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Mountain Pine Beetle Initiative
Working Paper 2006-06

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Abstract
The mountain pine beetle (*Dendroctonus ponderosae*), one of the most destructive bark beetles, has damaged large areas of lodgepole pine forests in British Columbia (Canada). It has been suggested that the beetle has a mutually beneficial relationship with the fungi that it carries. In this work, the fungal associates of the mountain pine beetle were extensively investigated. Fungi were isolated from the beetles, galleries and sapwood of infested lodgepole pines (*Pinus contorta* var. *latifolia*) at six epidemic sites in British Columbia. The isolated fungal species were more diverse than previously reported. We identified a total of 1042 isolates that belong to nine species. Among these, *Ophiostoma clavigeru*m, an *Ophiostoma minutum*-like species, and *Ophiostoma montium* were frequently isolated. Unexpectedly, the *Ophiostoma minutum*-like species was found at high frequency on the mountain pine beetle. *Leptographium longiclavatum*, *Entomocorticium dendroctoni* and an unidentified species of *Entomocorticium* also appeared to be specifically associated with the mountain pine beetle.

Key words: *Dendroctonus ponderosae*, diversity, mountain pine beetle, ophiostomatoid, Pinus contorta, lodgepole pine

Résumé
Le dendroctone du pin ponderosa (*Dendroctonus ponderosae*), l'un des scolytes les plus destructeurs, a endommagé de grandes zones de forêts de pins tordus latifoliés en Colombie-Britannique (Canada). Il a été suggéré que le scolyte avait une relation mutuellement avantageuse avec les champignons qu'il transporte. Dans le cadre du présent travail, les associés fongiques du dendroctone du pin ponderosa ont fait l'objet d'une enquête approfondie. On a isolé les champignons des scolytes, des galeries et de l'aubier de pins tordus latifoliés infestés (*Pinus contorta* var. *latifolia*) dans six endroits infestés en Colombie-Britannique. Les espèces fongiques isolées se sont révélées plus variées que ce qu'on avait précédemment signalé. En tout, 1042 isolats appartenant à 9 espèces ont été identifiés. Parmi eux, on a fréquemment isolé *Ophiostoma clavigerum*, une espèce semblable à *Ophiostoma minutum*, et *Ophiostoma montium*. Étonnamment, on a très souvent trouvé l'espèce semblable à *Ophiostoma minutum* sur le dendroctone du pin ponderosa. *Leptographium longiclavatum*, *Entomocorticium dendroctoni* et une autre espèce non identifiée d'*Entomocorticium* se sont également avérées spécifiquement associées au scolyte.

Mots clés: *Dendroctonus ponderosae*, diversité, dendroctone du pin ponderosa, ophiostomatoïde, *Pinus contorta*

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Introduction

Lodgepole pine (*Pinus contorta* var. *latifolia*) forests represent 25% of the total growing stock in British Columbia (BC; COFI 2005). However, large number of mature lodgepole pines has been killed by mountain pine beetle, *Dendroctonus ponderosae*, and its fungal associates. Although outbreaks of mountain pine beetle have occurred in BC in the past, the present infestation is the most severe infestation ever recorded. The current mountain pine beetle epidemic started near Tweedsmuir Park and has spread to northern BC (Houston and Prince George) and the Alberta border. As of 2004, it had spread over 7 million ha, infesting 283 million m^3 of lodgepole pine (BC Ministry of Forests 2005).

Usually, mountain pine beetle attacks its host in July and August. At this time, trees are often stressed by water deficiency. Mountain pine beetle feeds on fungi and tree tissues (Harrington 2005). Newly enclosed adults graze on fungi and acquire fungal spores in their mycangia and guts and on their exoskeletons before they emerge from the pupal chambers and attack new hosts. It appears that the mountain pine beetle and its fungal associates have a mutually beneficial relationship (Whitney 1982; Paine et al. 1997; Six 2003a; Harrington 2005).

The known fungal associates of mountain pine beetle are ascomycetes in the genera Ophiostoma and Leptographium. They produce asexual and sexual spores in slimy masses that attach to insect bodies and are dispersed to new hosts that represent fresh nutrient sources (Harrington, 1993). Two fungal species, *Ophiostoma montium* and *O. clavigerum*, have been consistently isolated from the mycangia and exoskeletons of mountain pine beetle as well as from infested pines (Robinson 1962; Whitney and Farris 1970; Solheim 1995; Six 2003b; Kim et al. 2005). Recently, *Leptographium longiclavatum* associated with mountain pine beetle has also been reported (Lee et al. 2005). In comparison, *Entomocorticium dendroctoni*, *Ophiostoma minutum* and *O. minus* have been occasionally found in mountain pine beetle galleries, and their association with mountain pine beetle has been suggested (Robinson 1962; Whitney et al. 1987). However, most previous work was carried out with a limited number of isolation sites, and fungi were isolated only from the beetle or infested trees. Recent outbreaks have occurred over a wide range in BC, in areas which have not been affected by mountain pine beetle epidemics in the past century and which have different climates from those previously studied. Therefore, the mycoflora in the current epidemic area in BC might be different from what has been reported.

