sampled × 100.

© 2005 NRC Canada

2, *Entomocorticium dendroctoni* H.S. Whitney, *Fomitopsis pinicola* (Sw.) P. Karst, *Heterobasidion annosum* (Fr.) Bref., *Sistotrema brinkmannii* (Bres.) J. Erikss., *Peniophora* sp. D22, and *Trichaptum abietinum* (Dicks.) Ryvarden.

Among the basidiomycetes, *S. brinkmannii* and *H. annosum* were easily identified by the presence of morphologically specific characteristics. On artificial media, *S. brinkmannii* produces basidia, while *H. annosum* has the oedocephaloid conidiophores (Nobles 1965; Stalpers 1978). *Trichaptum abietinum* did not grow on benomyl MEA, but it was easily identified by the fruiting bodies found on the tree bark (Fig. 1D). *Entomocorticium* sp. type 2 and *Peniophora* sp. D22 isolates were identified to the genus level using morphological characteristics. Their DNA sequences suggest that they may be a new species; however, additional DNA analyses need to be done on these genera (Hsiau and Harrington 2003; Whitney et al. 1987). In addition, a number of basidiomycetes (37) that are as yet unidentified were present mainly in the red and grey trees.

We found approximately three and two times more basidiomycetous fungal isolates in red and grey trees, respectively, than we found in green trees. At Riske Creek, no basidiomycetous fungi were isolated either from the sapwood or the boundary between the sapwood and the heartwood in green trees. At all sites, the data show that *Entomocorticium* sp. type 2 was the dominant species in most cases. *Entomocorticium dendroctoni* was only isolated in small numbers at Manning Park and Radium. *Peniophora* sp. D22 was the dominant species in grey trees at all sites except for Manning Park. In grey trees, *T. abietinum*, a sap rot, was found at Cranbrook (Fig. 1C), and *A. chailletii* was only present at Manning Park.

The effect of site on fungal diversity was not significant for green, red, or grey trees ($F_{[3,21]}$ = 2.16, 0.28, and 0.7, respectively, α = 0.05) or when data for green, red, and grey trees were combined ($F_{[3,21]}$ = 1.58, α = 0.05). Given this, we pooled data for all trees at all sites (Table 3). Among all sites, species richness was highest in grey trees from Cranbrook, followed by green and red trees in Manning Park (Table 3). When fungal communities from each phase were compared, diversity and species richness were slightly higher in grey trees. *Entomocorticium* sp. type 2 was one of the dominant species in all phases. *Fomitopsis pinicola* was the dominant species in green and red trees at Manning Park, while *Peniophora* sp. D22 was the dominant species in grey trees at three sites.

Heart rot was also observed in approximately 22% of the trees sampled at all sites (Table 1; Fig. 1E). Among the heart rot fungi found, only four species, *Postia sericeomollis* (Romell) Jülich, *Metulodontia nivea* (P. Karst.) Parmasto, *Phellinus pini* (Brot.) Bondartsev & Singer, and *Peniophora* sp. D22, were identified. Molds were widely present, especially on the sapwood surface of red and grey trees only. *Aspergillus*, *Penicillium*, *Aureobasidium*, *Trichodema*, and *Mucor* isolates were not further characterized.

Discussion

Mature lodgepole pine trees older than 80 years are highly susceptible to attack by MPB (http://www.for.gov.bc.ca/hts/pubs/beetledoc_oct29LO.pdf). However, most of the trees harvested at the four sites for this work were less than 80 years old, and we observed MPB attack in trees as young as 50 years. It is likely that at some sites, because of the MPB population increase, many older trees have already been attacked by MPB, and only younger trees are available to the beetles (http://www.mountainpinebeetle.com).

