Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta

A. V. Rice, M. N. Thormann, and D. W. Langor

Abstract: Mountain pine beetle (MPB) is the most serious pest of lodgepole pine in western Canada, and it is predicted to spread into boreal jack pine within the next few years. Colonization of host trees by MPB-associated blue-stain fungi appears to be required for successful beetle reproduction. Three species of blue-stain fungi, *Grosmannia clavigera* (Robinson-Jeffery and Davidson) Zipfel, de Beer, and Wingfield (≡ *Ophiostoma clavigerum* (Robinson-Jeffery and Davidson) Harrington), *Ophiostoma montium* (Rumbold) von Arx, and *Leptographium longiclavatum* Lee, Kim, and Breuil, are associated with MPB in Alberta. In inoculation experiments, all three fungi caused lesions on lodgepole pine, jack pine, and their hybrids. On average, lesions were longer on jack pine and hybrids than on lodgepole pine, suggesting that fungal development will not be a barrier to MPB success in these trees. Differences in lesion length caused by the three fungal species were minimal, with significant differences observed only on hybrid pine and between *O. montium* and the other fungal treatments. On average, lesions caused by combinations of the three fungi (pair-wise and all together) did not differ significantly in length from those caused by the fungi singly, and none of the fungal species competitively excluded any of the others. These observations suggest that all three species are pathogenic to boreal pines and that the virulence of all three species is comparable.

Key words: boreal, *Grosmannia clavigera*, *Leptographium longiclavatum*, lodgepole pine, jack pine, jack × lodgepole pine hybrids, *Ophiostoma montium*.

Résumé : Le dendroctone du pin (MPB) est le plus important ravageur du pin lodgepole dans l'ouest canadien, et on prédit qu'il s'étendra au pin gris dans la forêt boréale au cours des prochaines années. La colonisation de l'arbre hôte, par les champignons du bleuissement qui lui sont associés, semble nécessaire pour la reproduction de l'insecte. On retrouve trois espèces de champignons du bleuissement, associés au MPB en Alberta : *Grosmannia clavigera* (Robinson-Jeffery and Davidson) Zipfel, de Beer, and Wingfield (≡ *Ophiostoma clavigerum* (Robinson-Jeffery and Davidson) Harrington), *Ophiostoma montium* (Rumbold) von Arx, et *Leptographium longiclavatum* Lee, Kim, and Breuil. L'inoculation expérimentale montre que les trois champignons causent des lésions sur le pin lodgepole, le pin gris et leurs hybrides. Dans l'ensemble, les lésions sont plus longues sur le pin gris et ses hybrides que sur le pin lodgepole, ce qui suggère que le développement du champignon ne limitera pas le succès du MPB sur essences. Les différences de longueur des lésions causées par les trois espèces fongiques sont minimales, les seules différences significatives étant observées sur le pin hybride et entre le *O. montium* et les autres traitements fongiques. Dans l'ensemble, les lésions causées par des combinaisons de ces trois champignons (en paires ou tous ensemble) ne diffèrent pas significativement en longueur, de celles causées par les champignons individuels, et aucun de ces champignons n'exclut les autres par compétition. Ces observations suggèrent que les trois espèces de champignon sont pathogènes pour les pins de la forêt boréale et que la virulence des trois espèces est comparable.

Mots-clés : *Grosmannia clavigera*, *Leptographium longiclavatum*, pin lodgepole, pin gris, pin hybrides gris × lodgepole, *Ophiostoma montium*.

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Introduction

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is the most serious insect pest of pines in the mountain regions of western Canada and the United States. It has a broad host range, including all of the native pines of western Canada: lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelmann), whitebark pine (*P. albicaulis* Engelmann), ponderosa pine (*P. ponderosa* Douglas *ex* Lawson), western white pine (*P. monticola* Douglas ex D. Don), and limber pine (*P. flexilis* James) (Safranyik et al. 1974; Tsuneda and Hiratsuka 1984; Yamaoka et al. 1990; Cerezke 1995; Solheim and Krokene 1998; Carroll et al. 2003; Ono 2003; Lim et al. 2005; Lee et al. 2006). Many non-native pines planted within the range of the MPB also have been attacked and killed (Amman and Cole 1983).