To obtain accurate information on the fungal diversity involved in the current mountain pine beetle outbreak, we investigated fungi collected at six sites in BC. We examined, first, fungal species consistently associated with the mountain pine beetle across the large epidemic area in BC, and, second, whether the fungal species and their frequencies, as isolated from beetles, beetle galleries and infested trees, were different or not.

Materials and methods

Sampling strategies

Six epidemic sites (Fort St. James, Houston, Kamloops, Princeton, Tweedsmuir Park, and Williams Lake) were selected to cover the current outbreak regions in Canada (Figure 1). Samplings were conducted before the emergence of teneral adult beetles from the hosts. A total of 23 trees (3 to 5 trees/site), which were attacked by mountain pine beetle in the same or previous summer of the sampling year (early green phase and late green phase of trees, respectively), were harvested in 2001 and 2002 (Table 1). One bolt (50 cm to 80 cm in length) from each tree was cut at breast height and placed in a plastic bag in the field and then transported to the laboratory. The bolts were kept at 4 ºC for 1 to 3 days before fungal isolations were conducted.

The trees harvested ranged in age from 75 to 140 years, with diameters varying from 20.5 cm to 35 cm. Most of the trees were heavily attacked with 70 to 160 pitch tubes/m2, and we often observed that the mountain pine beetle larval galleries of adjacent broods overlapped at their ends. Generally, the entire sapwood was stained, and secondary beetles such as *Ips* and ambrosia were commonly found in most of the trees.

Figure 1. Map of sampling sites in British Columbia, Canada.

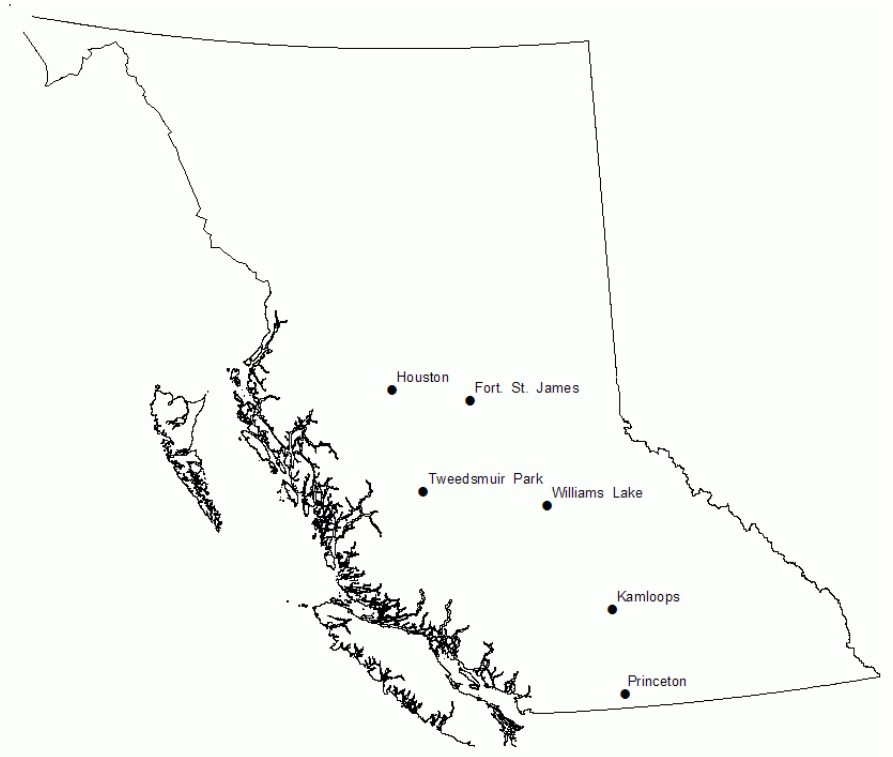

Table 1. Characteristics of the MPB-infested trees used for samplings.