Ophiostomatoid fungi, diversity, growth, damage, and survival

A limited number of species were present in the early or late green attacked trees. Most of the common species found in this work and reported by other authors were *O. clavigerum*, *O. minutum*, *O. montium*, and *O. nigrocarpum* (De Beer et al. 2003; Kim et al. 2003; Solheim 1995; Upadhyay 1981; Whitney 1971). *Ambrosiella*, *Graphium*, and *Leptographium* species have not been reported in previous work. The new *Leptographium* species we found is morphologically similar to the *O. clavigerum* anamorph. However, this new species did not produce synnemata-like structures, grew more slowly on artificial media, and showed some genetic differences with *O. clavigerum* (Lee et al. 2003). On stained wood, *O. clavigerum* was always isolated in areas ahead of the *Leptographium* species and *O. montium* (Lee et al. 2003). Although *Ambrosiella* species were isolated even in the early green trees, they were more frequently isolated in red trees. It is likely that this fungus was introduced into the trees by ambrosia beetles. These secondary beetles were sometimes observed a few weeks after the initial MPB attack. However, while secondary beetles (e.g., *Ips* species) and wood borers (e.g ambrosia beetles) were present in green trees in small numbers, more were observed in red and grey trees. Since these insects are associated with different fungal species, this could, at least in part, explain the increase in fungal diversity during these later phases.

In early work, researchers have reported the presence of *Ophiostoma ips* (Rumbold) Nannf. in pine affected by MPB; however, we did not isolate this fungal species, only *O. montium* (Furniss et al. 1995; Fox et al. 1993; Six 2003). As we reported previously, while these two species have often been misidentified, they can be differentiated by growth at 35 °C and PCR amplification and sequencing of partial β-tubulin and rDNA genes (Kim et al. 2003). *Leptographium terebrantis* was present in red and grey trees at some sites. It has been isolated from other pine species and seemed to be associated with *Dendroctonus frontalis*, *Dendroctonus pseudotsugae*, *Dendroctonus terebrans*, and *Dendroctonus valens*, but not with *Dendroctonus ponderosae* (MPB) (Jacobs and Wingfield 2001).

Many ophiostomatoid fungi stain the sapwood of various trees species. Pines are particularly susceptible to fungal discoloration (Seifert 1993). The wood defect is caused by melanin, a brown to black pigment that is produced in fungal hyphae, conidiophores, and perithecia (Brisson et al. 1996; Zink and Fengel 1988). When a lodgepole pine is attacked by MPB, stain due to fungal growth can spread throughout the sapwood within 2–6 weeks (Solheim 1995). In this work, the fungi involved in the early green tree discoloration included *O. clavigerum*, *O. montium*, and *Leptographium* species. These species are regarded as "deep" stainers, since their hyphae penetrate the sapwood. The sap-staining fungi *L. terebrantis* and *O. minus* were also isolated, but only in red and grey trees. The other ophiostomatoid fungi isolated, *O. olivaceum*, *O. minutum*, and *O nigrocarpum*, have little importance as wood stainers (Griffin 1968; Hunt

© 2005 NRC Canada

Table 4. GenBank accession numbers for sequences of fungi isolated from lodgepole pine trees infested with the mountain pine beetle.

Fungus	Isolate	Ampification primers	Accession No.	Closest match in BLAST	Accession No. of match	% similarity[a]
Ophiostomatoid fungi						
Ambrosiella sp.	MR17EW1	LROR/LR5	AY672929	*A. macrospora*	AY282873	94
Leptographium terebrantis	MY23AW3	T10/BT12	AY672911	*L. terebrantis*	AY267826	100
Ophiostoma clavigerum	S4GAW1	T10/BT12	AY672912	*O. clavigerum*	AY267828	100
Ophiostoma minus	MY28EW1	T10/BT12	AY672913	*O. minus*	AY548743	100
Ophiostoma montium	MG8AW1	T10/BT12	AY672914	*O. montium*	AY194960	100
Ophiostoma nigrocarpum	MPBON-1	ITS5/ITS4	AY672915	*O. nigrocarpum*	AY484474	99
Ophiostoma olivaceum	MY25AW3	ITS5/ITS4	AY672916	*O. olivaceum* 850A[b]	**AY672917**[c]	99
Basidiomycetes						
Amylostereum chailletii	MY28AW2	ITS5/ITS4B	AY672918	*A. chailletii*	AF218393	100
Entomocorticium sp. type 2	MPBType2	ITS5/ITS4B	AY672919	*Entomocorticium* sp. H	AF119512	99
Entomocorticium dendroctoni	MG8EW1	ITS5/ITS4B	AY672920	*E. dendroctoni*	AF119506	99
Fomitopsis pinicola	MR12EW3	ITS5/ITS4B	AY672921	*F. pinicola*	AJ560638	97
Heterobasidion annosum	MG2AG2	ITS5/ITS4B	AY672922	*H. annosum*	X70023	99
Peniophora sp. D22	MY32EW2	ITS5/ITS4B	AY672923	*P. pseudo-pini*	AF119514	97
Sistotrema brinkmannii	MR20EW1	ITS5/ITS4B	AY672924	*S. brinkmannii*	AY089729	99
Trichaptum abietinum	MPBTA-1	LROR/LR5	AY672927	*T. abietinum*	AF518659	99
Heart rot fungi						
Metulodontia nivea	MPBHR-1	LROR/LR5	AY672928	*M. nivea*	AF506423	99
Phellinus pini	MPBHR-3	ITS5/ITS4B	AY672925	*P. pini*	AF420589	99
Postia sericeomollis	MPBHR-2	ITS5/ITS4B	AY672926	*P. sericeomollis*	AJ006667	95