British Columbia (BC) is currently experiencing the worst

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outbreak of MPB ever reported (Lee et al. 2006), with millions of lodgepole pines killed annually (Huber and Borden 2001; Carroll et al. 2003; Kim et al. 2005; British Columbia Ministry of Forests 2006; Lee et al. 2006). By 2005, more than 8.7×10^6 ha of lodgepole pine forests were affected, an increase of well over 1×10^6 ha since 2004 (British Columbia Ministry of Forests 2005, 2006).

The beetle is currently spreading eastward into Alberta (AB) (Ono 2003). Historically, two outbreaks of MPB have been reported in southern AB (1940–1943 and 1977–1985) (Cerezke 1995; Ono 2003). In 1997, MPB was observed in lodgepole pine in the Willmore Wilderness Area, AB (53°45′N), a northerly increase of more than 2° of latitude in the beetle's range since 1985 (Ono 2003). Since then, beetle populations have increased rapidly in this area and have spread north and east despite management efforts. In 2006, MPB entered northwestern AB, presumably spreading from source populations in northeastern BC, and became established in the boreal forest of the Peace District (at least as far north as Peace River, 56°15′N), where it infested lodgepole pine, lodgepole × jack pine hybrids, and introduced Scots pine (*Pinus sylvestris* L.) (D.W. Langor, unpublished data, 2006). This northeasterly range expansion into boreal Canada is predicted to accelerate with global warming (Carroll et al. 2003). There is concern that MPB may be able to infest jack pine (*Pinus banksiana* Lambert) and use this species as a conduit to spread to eastern Canada and possibly the southeastern United States, where many other native pine species grow. This spread would have potentially disastrous environmental, social, and economic consequences (Ono 2003). With successful colonization of hybrid pine populations in northwestern AB in 2006, the threat of MPB invasion of boreal jack pine appears imminent. The ability of MPB to attack and kill natural jack pine populations is unknown, but evidence suggests that the beetles can infest jack pine. Artificial rearing experiments (Safranyik and Linton 1982; Cerezke 1995; D.W. Langor, unpublished data, 2006) indicate that MPB can survive and reproduce in cut sections of jack pine, and that survival and fecundity are comparable to that observed in cut sections of lodgepole pine (Cerezke 1995). Furthermore, MPB attacked and killed a small number of mature (51-year-old) jack pines in an Idaho arboretum, although their reproductive success in those trees is unknown (Furniss and Schenk 1969).

The ability of MPB to colonize successfully trees appears to be largely dependent on a symbiotic relationship with ophiostomatoid fungi. Three blue-stain ascomycetes, *Grosmannia clavigera* (Robinson-Jeffery and Davidson) Zipfel, de Beer and Wingfield [≡ *Ophiostoma clavigerum* (Robinson-Jeffery and Davidson) Harrington], *Ophiostoma montium* (Rumbold) von Arx, and *Leptographium longiclavatum* Lee, Kim and Breuil, are associated with the mycangia of the MPB (Tsuneda and Hiratsuka 1984; Yamaoka et al. 1990, 1995; Solheim and Krokene 1998; Six 2003; Kim et al. 2005; Lee et al. 2005, 2006; Lim et al. 2005), although other blue-stain fungi have been reported from the exoskeleton and beetle galleries (Six 2003; Lee et al. 2006). The mycangial fungi have been suggested to aid the beetle in overwhelming the host trees' defences (Raffa and Berryman 1983), provide necessary nutrition throughout the beetles' life cycle (Six and Paine 1998; Adams and Six 2007), and perhaps provide a more favourable environment for beetle development (see Lee et al. 2006). Mycangial fungi are essential to MPB success, because the beetles appear to be unable to reproduce successfully in their absence (Six and Paine 1998). Although both the beetles and their mycangial blue-stain fungi on their own can kill the host trees (Mathre 1964; Basham 1970; Shrimpton 1973; Strobel and Sugawara 1986; Yamaoka et al. 1995), it has been suggested that the combined action of the beetles and fungi is responsible for rapid host death (Amman et al. 1989; Yamaoka et al. 1990, 1995; Nebeker et al. 1993; Solheim and Krokene 1998; Kim et al. 2005; Lee et al. 2006), although other studies, on related bark beetles, indicate that extensive fungal growth occurs after host tree death (Hobson et al. 1994). Successful colonization of jack pine by the MPB mycangial blue-stain fungi is considered to be a factor required for successful attack by MPB. In preliminary research, we found that both *G. clavigera* and *O. montium* caused longer lesions on jack × lodgepole pine hybrids and jack pine than they did on lodgepole pine. This suggests that these trees are suitable hosts for MPB-associated blue-stain fungi, and that these trees might be more vulnerable than lodgepole pines (Rice et al. 2007). However, this study was conducted on a relatively small number of trees using a single site for each host species and did not include *L. longiclavatum*. Thus, to provide a more complete assessment of the possible susceptibility of jack pine to MPB-associated blue-stain fungi, we assessed the relative virulence of all three fungi over a wider range of tree populations.