Site	No. of trees	Location (latitude, longitude)	Date sampled	Tree phase*
Kamloops	2	N 50° 65′, W 120° 36′	June 6, 2001	Late green
	1	N 50° 45′, W 120° 31′	August 16, 2001	Early green
	1	N 50° 45′, W 120° 31′	August 31, 2001	Early green
	1	N 50° 45′, W 120° 31′	May 20, 2002	Late green
Williams Lake	3	N 52° 26′ 21″, W 122° 03′ 15″	July 9, 2001	Late green
Princeton	3	N 49° 14′ 58″, W 120° 34′ 43″	July 13, 2001	Late green
Tweedsmuir Park	4	N 52° 43′ 41″, W 125° 30′ 23″	July 19, 2001	Late green
Houston	4	N 54° 08′, W 126° 40′	July 17, 2002	Late green
Fort St. James	4	N 54° 38′ 66″, W 124° 25′ 14″	August 8, 2002	Late green

*'Late green' indicates that trees were attacked in the previous summer and 'early green' means that the trees were attacked in the same summer of the sampling year (Kim et al. 2005)

Isolation of fungi from mountain pine beetle, galleries and sapwood

Bolts were debarked, and beetles were collected from galleries that were not intermingled with other galleries. Fungi on body surfaces of larvae, pupae, and adults were isolated using serial dilution with 0.01% (v/v) Tween-20, and were cultured on 2% malt extract agar (MEA) with ampicillin (50 μg/mL), as described by Lee et al. (2005). Fungi were sampled from the same galleries from which the beetles were collected, by aseptically transferring small amounts of frass onto 2% MEA with ampicillin. After 3 to 30 days of incubation at room temperature, either hyphal tips or the conidial mass on top of conidiophores were sub-cultured and grown on 2% MEA for identification. Fungi were also isolated from sapwood, as described by Kim et al. (2005).

Fungal mycelia removed from the edge of a young colony were placed in micro-tubes (Sarstedt, Montreal, Québec) with either 1 mL of water for storage at 4 ºC, or 1 mL of 20% (v/v) glycerol (Sigma-Aldrich, Oakville, ON) solution for storage at –80 ºC.

Fungal identification

The filamentous fungal isolates were identified by the morphology of asexual structures (Rumbold 1941; Batra 1967; Robinson-Jeffrey and Davidson, 1968; Upadhyay 1981; Jacobs and Wingfield 2001). Identification was confirmed by sequencing the ribosomal DNA (rDNA) or β-tubulin gene of representative isolates (Table 2). Fungal DNA extraction and PCR were conducted following Lee et al. (2003), and sequencing was carried out as described by Lim et al. (2005). The identification of *O. clavigerum* was confirmed using an *O. clavigerum*-specific PCR-RFLP marker (Lee et al. 2003). Fungal growth rates were measured at 23 ºC on 2% MEA as described by Lee et al. (2005).

Table 2. GenBank accession numbers and growth rates of the fungi isolated from *D. ponderosae*.

* K: Kamloops, P: Princeton, TP: Tweedsmuir Park, WL: Williams Lake

Taxon	Strain	Site*	Primer used for amplification†	Accession no.‡	Closest match in BLAST	Accession of match	Identity § %	Growth rate mm/day
Entomocorticium dendroctoni Whitney	SL-A69	TP	ITS5/ITS4	**DQ118419**	*E. dendroctoni*	AF119506	99.6	0.7 ± 0.2
	SL-P44	P	ITS5/ITS4	**DQ118418**	*E. dendroctoni*	AF119506	99.6	0.5 ± 0.2
Entomocorticium sp.	SL-A3	TP	ITS5/ITS4	**DQ118416**	*Entomocorticium* sp. H	AF119512	99.1	3.6 ± 0.3
	SL-W002	WL	ITS5/ITS4	**DQ118417**	*Entomocorticium* sp. H	AF119512	99.1	3.5 ± 0.2
Leptographium longiclavatum Lee *et al.*	SL-K215	K	T10/BT12	AY288931	--	--	--	6.3 ± 0.9
	SL-W001	WL	T10/BT12	AY288936	--	--	--	7.1 ± 0.8
Leptographium terebrantis Barras & Perry	SL-A57	TP	T10/BT12	**DQ118421**	*L. terebrantis*	AY263192	100	12.8 ± 0.4
Ophiostoma clavigerum (Robins-Jeff. &Davids.) Harrington	SL-K1	K	T10/BT12	AY263210	*O. clavigerum*	AY263194	100	14.3± 0.7
Ophiostoma minutum-like sp.	SL-K70	K	ITS5/ITS4	**DQ128175**	*O. minutum*	**DQ128173**	93.1	1.5 ± 0.3
	SL-W15	WL	ITS5/ITS4	**DQ128174**	*O. minutum*	**DQ128173**	92.9	1.9 ± 0.3
Ophiostoma montium (Rumbold) von Arx	SL-K77	K	ITS1-F/ITS4	AY194942	*O. montium*	AY194941	100	6.7 ± 0.3
Ophiostoma nigrocarpum-like sp.	SL-A54	TP	ITS5/ITS4	**DQ118420**	*O. nigrocarpum*-like	AF484452	99.8	1.6 ± 0.2
Pichia capsulate (Wick.) Kurtzman	SL-WY2	WL	LR0R/LR3	**DQ128167**	*P. capsulata*	U70178	99.8	--
Pichia holstii (Wick.) Kurtzman	SL-W2Y4	WL	LR0R/LR3	**DQ128171**	*P. holstii*	U75722	99.2	--
Pichia scolyti (Phaff & Yoney.) Kreger	SL-W2Y3	WL	LR0R/LR3	**DQ128172**	*P. scolyti*	U45788	99.8	--
	SL-PY1	P	LR0R/LR3	**DQ128170**	*P. scolyti*	U45788	99.8	--
Unidentified yeast	SL-WY4	WL	LR0R/LR3	**DQ128168**	*P. ofunaensis*	U45829	98.3	--
	SL-W2Y1	WL	LR0R/LR3	**DQ128169**	*P. ofunaensis*	U45829	96.8	--