[a]Similarity scores from pairwise alignments of sample sequences with closest BLAST match or reference strains.
[b]Collected by K.A. Seifert and B.T. Grylls and provided by Dr. A. Uzunovic (Forintek Canada Corp., Western lab).
[c]Accession in bold type is from reference strains sequenced during the present study.

1956; Whitney and Cobb 1972). Similarly, the *Graphium* and *Ambrosiella* species isolated did not cause wood discoloration, although some *Ambrosiella* species can also discolor wood to some degree.

The minimum wood moisture content for fungal growth is about 20% of the dry wood mass (Seifert 1993; Zabel and Morell 1992). Most sapstaining fungi grow well at moisture contents between 40% and 80%, but can survive at lower moisture levels (Seifert 1993; Zabel and Morrell 1992). In the current work, sapwood moisture contents of lodgepole pine were 120%–130% based on oven-dry mass of the wood for trees not infested with MPB, but had decreased to 40%–80% within 1 year of an MPB attack. Significantly, moisture contents were lower in stained regions than in living wood that was free of fungi. As fungal growth proceeds, it disrupts the water transportation from the roots to the crown of the tree, reducing sapwood moisture content. This may affect the frequency of the sap-staining fungal isolation, as shown in our results. In green trees, although fungal diversity was lower, the frequency of staining fungi isolated was much higher than in red or grey trees, which had lower moisture contents. The two dominant species of the green trees, *O. clavigerum* and *O. montium*, colonized standing trees effectively under the high moisture–resin environmental conditions prevailing in the sapwood at the point when MPB became established. Wood has low oxygen content when its moisture content is high (Solheim 1991). In contrast to many other staining fungi, *O. clavigerum* grows well in such environments, as do *Ophiostoma polonicum* Siemaszko, *Ceratocystis rufipenni* M.J. Wingf., T.C. Harr. & H. Solheim, and *Ophiostoma europhioides* (E.F. Wright & Cain) H. Solheim (Solheim 1991; Solheim and Krokene 1998). Solheim (1991) has suggested that this ability to tolerate low oxygen levels is one of the principle factors favoring one fungus as an effective primary invader over the other early colonizers of living trees. In red trees, the frequency of isolation of *O. clavigerum* decreased, while that of *O. montium* slightly increased at many sites. Some species, like *L. terebrantis*, *O. minus*, and *O. olivaceum*, were only isolated in red or grey trees, where moisture contents were lower. When the wood moisture content had reached 18% or less, mainly for the grey trees, some species, including the dominant *O. clavigerum*, *O. montium* and *Ambrosiella* sp., were not isolated. This suggests that not only the growth but also the survival of some species is reduced at low moisture contents. It is well established in the wood industry that kiln-drying lumber to moisture contents below 20% prevents fungal growth (Forest Products Laboratory 2002).