The MPB-associated blue-stain fungi often occur in combination on individual beetles (Six 2003; Lee et al. 2006) and on different beetles attacking the same tree. Interactions, including competition and facilitation, among the fungi inside the host tree may affect virulence as well as beetle development and success, but the nature of these interactions is not known. The only study reporting combined inoculation of trees with *G. clavigera* and *O. montium* found that *O. montium* inhibited *G. clavigera* on ponderosa pine seedlings (Owen et al. 1987). Although the experimental conditions of that study did not accurately reflect natural conditions whereby mature trees in natural stands are attacked, their results suggest that competition between the mycangial symbionts is likely. Therefore, we inoculated jack pine, lodgepole pine, and their hybrids with the fungi, alone and in combination, to compare virulence and assess the outcomes to test the hypothesis that the three fungi compete with or inhibit each other.

Materials and methods

Fungal isolates

The fungi used in this study were isolated from the sapwood of MPB-infested lodgepole pine trees harvested from the Willmore Wilderness Area in January 2006. The isolates are deposited as live cultures at the Northern Forestry Centre Culture Collection (NOF). One isolate each of *G. clavigera* (NOF 2948), *L. longiclavatum* (NOF 2954), and *O. montium* (NOF 2951) were used in this study.

Study sites

Three sites across central Alberta were chosen for tree in-

Table 1. Mountain pine beetle (MPB)-associated blue-stain fungi recovered from lesions inoculated with combinations of three species of MPB-associated blue-stain fungi.

Treatment	Lodgepole pine	Jack pine	Hybrid pine
GO	4 G; 6 GO	3 G; 1 O; 6 GO	3 G; 2 O; 5 GO
GL	4 G; 5 GL; 1 neither	1 G; 9 GL	3 L; 7 GL
LO	5 O; 1 L; 4 LO	2 L; 1 O; 7 LO	4 L; 1 O; 5 LO
GLO	2 O; 1 L; 3 GO; 2 GL; 1 LO; 1 GLO	2 GO; 1 GL; 1 LO; 6 GLO	1 G; 1 L; 2 LO; 1 GO; 1 GL; 4 GLO

Note: G, *Grosmannia clavigera*; L, *Leptographium longiclavatum*; O, *Ophiostoma montium*; GO, *G. clavigera* and *O. montium*; GL, *G. clavigera* and *L. longiclavatum*; LO, *L. longiclavatum* and *O. montium*; GLO, all three fungi together. Numbers indicate the number of times a particular fungus or combination of fungi was recovered from a particular treatment. There are 10 inoculation points per species combination per host.

oculations. The pine forests at each site were mature (at least 50 years old), and inoculated trees had a diameter at breast height of at least 20 cm. Ten lodgepole pine trees were inoculated near the Berland River between Hinton and Grande Cache (53°45.361′N, 118°20.207′W), 10 hybrid pines were inoculated at a site northeast of Blue Ridge (54°13.127′N, 115°16.456′W), and 10 jack pine trees were inoculated near Tawatinaw (54°16.647′N, 113°28.171′W). Inoculation of the tree species at a single site is not possible, because their geographic ranges do not overlap in natural forests.