† ITS1-F (Gardes and Bruns 1993), ITS4 and ITS5 (White et al. 1990), LR0R and LR3 (Vilgalys and Hester 1990), T10 and BT12 (Lee et al. 2003).

‡ Accession numbers in bold were sequenced during this work.

§ Identity (%) was derived from the paiwise alignment of each isolate sequence with the closest BLAST match in GenBank or a reference strain (DQ128173, *O. minutum* CBS 145.59; AF484452, *O. nigrocarpum*-like C 314)

Statistical analyses
As in our previous work (Kim et al. 2005), the Simpson diversity index (Simpson 1949) was used to indicate fungal diversity as sample sizes in this study were relatively small (Mouillot and Leprêtre 1999). The index is defined as:

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

where Pi is the relative abundance of a species *i*, and *S* is the species richness, which is defined as the number of competing species present in the community. Fungal dominance was determined by Camargo's index (1/S) (Camargo 1992), where S represents species richness. A species was defined as dominant if *Pi* > 1/*S.*

Results

A total of 1 042 fungal isolates were obtained from mountain pine beetle adults, pupae, larvae, galleries, and sapwood collected from 23 lodgepole pines. Nine fungal species were isolated: *Ophiostoma montium*, *O. clavigerum*, an *O. minutum*-like species, an *O. nigrocarpum*-like species, *Leptographium longiclavatum*, *L. terebranti*s, *Entomocorticium dendroctoni*, and two unknown fungi, an *Entomocorticium* species and an *Ambrosiella* species.

Fungal diversity on the mountain pine beetle
From the exoskeletons of the mountain pine beetle adults, we obtained 516 fungal isolates comprising eight species (Table 3). The most dominant species was *Ophiostoma montium*, whose average isolation frequency was 68% (ranging from 44% at Fort St. James to 92% at Williams Lake) of the total number of fungal isolates. In total, 85% of the beetles (from 67% at Tweedsmuir Park to 100% at Princeton and Williams Lake) yielded *O. montium*. Unexpectedly, the second most dominant isolate was the *O. minutum*-like species, which was isolated from 32% of the beetles with an average isolation frequency of 16% (from 0% at Princeton to 38% at Fort St. James). *Ophiostoma clavigerum* was also commonly isolated. However, it was isolated from fewer beetles (24%) than the *O. minutum*-like species, and its average frequency was lower (9%). In contrast to the *O. minutum*-like species, which was dominant at two sites, *O. clavigerum* was not dominant at any site. When the data were pooled, the dominant species on the mountain pine beetle exoskeletons were *O. montium* and the *O. minutum*-like species. *Entomocorticium* sp. and *E. dendroctoni* were often isolated, while *Leptographium longiclavatum*, *Ambrosiella* sp., and *Leptographium terebrantis* were found occasionally. At the sites where larvae, pupae and adults were found together (Kamloops, Tweedsmuir Park, and Williams Lake), the fungi obtained at each developmental stage were similar (Table 4).

Often, more than one filamentous fungal species was isolated from one beetle. Many beetles yielded two species (Tweedsmuir Park, 83% of beetles; Houston, 39%; Kamloops, 29%; Williams Lake 25%), or even three (Houston, 15% of beetles; Princeton, 10%; Williams Lake, 1%).

Yeasts were present in higher numbers than filamentous fungi. At Princeton, Williams Lake and Kamloops, the average number of yeast colonies per adult beetle was approximately 3×105, 5×105, and 7×105, respectively. Yeasts were obtained at all beetle developmental stages, but were more abundant on eggs (data not shown). Similarly to the filamentous fungi, more than one yeast species was isolated from most beetles. The 26S rDNA of the yeasts isolated in this study had high sequence identity (> 99.2 %) with those of *Pichia capsulat*a, *P. holstii*, and *P. scolyti* (Table 2).

Table 3. Number of fungal isolates from *D. ponderosae* and number of beetles yielding each fungal species at six sites in British Columbia.

[*] The total number of fungi on the beetle = the number of fungal isolates written in the table × 10^4.