Basidiomycetes, diversity, and damage

In this work, approximately 16% of the basidiomycetes isolated were found in green trees; however, decay fungi were rarely detected in these trees. In contrast to staining fungi, the frequency of basidiomycetes increased in the red trees. As with staining fungi, some basidiomycetes have mutualistic relationships with insect species (Hsiau and Harrington 2003; Whitney et al. 1987). Four species isolated, *Entomocorticium* sp. type 2, *E. dendroctoni*, *F. pinicola*, and *H. annosum*, occurring at over 64% frequency, have been reported to be associated with bark beetles (Castello et al. 1976; Hsiau and Harrington 2003; Hunt and Cobb 1982; Kim et al. 2004; Whitney et al. 1987). However, three isolated species, *Entomocorticium* sp. type 2, *F. pinicola*, and *H. annosum*, have not been previously reported (Kim et al. 2004). Fungi from the above groups were sometimes iso-

© 2005 NRC Canada

lated from MPB body surfaces (data not shown). Another species, *A. chailletii*, present in the grey trees at Manning Park, is also a well-known mycangial fungus of wood wasps (Slippers et al. 2003; Tabata et al. 2000)

The rates at which many of these fungi degrade wood are unknown. Additionally, rates are likely to vary both within and among fungal species as moisture contents change in MPB-killed trees over time. Among the basidiomycetous fungi, which include both decay and nondecay fungi, the *Entomocorticium* sp. type 2 was the dominant species at all sites; however, we do not know at this time whether *Entomocorticium* sp. type 2 is a decay species. To assess the decay potential of this species, mass losses were measured for small wood blocks that had been inoculated with the fungus for 12 weeks (*Annual Book of ASTM Standards* D 1413-76 2000). Under such conditions, *Entomocorticium* species caused only a slight mass loss of <2.0%, suggesting that this species does not damage wood. It is likely that the *Entomocorticium* species serve as a food source for its beetle vectors, as reported previously (Bridges 1983; Coppedge et al. 1995; Hsiau and Harrington 2003; Klepzig et al. 2001; Tsuneda et al. 1993; Whitney et al. 1987). However, we also isolated species like *F. pinicola* and *H. annosum*, which cause serious wood decay and are well-known root-rot basidiomycetes (Hunt and Cobb 1982; Johannesson and Stenlid 2003; Whitney et al. 1987). *Trichaptum abietinum*, which was only present at Cranbrook in grey trees, has been reported in a wide range of hosts in British Columbia and seems to damage only sapwood (Allen et al. 1996). However, to support harvest management decisions that minimize fiber yield losses, the rates of decay damage at different moisture contents need to be better characterized**.**

In conclusion, during the first year of infestation of lodgepole pine by MPB, the sap-staining fungi *O. clavigerum* and *O. montium* were the dominant species at all sites. Red and grey trees had significantly decreased wood moisture contents. In these two phases the frequencies of ophiostomatoid isolates decreased, while the fungal diversity and the number of secondary beetles increased. More basidiomycetes, including decay fungi, were isolated in red than in green trees. The work presented here covers four sites within a large epidemic region. It offers data on the succession of staining and decay fungi following MPB attack and serves as a pilot-scale trial for the work required to support decision-making on which trees should be rapidly harvested and which trees could be left on the ground.

Acknowledgements

This work was supported by Natural Resources Canada through fund from the Mountain Pine Beetle Initiative. We would like to thank Karen Leung (co-op student) that participated in the fungal isolation and Russell Chedgy for assistance in preparing some of the statistical analyses.

References

Adair, S., Kim, S.H., and Breuil, C. 2002. A molecular approach for early monitoring of decay basidiomycetes in wood chips. FEMS Microbiol. Lett. **211**: 117–122.

Allen, E., Morrison, D., and Wallis, G. 1996. Common tree diseases of British Columbia. Canadian Forest Service, Victoria, B.C.

ASTM. 2000. Annual book of ASTM standards. ASTM International Press, West Conshohocken, Penn.

Barnett, H.L., and Hunter, B.B. 1987. Illustrated genera of imperfect fungi. Macmillan Publishing Company, New York.

Batra, L.R. 1967. Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. Mycologia, **59**: 976–1017.

Bridges, J.R. 1983. Mycangial fungi of *Dendroctonus frontalis* (Coleoptera: Scolytidae) and their relationship to beetle population trends. Environ. Entomol. **12**: 858–861.

Brisson, A., Gharibian, S., Eagen, R., Leclerc, D.F., and Breuil, C. 1996. Localization and characterization of the melanin granules produced by the sap-staining fungus *Ophiostoma piceae*. Mater. Org. **28**: 105–118.