Inoculation

Holes (5 mm diameter, 10 mm deep) were drilled through the bark and phloem in each tree. Inoculum, consisting of active mycelium growing on 2% malt extract agar (MEA; 20 g Difco malt extract (Difco Laboratories, Detroit, Mich.), 15 g agar (Fisher Scientific, Fair Lawn, N.J.), 1 L dH_2O), or sterile MEA as a control, was inserted into holes using a flame-sterilized probe and placed on the surface of the sapwood. A sterile dowel (5 mm diameter, 5–7 mm long) was placed into each hole to cover the inoculum. As controls, one hole per tree received a sterile agar plug and another did not receive any medium; both were plugged with dowels. The fungal treatments consisted of each of the three fungi alone, each of the three possible pair-wise combinations, and all three fungi together. The nine holes (seven fungal treatments and two controls) were at least 5 cm apart in a ring encircling the tree at breast height. Parafilm® strips (American National Can, Neenah, Wisc.) were wrapped around the trees at the inoculation sites to reduce contamination and desiccation. Trees were inoculated in August, corresponding with the time when most beetles complete host colonization in Alberta (Langor 1989). Trees were harvested 6 weeks after inoculation in September 2006. The trees were felled, and bolts (>1.2 m long) were cut from around the inoculation site (with at least 50 cm above and below the inoculation points) and transported to the laboratory. Bark and phloem were stripped from the bolts within 36 h of harvesting, and the lengths of the lesions were measured at each inoculation point. Lesion length is a measure of the vertical effect of the fungi on the host and commonly has been used as an indicator of relative virulence (e.g., Solheim and Krokene 1998; Masuya et al. 2003; Lieutier et al. 2004). Samples of sapwood were removed aseptically from three random points within the lesions then surface sterilized and plated onto MEA to recover the fungi.

Statistical analyses

Differences in fungal lesion lengths were compared using an analysis of variance (ANOVA), using a general linear model consisting of a two-way comparison of the main effects: tree species and fungal treatment. Treatments were *G. clavigera*, *L. longiclavatum*, *O. montium*, *G. clavigera* and *O. montium*, *G. clavigera* and *L. longiclavatum*, *L. longiclavatum* and *O. montium*, all three fungi together, agar plug, and the empty control. Significant main effects were compared using Tukey adjustment of the least squared means. Examination of the residual values from the ANOVA using the Shapiro–Wilk statistic, stem leaf and box plots, normal probability plot, and plot of residual against predicted values indicated that the lesion length values followed a normal distribution ($N = 270$, $W = 0.84$). No data were excluded. All analyses were performed using SAS (SAS Institute Inc., Cary, N.C.).

Results

At the time of harvest, necrotic lesions were observed on the sapwood surrounding the inoculation points (Fig. 1). Discolouration (typically less than 30 mm long) was observed around control points, but resin responses by the trees were not observed (Figs. 1A and 1B). The lesions around all of the fungal inoculation points were longer than the discolouration observed around the control points and involved both discolouration of the sapwood and a resin response by the trees (Figs. 1B and 1C). All fungal species were recovered from their individual inoculation points and lesions but not from the discoloured area around the controls.

Fungi were also recovered from all but one of the lesions formed around points where the fungal species were inoculated in combination (Table 1). For the *G. clavigera* and *O. montium* combined inoculation, both species were recovered from at least half of the inoculation points in each host. When only one species was recovered, it was more commonly *G. clavigera*; *O. montium* alone was recovered from only three inoculation points in jack pine and hybrids (Table 1). For the *G. clavigera* and *L. longiclavatum* combined inoculations, both species were recovered from at least half of the inoculation points in each host. *Grosmannia clavigera* alone was recovered from five inoculation points, and *L. longiclavatum* alone was recovered from three. For the *O. montium* and *L. longiclavatum* inoculations, both species were recovered from at least half of the inoculation points in jack pine and hybrids and from four points in lodgepole pine. When only one species was recovered, it was mainly *O. montium* in lodgepole pine and mainly *L. longiclavatum* in jack pine. When all three fungi were inoculated together, all three species were recovered from 11