	Number of Isolates[*]						
Taxon	**Fort St. James**	**Houston**	**Kamloops**	**Princeton**	**Tweedsmuir Park**	**Williams Lake**	**Total isolates**
Ambrosiella sp.	--	3 (2)	--	--	--	--	3 (2)
Entomocorticium dendroctoni Whitney	--	--	--	17 (2)	3 (1)	--	20 (3)
Entomocorticium sp.	--	--	1 (1)	2 (2)	8 (2)	--	11 (5)
Leptographium longiclavatum Lee *et al.*	--	--	2 (1)	--	--	--	2 (1)
Leptographium terebrantis Barras & Perry	--	--	--	--	1 (1)	--	1 (1)
Ophiostoma clavigerum (Robins-Jeff. & Davids.) Harrington	11 (3)	20 (6)	9 (3)	--	2 (2)	2 (2)	44 (16)
Ophiostoma minutum-like sp.	23[a] (6)	25 (5)	29[a] (7)	--	2 (2)	5 (2)	84[a] (22)
Ophiostoma montium (Rumbold) von Arx	27[a] (5)	57[a] (11)	91[a] (16)	77[a] (10)	24[a] (4)[†]	75[a] (12)	351[a] (58)
No. of total fungal isolates	61	105	132	96	40	82	516
No. of total MPB	7	13	20	10	6	12	68
Species richness (*S*)	3	4	5	3	6	3	8
Camargo's index (1/*S*)	0.33	0.25	0.20	0.33	0.17	0.33	0.13
Simpson's index of diversity (*C*)	0.63	0.58	0.47	0.33	0.59	0.16	0.50

[†] Values in parentheses are the number of MPB yielding each fungal species.

[a] Dominant species. Species was considered as dominant if $Pi > 1/S$, where Pi is the relative abundance of a species i and S is the species richness, which is the number of competing species present in the community (Camargo 1992).

Table 4. Number of fungal isolates from larvae, pupae and adults of *D. ponderosae.*[*]

	Number of Isolates[*]											
	Kamloops			Tweedsmuir Park			Williams Lake			Total		
Taxon	**Larvae**	**Pupae**	**Adults**	**Larvae**	**Pupae**	**Adults**	**Larvae**	**Pupae**	**Adults**	**Larvae**	**Pupae**	**Adults**
Entomocorticium dendroctoni Whitney	2	--	--	--	--	3	--	--	--	2	--	3
Entomocorticium sp.	2	--	1	9	9	8	--	--	--	11	9	9
Ophiostoma clavigerum (Robins-Jeff. &Davids.) Harrington	10	--	9	--	4	2	--	--	2	10	4	13
Ophiostoma minutum-like sp.	8	4	29	3	18	2	--	3	5	11	25	36
Ophiostoma montium (Rumbold) von Arx	96	26	91	5	15	24	14	23	75	115	64	190
Ophiostoma nigrocarpum-like sp.	--	--	--	--	1	--	--	--	--	--	1	--
Leptographium longiclavatum Lee *et al.*	--	--	2	--	--	--	--	--	--	--	--	2
Leptographium terebrantis Barras & Perry	--	--	--	--	--	1	--	--	--	--	--	1
No. of *D. ponderosae*	14	4	20	2	5	6	2	3	12	18	12	38
No. of fungi	118	30	132	17	47	40	14	26	82	149	103	254

[*] The total number of fungi on the beetle = the number of fungal isolates written in the table × 10^4.

Table 5. Fungal isolates from gallery and wood at six sites in British Columbia.

	Number of Isolates													
	Fort. St. James		Houston		Kamloops		Princeton		Tweedsmuir Park		Williams Lake		Total isolate	
Taxon	**G***	**W†**	**G**	**W**	**G**	**W**	**G**	**W**	**G**	**W**	**G**	**W**	**G**	**W**
Ambrosiella sp.	--	--	--	--	--	--	--	2	--	--	--	--	--	2
Entomocorticium sp.	--	--	--	1	--	1	--	--	--	--	--	--	--	2
Leptographium longiclavatum Lee *et al.*	3	--	1	--	4	7	--	--	--	--	--	--	8	7
Leptographium terebrantis Barras & Perry	--	--	--	--	--	1	--	--	--	--	--	1	--	2
Ophiostoma clavigerum (Robins-Jeff. & Davids.) Harrington	8[a]	13[a]	5[a]	2	27[a]	54[a]	2	6[a]	7[a]	3	4	8[a]	53[a]	86[a]
Ophiostoma minutum-like sp.	2	--	1	--	--	1	--	--	--	--	--	1	3	2
Ophiostoma montium (Rumbold) von Arx	7[a]	4	4[a]	5[a]	25[a]	32[a]	4[a]	5[a]	1	14[a]	5[a]	3	46[a]	63[a]
No. of total fungal isolates	20	17	11	8	56	96	6	13	8	17	9	13	110	164
Species richness (*S*)	4	2	4	3	3	6	2	3	2	2	2	4	4	7
Camargo's index (1/*S*)	0.25	0.50	0.25	0.33	0.33	0.17	0.50	0.33	0.50	0.50	0.50	0.25	0.25	0.14
Simpson's index of diversity (*C*)	0.69	0.36	0.65	0.53	0.56	0.57	0.44	0.49	0.22	0.29	0.49	0.56	0.59	0.56