Caird, R.W. 1935. Physiology of pines infested with bark beetles. Bot. Gaz. **96**: 709–733.

Camargo, J.A. 1993. Must dominance increase with the number of subordinate species in competitive interactions? J. Theor. Biol. **161**: 537–542.

Castello, J.D., Shaw, C.G., and Furniss, M.M. 1976. Isolation of *Crytoporus volvatus* and *Fomes pinicola* from *Dendroctonus pseudotsugae*. Phytopathology, **66**: 1431–1434.

Coppedge, B.R., Stephen, F.M., and Felton, G.W. 1995. Variation in female southern pine beetle size and lipid content in relation to fungal associates. Can. Entomol. **127**: 145–154.

Craighead, F.C. 1928. Interrelation of tree killing bark beetles (*Dendroctonus*) and blue stain. J. For. **26**: 886–887.

De Beer, Z.W., Harrington, T.C., Vismer, H.F., Wingfield, B.D., and Wingfield, M.J. 2003. Phylogeny of the *Ophiostoma stenoceras – Sporothrix schenckii* complex. Mycologia, **95**: 434–441.

Eaton, R.A., and Hale, M.D.C. 1993. Wood: decay, pests and protection. Chapman & Hall, London.

Forest Products Laboratory. 2002. Wood handbook: wood as an engineering material. Gen. Tech. Rep. FPL-GTR-113.

Fox, J.W., Wood, D.L., and Akers, R.P. 1993. Survival and development of *Ips paraconfusus* Lanier (Coleoptera: Scolytidae) reared axenically and with tree-pathogenic fungi vectored by cohabiting *Dendroctonus* species. Can. Entomol. **125**: 1157–1167.

Furniss, M.M., Harvey, A.E., and Solheim, H. 1995. Transmission of *Ophiostoma ips* (Ophiostomales: Ophiostomaceae) by *Ips pini* (Coleoptera: Scolytidae) in ponderosa pine in Idaho. Ann. Entomol. Soc. Am. **88**: 653–660.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. **2**: 113–118.

Gibbs, J.N. 1993. The biology of ophiostomatoid fungi causing sapstain in trees and freshly cut logs. *In Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. *Edited by* M.J. Wingfield, K.A. Seifert, and J.F. Webber. APS Press, St. Paul, Minn. pp. 153–160.

Griffin, H.D. 1968. The genus *Ceratocystis* in Ontario. Can. J. Bot. **46**: 659–718.

Grylls, B.T., and Seifert, K.A. 1993. A synoptic key to species of *Ophiostoma*, *Ceratocystis* and *Ceratocystiopsis*. *In Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. *Edited by* M.J. Wingfield, K.A. Seifert, and J.F. Webber. APS Press, St. Paul, Minn. pp. 261–268.

Harrington, T.C. 1993. Biology and taxonomy of fungi associated with bark beetles. *In* Beetle–pathogen interactions in conifer forests. *Edited by* T.D. Schowalter and G.M. Filip. Academic Press, Inc., San Diego. pp. 37–51.

Hsiau, P.T.W., and Harrington, T.C. 2003. Phylogenetics and adaptations of basidiomycetous fungi fed upon by bark beetles (Coleoptera: Scolytidae). Symbiosis, **34**: 111–131.

© 2005 NRC Canada

Hunt, J. 1956. Taxonomy of the genus *Ceratocystis*. Lloydia **19**: 1–58.

Hunt, R.S., and Cobb, F.W., Jr. 1982. Potential arthropod vectors and competing fungi of *Fomes annosus* in pine stumps. Can. J. Plant Pathol. **4**: 247–253.

Jacobs, K., and Wingfield, M.J. 2001. *Leptographium* species: tree pathogens, insect associates, and agents of blue-stain. Academic Press, Inc., New York.

Jacobs, K., Kirisits, T., and Wingfield, M.J. 2003. Taxonomic re-evaluation of three related species of *Graphium*, based on morphology, ecology and phylogeny. Mycologia, **95**: 714–727.

Johannesson, H., and Stenlid, J. 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. Mol. Phylogenet. Evol. **29**: 94–101.

Kim, J.-J., Kim, S.H., Lee, S., and Breuil, C. 2003. Distinguishing *Ophiostoma ips* and *O. montium* two bark beetles associated sapstain fungi. FEMS Microbiol. Lett. **222**: 187–192.