Fig. 1. Lesions produced around inoculation points on jack × lodgepole pine hybrids. Photographs were taken without magnification under fluorescent lighting using a Nikon Coolpix 7600 digital camera. (A) Discolouration or lesion produced around control inoculation points. Scale bar = 5 mm. (B) Lesions produced around an agar control (C), and inoculations of *Ophiostoma montium* (O) and *Grosmannia clavigera* (G). Note that the lesions around the fungi involve both discolouration and a resin response by the tree. Scale bar = 10 mm. (C) Lesions produced around inoculations of *O. montium* (O), *G. clavigera* (G), and *Leptographium longiclavatum* (L). Note all of the lesions were similar in length on this tree. Scale bar = 5 mm.

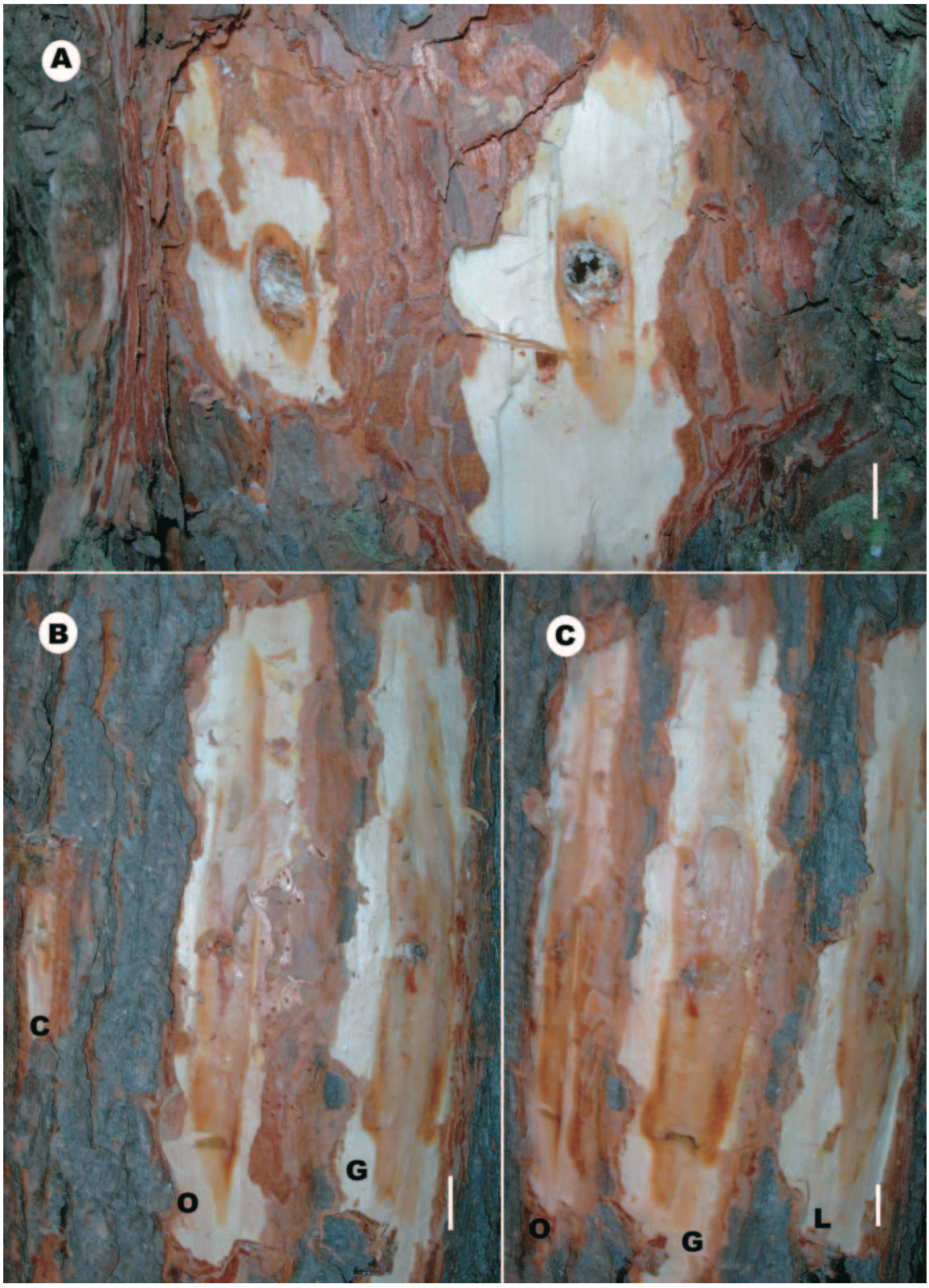

Table 2. Results of analysis of variance of lesion lengths for tree species and fungal treatments.