* gallery, † wood

[a] Dominant species. Species was considered dominant if $Pi > 1/S$, where Pi is the relative abundance of a species i and S (Species richness) is the number of competing species present in the community (Camargo 1992).

Fungal diversity in the beetle galleries and the stained sapwood
A total of 274 isolates were collected from galleries and sapwood (Table 5). *Ophiostoma montium* and *O. clavigerum* were frequently isolated from both galleries and sapwood. In contrast to the results from the beetle exoskeletons, the *O. minutum*-like species was isolated at low frequency, while *Leptographium longiclavatum* was often isolated. Other species, including *Entomocorticium* sp., *Ambrosiella* sp., and *Leptographium terebrantis*, were occasionally isolated in the sapwood. However, *Entomocorticium dendroctoni* was not found in sapwood. Yeasts were often found in galleries and occasionally in sapwood (data not shown).

Discussion

Through an extensive survey, we isolated more diverse fungal associates of the mountain pine beetle than previously reported. While *Ophiostoma montium* and *O. clavigerum* were frequently isolated from the mountain pine beetle in accordance with previous studies (Robinson 1962; Whitney and Farris 1970; Six 2003b), we also isolated an *O. minutum*-like species, *Leptographium longiclavatum*, *Entomocorticium* sp., and *E. dendroctoni*. The isolation methods in each study might have affected the species found. In this work, the beetles were washed and the diluted washes were plated onto media. In previous research, the beetles were either streaked onto or allowed to walk on the surface of the media; with such methods, it is less likely that all spores in the cavities of the beetle exoskeletons would be removed. Since sampling fungi in mycangia without cross contamination from fungi present on the exoskeletons was difficult, we only isolated fungi from beetle body surfaces.

Unexpectedly, the frequency of the *Ophiostoma minutum*-like species on the mountain pine beetle exoskeletons was high; often this fungus was isolated more frequently than *O. clavigerum*. The *O. minutum*-like species appears to belong to a complex phylogenetic group that is often reported as *Ophiostoma minutum* Siem. or as *Ceratocystiopsis minuta* (Siem.) Upadhyay & Kendrick (Hausner et al. 1993, 2003). To our knowledge, *Ophiostoma minutum* has been isolated occasionally from infested lodgepole pines (Robinson 1962), but not from mountain pine beetle exoskeletons. It has also been isolated from phoretic mites carried by other bark beetles (Moser and Macias-Samano 2000). We often observed mites in the mountain pine beetle galleries, but further work needs to be done to determine whether the mountain pine beetle is associated with phoretic mites carrying the *O. minutum*-like species.

Consistent with Robinson's (1962) data, we isolated *O. montium* more frequently than *O. clavigerum* from beetle exoskeletons. This was also the case when *O. clavigerum* was more prevalent than *O. montium* in the galleries, even though, for such cases, the beetles had more opportunity to contact *O. clavigerum* than *O. montium*. The lower frequency of *O. clavigerum* on beetles could be due to its large clavate spores, which may not be able to adhere to the beetles as stably as the small conidia of *O. montium*.

Leptographium longiclavatum was isolated from the mountain pine beetle exoskeletons and infested sapwood. In previous work, it has been found in mountain pine beetle mycangia (Lee et al. 2005). This species appeared to be affected by the moisture content of its environment, as it was isolated more often from early green phase trees than from the drier late green phase trees that had been infested the previous summer (Kim et al. 2005). Like *Ophiostoma clavigerum*, *Leptographium longiclavatum* has long conidia, and may be more easily grazed by the beetles and carried preferentially in the mycangia rather than on the exoskeleton (Harrington and Zambino 1990; Hsiau and Harrington 1997; Six 2003b).

In this work, we isolated two basidiomycetes. *Entomocorticium dendroctoni* was isolated from mountain pine beetle exoskeletons, although it has been only reported in beetle galleries (Whitney et al. 1987). The *Entomocorticium* sp. had a faster growth rate than *E. dendroctoni*, and its rDNA showed only 97.8% to 98.1% sequence identity to that of *E. dendroctoni*. *Entomocorticium* species have been suggested to be good nutritional sources for mountain

pine beetle and other *Dendroctonus* species (Barras and Perry 1972; Whitney and Cobb 1972; Whitney et al. 1987).