Kim, J.-J., Lee, S., Breuil, C., Humble, L.M., and Allen, E.A. 2004. Diversity of fungi isolated from lodgepole pine attacked by the Mountain Pine Beetle. *In* Fungal Diversity in the Southern Appalachians: Annual Meeting of the 2004 Mycological Society of America, Asheville, North Carolina, 17–21 July 2004. Inoculum (Suppl. to Mycologia), **55**(4): 21.

Kim, S.H., Uzunovic, A., and Breuil, C. 1999. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood using PCR. Appl. Environ. Microbiol. **65**: 287–290.

Klepzig, K.D., Moser, J.C., Lombardero, M.J., Ayres, M.P., Hofstetter, R.W., and Walkinshaw, C.J. 2001. Mutualism and antoagonism: ecological interations among bark beetles, mites, and fungi. *In* Biotic interations in plant–pathogen associations. *Edited by* M.J. Jeger and N.J. Spence. CABI Publishing, New York. pp. 237–269.

Lee, S., Kim, J.-J., Fung, S., and Breuil, C. 2003. A PCR-RFLP marker distinguishing *Ophiostoma clavigerum* from morphologically similar *Leptographium* species associated with bark beetles. Can. J. Bot. **81**: 1104–1112.

Nelson, R.M. 1934. Effect of bluestain fungi on southern pines attacked by bark beetles. Phytopathol. Z. **7**: 327–353.

Nelson, R.M., and Beal, J.A. 1929. Experiments with bluestain fungi in southern pines. Phytopathology, **19**: 1102–1106.

Nobles, M.K. 1965. Identification of cultures of wood inhabiting Hymenomycetes. Can. J. Bot. **43**: 1097–1139.

Okada, G., Seifert, K.A., Takematsu, A., Yamaoka, Y., Miyazaki, S., and Tubaki, K. 1998. A molecular phylogenetic reappraisal of the *Graphium* complex based on 18S rDNA sequences. Can. J. Bot. **76**: 1495–1506.

Okada, G., Jacobs, K., Kirisits, T., Louis-Seize, G.W., Seifert, K.A., Sugita, T., Takematsu, A., and Wingfield, M.J. 2000. Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of *Graphium*-like synnematous fungi. Stud. Mycol. **45**: 169–188.

Paine, T.D. 1984. Influence of the mycangial fungi of the western pine beetle on water conduction through ponderosa pine seedlings. Can. J. Bot. **62**: 556–558.

Paine, T.D., Raffa, K.F., and Harrington, T.C. 1997. Interactions among Scolytidae bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol. **42**: 179–206.

Reid, R.W. 1961. Moisture changes in lodgepole pine before and after attack by mountain pine beetle. For. Chron. **37**: 368–375.

Reid, R.W., Whitney, H.S., and Watson, J.A. 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Bot. **45**: 1111–11126.

Robinson, R.C. 1962. Blue stain fungi in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) infested by the mountain pine beetle (*Dendroctonus monticolae* Hopk.). Can. J. Bot. **40**: 609–614.

Rollins, F., Jones, K.G., Krokene, P., Solheim, H., and Blackwell, M. 2001. Phylogeny of asexual fungi associated with bark and ambrosia beetles. Mycologia, **93**: 991–996.

SAS Institute Inc. 2001. SAS/STAT statistical software (version 8.2) [computer program]. SAS Institute Inc., Cary, N.C.

Scheffer, T.C. 1973. Microbiological degradation and the causal organisms. *In* Wood deterioration and its prevention by preservative treatment. Volume I. *Edited by* D.D. Nicholas. Syracuse University Press, Syracuse, N.Y. pp. 31–106.

Seifert, K.A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. *In Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. *Edited by* M.J. Wingfield, K.A. Seifert, and J.F. Webber. APS Press, St. Paul, Minn. pp. 141–151.

Simpson, E.H. 1949. Measurement of species diversity. Nature (Lond.), **163**: 688.

Six, D.L. 2003. A comparison of mycangial and phoretic fungi of individual mountain pine beetles. Can. J. For. Res. **33**: 1331–1334.