Source	df	*F*	*p*
Model	26	27.43	<0.0001
Tree sp.	2	36.65	<0.0001
Fungus	8	77.58	<0.0001
Tree sp. × fungus	16	1.36	0.1627

of the 30 inoculation points. All three combinations of two species were recovered from each host, and each of the three species was recovered individually at one or two inoculation points in lodgepole pine or hybrids.

Tree species and fungal species had significant influences on mean lesion lengths, but there were no significant interactions (Table 2). For all fungal treatments combined, mean lesion lengths on each of the three tree species differed significantly from each other ($p < 0.0001$), with the longest lesions on hybrid pines (mean ± SE; 143 ± 8.4 mm), the shortest lesions on lodgepole pine (98 ± 5.4 mm), and intermediate lesions on jack pine (120 ± 6.1 mm). The lesions induced by *G. clavigera* and *O. montium* plus *L. longiclavatum* were significantly longer on hybrid pines than on lodgepole pines ($p \leq 0.0176$) (Table 3). Although none of the other fungal treatments differed significantly among hosts, they all exhibited the same trend of producing the longest lesions on hybrids and the shortest on lodgepole pine (Table 3). Every fungal treatment produced significantly longer lesions than the controls ($p \leq 0.0026$). With data from all tree species combined, lesions caused by *O. montium* were significantly smaller than those caused by the other fungal treatments ($p \leq 0.0212$). Although all other fungal treatments consistently produced longer lesions than *O. montium* in each of the three host species, the only significant difference was between *O. montium* and *G. clavigera* on hybrid pines ($p = 0.0108$) (Table 3).

Discussion

All three blue-stain fungi caused lesions on the sapwood of jack pine, lodgepole pine, and jack × lodgepole pine hybrids in northern Alberta when artificially inoculated alone or in combination with each other. These results are comparable with our preliminary study (Table 4). Furthermore, we confirm the findings that lesions are longer on jack and hybrid pines than on lodgepole pine. This suggests that jack and hybrid pines are at least as susceptible to the fungi as lodgepole pines, and that the success of the MPB in these species will not be restricted by the growth of their mycangial fungi.

Lesions produced by *G. clavigera* and *O. montium* in this study were longer on average than those we observed in 2005. Although this trend was observed on all three host trees, it was most pronounced on the hybrid pines, on which the lesions caused by *G. clavigera* and *O. montium* averaged nearly 75 mm and 65 mm longer, respectively, in 2006 than in 2005. On the other hosts, the differences in mean lesion length between the years ranged from 10 to 40 mm. There were no differences in lesion length observed among the empty control inoculations between the two years. The problems with contamination of the agar controls observed in the previous study were eliminated in this study. These year-to-year differences may result from more favourable (warmer) environmental conditions for fungal growth, genetic differences, or site-specific effects. These differences were most pronounced on the hybrid pines, where genetic variation is high. Intraspecific variation in virulence has been observed for *G. clavigera* (Rice et al. 2007), and some of the year-to-year differences in magnitude might be explained by this variability. However, despite differences in lesion length among isolates of this species, the same trend was observed for all isolates.

Previous inoculation experiments (Reid et al. 1967; Owen et al. 1987; Yamaoka et al. 1990; Solheim and Krokene 1998; Rice et al. 2007) have found that *G. clavigera* is more virulent than *O. montium* on lodgepole pine and other traditional hosts. The same trend was observed on jack × lodgepole pine hybrids, but not on jack pine (Rice et al. 2007). Our observations suggest that *G. clavigera* does induce longer lesions than *O. montium* on all three host species, although only significantly so on hybrids. These observations provide further support for the assertions that there is little difference in virulence between *G. clavigera* and *O. montium* on jack pine and that both species have the potential to be important pathogens of this tree species (Rice et al. 2007). The virulence of the third MPB associate, *L. longiclavatum*, had not been reported previously. We found no significant differences in lesion lengths caused by this species and *G. clavigera*, suggesting that this species is at least as virulent as *G. clavigera* on all three hosts.