In contrast to all the above fungal species, which appear to be specifically associated with the mountain pine beetle, *Leptographium terebrantis*, *Ambrosiella* sp., and the *Ophiostoma nigrocarpum*-like species seemed to be incidental associates. The presence of these fungi on mountain pine beetle is likely due to cross-contamination with fungal associates of other cohabiting Scolytid beetles (e.g. Ips and ambrosia beetles), which were frequently observed in sampled trees. At this point, information available on fungal associates of such other beetles is limited. *Ophiostoma minus* and *O. huntii*, which have been found in old mountain pine beetle galleries (Robinson 1962; Robinson-Jeffrey and Grinchenko 1964), were not isolated in this work.

The fungi that we isolated can be grouped into fast-growing sapstaining fungi and slow-growing non-staining fungi. It is likely that non-staining fungi, such as the *O. minutum*-like species, *Entomocorticium* species and yeasts, found mainly on the beetle and rarely in sapwood, might be better nutritional sources for the mountain pine beetle than are staining fungi, which contain melanin, a phenolic derivative. Whitney et al. (1987) has shown that the sapstaining fungi *Ophiostoma montiu*m and *O. clavigerum* were less efficient in supporting beetle reproduction than *E. dendroctoni*. Yeasts, commonly found on the mountain pine beetle in current and previous studies (Whitney 1971; Lim et al. 2005), may also be an important nutritional source. In addition, they may contribute to successful brood development by preventing excess numbers of beetles in individual trees, since some yeasts can convert the mountain pine beetle aggregation pheromone, *trans*-verbenol, into the anti-aggregation pheromone, verbenone (Hunt and Borden, 1990). Similarly, pathogenic species *Ophiostoma clavigerum*, *O. montium* and *Leptographium longiclavatum*, by invading the sapwood and interrupting water transportation, decrease the moisture content in the tree and may provide a better environment for beetle brood development (author's unpublished data for *L. longiclavatum*; Strobel and Sugawara 1986; Yamaoka et al. 1995; Webb and Franklin 1978).

In conclusion, in contrast to previous work, we showed that more diverse fungal species were associated with the mountain pine beetle. We also found that species dominant on the mountain pine beetle exoskeletons differed from those dominant in galleries and sapwood, with the difference mainly due to the *Ophiostoma minutum*-like species. Comparison of fungus frequencies from different parts of the mountain pine beetle, such as the exoskeleton, mycangia and gut, would suggest which fungi might be more preferentially consumed by the beetle. Further work will be needed to clarify interactions among fungi and the impact of each fungus species on the mountain pine beetle fitness and on host defence mechanisms.

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References

Barras, S.J.; Perry, T. 1972. Fungal symbionts in the prothoractic mycangium of *Dendroctonus frontalis* (Coleoptera: Scolytidae). Zeitschrift fur Angewandte Entomologie. Zeitschrift Fur Angewandte Entomologie 71:95–104.

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

BC Ministry of Forests. 2005. British Columbia's mountain pine beetle action plan 2005-2010. Available from http://www.for.gov.bc.ca/hfp/mountain_pine_beetle/actionplan/2005/intro.htm [cited 14 July 2005].

Camargo, J.A. 1992. Can dominance influence stability in competitive interactions? Oikos 64:605–609.

Council of Forest Industries. 2005. Reports: COFI facts books. Part III. BC's forests and forest industry. Available from http://www.cofi.org/reports/factbooks.htm. [cited 14 July 2005].

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

Harrington, T.C. 1993. Biology and taxonomy of fungi associated with bark beetles. Pages 37–51 in: *Beetle-Pathogen Interactions in Conifer Forest* (eds T.D. Schowalter and G.M. Filip). Academic Press, San Diego.

Harrington, T.C. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. pages 257–291 in: *Insect-Fungal Associations: ecology and evolution* (eds F.E. Vega). Oxford University Press, Oxford, UK.

Harrington, T.C.; Zambino, P.J. 1990. *Ceratocystiopsis ranaculosus*, not *Ceratocystis minor* var. *barrasii*, is the mycangial fungus of the southern pine beetle. Mycotaxon 38:103–-115.

Hausner, G.; Reid, J.; Klassen, G.R. 1993. *Ceratocystiopsis* - a reappraisal based on molecular criteria. Mycological Research 97:625–633.

Hausner, G.; Eyjólfsdóttir, G.G.; Reid., J. 2003. Three new species of *Ophiostoma* and notes on *Cornuvesica falcata*. Canadian Journal of Botany 81:40–48.

Hsiau, P.T.; Harrington, T.C. 1997. *Ceratocystiopsis brevicomi* sp. nov., a mycangial fungus from *Dendroctonus brevicomis* (Coleoptera: Scolytidae). Symbiosis 34:111–131.