Six, D.L., and Paine, T.D. 1998. Effect of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus ponderosae* (Coleoptera: *Scolytidae*). Environ. Entomol. **27**: 1393–1401.

Slippers, B., Coutinho, T.A., Wingfield, B.D., and Wingfield, M.J. 2003. A review of the genus *Amylostereum* and its association with woodwasps. S. Afr. J. Sci. **99**: 70–74.

Solheim, H. 1991. Oxygen deficiency and spruce resin inhibition of growth of blue stain fungi associated with *Ips typographus*. Mycol. Res. **95**: 1387–1392.

Solheim, H. 1995. Early stages of blue-stain fungus invasion of lodgepole pine sapwood following mountain pine beetle attack. Can. J. Bot. **73**: 70–74.

Solheim, H., and Krokene, P. 1998. Growth and virulence of *Ceratocystis rufipenni* and three blue-stain fungi isolated from the Douglas-fir beetle. Can. J. Bot. **76**: 1763–1769.

Stalpers, J.A. 1978. Identification of wood-inhabiting fungi in pure culture. Stud. Mycol. **16**: 1–248.

Tabata, M., Harrington, T.C., Chen, W., and Abe, Y. 2000. Molecular phylogeny of species in the genera *Amylostereum* and *Echinodontium*. Mycoscience, **41**: 585–593.

Tsuneda, A., Murakami, S., Sigler, L., and Hiratsuka, Y. 1993. Schizolysis of dolipore-parenthesome septa in an arthroconidial fungus associated with *Dendroctonus ponderosae* and in similar anamorphic fungi. Can. J. Bot. **71**: 1032–1038

Upadhyay, H.P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. The University of Georgia Press, Athens, Ga.

Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. **172**: 4238–4236.

von Arx, J., and Hennebert, G.L. 1965. Deux champignons ambrosia. Mycopathol. Mycol. Appl. **25**: 309–315.

Wagner, T.L., Gagne, J.A., Doraiswamy, P.C., Coulson, R.N., and Brown, K.W. 1979. Development time and mortality of *Dendroctonus frontalis* in relation to changes in tree moisture and xylem water potential. Environ. Entomol. **8**: 1129–1138.

Wang, C.J.K., and Zabel, R.A. 1990. Identification manual for fungi from utility poles in the eastern United States. Allen Press, Inc., Lawrence, Kans.

© 2005 NRC Canada

White, T.J., Bruns, T.D., Lee, S., and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, Inc., San Diego. pp. 315–322.

Whitney, H.S. 1971. Association of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) with blue stain fungi and yeasts during brood development in lodgepole pine. Can. J. Entomol. **103**: 1495–1503.

Whitney, H.S., and Cobb, F.W., Jr. 1972. Non-staining fungi associated with the bark beetle *Dendroctonus brevicomis* (Coleoptera: Scolytidae) on *Pinus ponerosae*. Can. J. Bot. **50**: 1943–1945.

Whitney, H.S., and Farris, S.H. 1970. Maxillary mycangium in the mountain pine beetle. Science (Washington, D.C.), **167**: 54–55.

Whitney, H.S., Bandoni, R.J., and Oberwinkler, F. 1987. *Entomocorticium dendroctoni* gen. et sp. nov. (Basidiomycotina), a possible nutritional symbiote of the mountain pine beetle in lodgepole pine in British Columbia. Can. J. Bot. **65**: 95–102.

Wingfield, M.J., Capretti, P., and Mackenzie, M. 1988. *Leptographium* spp. as root pathogens of conifers. An International perspective. *In Leptographium* root diseases of conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. APS Press, St. Paul, Minn. pp. 113–128.

Yamaoka, Y., Hiratsuka, Y., and Maruyama, P.J. 1995. The ability of *Ophiostoma clavigerum* to kill mature lodgepole-pine trees. Eur. J. For. Pathol. **25**: 401–404.

Zabel, R.A., and Morrell, J.J. 1992. Wood microbiology. Decay and its prevention. Academic Press, Inc., London.

Zink, P., and Fengel, D. 1988. Studies on the colouring matter of blue-stain fungi. Part 3. Spectroscopic studies on fungal and synthetic melanins. Holzforschung, **44**: 163–168.

© 2005 NRC Canada