Individual MPB often carry multiple species of blue-stain fungi on their exoskeletons and in their mycangia (Six 2003; Lee et al. 2006). When a beetle attacks a tree, all three of these fungi could be inoculated together. We predicted that the interactions among these fungi could influence their virulence to the host tree. Also, if the fungi differentially benefit the beetles (see Six and Paine 1998; Lee et al. 2006; Adams and Six 2007), the interactions could also determine the development and success of the beetles and, thus, the outcome of the infestation. The only differences observed between the combined fungal inoculum and the individual fungal species was that the combinations involving *O. montium* (i.e., pair-wise combination with *G. clavigera* and *L. longiclavatum*, and all three fungi combined) produced longer lesions than *O. montium* alone. These differences were only significant when data from all three tree hosts were combined but not when host tree species were considered individually. Notably, there were no significant differences observed among the combined inocula or the combinations and *G. clavigera* or *L. longiclavatum*. These observations suggest that interactions among the MPB-associated blue-stain fungi do not have a significant effect, either positive or negative, on virulence to the host tree, though their direct effects on the beetle remain unknown and must be tested. These results also contradict those observed on ponderosa pine seedlings (Owen et al. 1987), where co-inoculation significantly reduced seedling mortality caused by *G. clavigera*. These differences could be due to the different ages of the hosts (seedlings versus mature trees) or to host-specific effects (ponderosa pine versus jack and lodgepole pines and their hybrids). None of the fungi

Table 3. Mean (SE) lesion lengths (mm) for each fungal treatment for each host.

Treatment	Lodgepole pine	Jack pine	Hybrid pine	Mean
E	16(1.4)a	20(1.5)a	24(2.1)a	20(1.1)a
A	22(1.0)a	27(6.0)a	34(7.6)a	28(3.2)a
O	85(5.3)b	120(6.8)b	135(12.1)b	114(6.2)b
G	130(4.9)A,b	156(13.4)AB,b	201(27.5)B,c	162(11.4)c
L	135(11.0)b	146(7.7)b	184(7.7)bc	155(6.4)c
GO	116(7.4)b	157(4.3)b	161(13.6)bc	144(6.4)c
GL	133(12.2)b	144(9.8)b	188(27.0)bc	155(10.9)c
LO	124(10.0)A,b	148(6.6)AB,b	188(14.1)B,bc	153(7.7)c
GLO	121(10.6)b	158(6.0)b	170(7.7)bc	150(6.1)c
Mean	98(5.4)A	120(6.1)B	143(8.4)C	120(12.1)

Note: E, empty hole; A, agar plug; O, *Ophiostoma montium*; G, *Grosmannia clavigera*; L, *Leptographium longiclavatum*; GO, *G. clavigera* and *O. montium*; GL, *G. clavigera* and *L. longiclavatum*; LO, *L. longiclavatum* and *O. montium*; GLO, all three fungal species. Post hoc Tukey results are indicated where there are significant differences in means. Upper-case letters indicate among-host comparisons within a fungal treatment, and lower-case letters indicate among-fungus comparisons on a host. Means followed by the same letter do not differ significantly.

Table 4. Mean (SE) lesion lengths (mm) caused by *Ophiostoma montium* and *Grosmannia clavigera* on each host species in 2005 (Rice et al. 2007) and 2006 (this study).