Hunt, D.W.A.; Borden, J.H. 1990. Conversion of verbenols to verbenone by yeasts isolated from *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Journal of Chemical Ecology 16:1385–1397.

Jacobs, K.; Wingfield, M.J. 2001. Leptographium Species: tree pathogens, insect associates and agents of blue-stain. Academic Press, NY, USA. 205 p.

Kim, J.J.; Allen, E.A.; Humble, L.M.; Breuil, C. 2005. Ophiostomatoid and basidiomycetous fungi associated with green, red and grey lodgepole pines after mountain pine beetle (*Dendroctonus ponderosae*) infestation. Canadian Journal of Forest Research 35:274–284.

Lee, S.; Kim, J.J.; Breuil, C. 2003. A PCR-RFLP marker distinguishing *Ophiostoma clavigerum* from morphologically similar *Leptographium* species associated with bark beetles. Canadian Journal of Botany 81:1104–1112.

Lee, S.; Kim, J.J.; Breuil, C. 2005. *Leptographium longiclavatum* sp. nov., a new species associated with the mountain pine beetle, *Dendroctonus ponderosae*. Mycological Research 109:1162–1170.

Lim, Y.W.; Kim, J.J.; Lu, M.; Breuil, C. 2005. Determining fungal diversity on *Dendroctonus ponderosae* and *Ips pini* affecting lodgepole pine using cultural and molecular methods. Fungal Diversity 19:79–94.

Moser, J.C.; Macias-Samano, J.E. 2000. Tarsonemid mite associates of *Dendroctonus frontalis* (Coleoptera: Scolytidae): Implications for the historical biogeography of *D. frontalis*. Canadian Entomologist 132:765–771.

Mouillot D.; Leprêtre, A. 1999. A comparison of species diversity estimators. Researches on Population Ecology 41:203-215.

Paine, T.D.; Raffa, K.F.; Harrington, T.C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology 42:179–206.

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.). Canadian Journal of Botany 40:609–614.

Robinson-Jeffrey, R.C.; Davidson, R.W. 1968. Three new *Europhium* species with *Verticicladiella* imperfect states on blue-stained pine. Canadian Journal of Botany 46:1523–1527.

Robinson-Jeffrey, R.C.; Grinchenko, A.H.H. 1964. A new fungus in the genus *Ceratocystis* occurring on blue-stained lodgepole pine attacked by bark beetles. Canadian Journal of Botany 42:527–532.

Rumbold, C.T. 1941. A blue stain fungus, *Ceratostomella montium* n.sp., and some yeasts associated with two species of *Dendroctonus*. The Journal of Agricultural Research 62:589–601.

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

Six. D.L. 2003a. Bark beetle-fungus symbioses. Pages 97–114 in *Insect Symbiosis* (eds. K. Bourtzis and A.M. Thomas). CRC press, FL, USA.

Six, D.L. 2003b. A comparison of mycangial and phoretic fungi of individual mountain pine beetle. Canadian Journal of Forest Research 33:1331–1334.

Solheim, H. 1995. Early stages of blue-stain fungus invasion of lodgepole pine sapwood following mountain pine beetle attack. Canadian Journal of Botany 73:70–74.

Strobel, G.A.; Sugawara, F. 1986. The pathogenicity of *Ceratocystis montia* to lodgepole pine. Canadian Journal of Botany 64:113–116.

Upadhyay, H.P. 1981. *A Monograph of Ceratocystis and Ceratocystiopsis*. The University of Georgia Press. Athens, GA, USA.

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

Webb, J. W.; Franklin, R.T. 1978. Influence of phloem moisture on brood development of the southern pine beetle (Coleoptera: Scolytidae). Environmental Entomology 7:405–410.

White, T.J.; Bruns, T.D.; Lee, S.; Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in *PCR Protocols, A Guide to Methods and Applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press, San Diego, CA.

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

Whitney, H.S. 1982. Relationships between bark beetles and symbiotic organisms. Pages 183–211 in *Bark Beetles in North American Conifers* (eds. J.B. Mitton and K.B. Sturgeon). University of Texas Press, Austin. Texas.

Whitney, H.S.; Cobb, F.W. Jr. 1972. Non-staining fungi associated with the bark beetle *Dendroctonus brevicomis* (Coleoptera: Scolytidae) on *Pinus ponderosa*. Canadian Journal of Botany 65:95–102.

Whitney, H.S.; Farris, S.H. 1970. Maxillary mycangium in the mountain pine beetle. Science 167:54–55.

Whitney, H.S.; Bandoni, R.J.; 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. Canadian Journal of Botany 65:95–102.

Yamaoka Y.; Hiratsuka, Y.; Maruyama, P.J. 1995. The ability of *Ophiostoma clavigerum* to kill mature lodgepole pine trees. European Journal of Forest Pathology 25:401–404.

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