Tree	2005 *O. montium*	2006 *O. montium*	2005 *G. clavigera*	2006 *G. clavigera*
Lodgepole pine	63(3)	85(5)	82(4)	130(5)
Jack pine	108(8)	120(7)	115(9)	156(13)
Hybrid pine	68(5)	135(12)	128(12)	201(28)

*Shows significant between-year differences as indicated by ANOVA (a general linear model consisting of a three-way comparison of fungal species, host species, and year).

consistently excluded another when they were inoculated in combination. Each of the three fungi was recovered from the lesions surrounding most of its combined inoculations, although *G. clavigera* was recovered on its own more often than *L. longiclavatum* or *O. montium* in pair-wise combinations. These results suggest that the three mycangial fungi are about equally competitive and are unable to competitively exclude one another from an infection site. The competitive equality of the MPB-associated fungi suggests that the spatial and temporal distribution of these fungi within and among beetle populations is due to factors other than competition among the fungi, such as temperature (see Adams and Six 2007).

This situation is comparable to that observed for the two mycangial fungi of the southern pine beetle (Klepzig and Wilkens 1997; Klepzig et al. 2004). In that system, Klepzig and Wilkens (1997) found that the two species of mycangial fungi were competitively similar to each other. Both mycangial fungi were outcompeted by the virulent plant pathogen and phoretic fungus, *Ophiostoma minus* (Hedgc.) Syd. & P. Syd., under most conditions (Klepzig and Wilkens 1997; Klepzig et al. 2004). Although it was completely excluded under most conditions (Klepzig and Wilkens 1997), the ophiostomatoid mycangial associate (*Ceratocystiopsis ranaculosa* T.J. Perry & J.R. Bridges) was able to compete almost as well as *O. minus* under selected moisture conditions (Klepzig et al. 2004). Other blue-stain fungi, including an *Ophiostoma minus*-like fungus, have been recovered from the exoskeletons and galleries of MPB (Six 2003; Lee et al. 2006). The interactions between these fungi and the mycangial associates of the MPB should be explored, since competitive exclusion of the mycangial fungi by a phoretic associate could suggest a possible avenue for biocontrol research. Notably, *O. minus* is also directly antagonistic to the southern pine beetle (e.g., Klepzig et al. 2001; Hofstetter et al. 2006). A similar relationship between MPB and *O. minus* or other phoretic fungi would suggest another avenue for biocontrol of MPB.

Our results suggest that both jack pine and jack × lodgepole pine hybrids in boreal Alberta are susceptible to infection by all of the MPB-associated blue-stain fungi, and that these trees could be more suitable than lodgepole pine as hosts for these fungi. This study was conducted using different stands of each of the three host trees than those used in our previous study. The fact that the same pattern of virulence for *O. montium* and *G. clavigera* was observed on jack pine at two distant sites (Tawatinaw and the Logan River area northwest of Lac La Biche, about 200 km apart) suggests that the pattern is common to jack pine. The two hybrid stands were located close to each other, but were separated by a large stand of trembling aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss). The trees in the two stands differed from each other in phenotype (resemblance to parent species) and presumably genotype. Our observations suggest that *O. montium* is extremely sensitive to genetic variation within the hybrid pines, causing much longer lesions on hybrids that resemble jack pine than on those that resemble lodgepole pine, and that susceptibility of hybrid pines to *O. montium* may vary considerably among stands.

All three MPB-associated fungi successfully colonized jack pine and jack × lodgepole pine hybrids, but the success of the fungi does not guarantee the success of their associated beetles. The brood success of MPB also depends on factors, such as phloem thickness, that may not influence the fungi (Amman 1972; Langor 1989). On average, the phloem of jack pines is thinner than that of similar-sized lodgepole pines, whereas phloem thickness in hybrids is highly variable (D. Williams, personal communication 2006). It is possible that MPB brood success in jack pines will be limited by phloem thickness. However, evidence from artificial brood experiments (Safranyik and Linton 1982; Cerezke 1995; D.W. Langor, unpublished data, 2006) and infection of arboretum trees (Furniss and Schenk 1969), coupled with the colonization of hybrid pines by MPB in 2006, suggest that these trees are suitable hosts for MPB. Ultimately, the ability of MPB to select, attack, overwinter, and breed in jack pines will be determined only by the spread of the beetle into natural jack pine stands. We can conclude that the growth of their associated blue-stain fungi will not limit the success of the beetles. It is conceivable that the consistently better performance of the fungi in the hybrid and jack pines may hasten the spread of MPB through the boreal region.

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