

Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole × jack pine hybrids in Alberta

Adrienne V. Rice, Markus N. Thormann, and David W. Langor

Abstract: Mountain pine beetles (*Dendroctonus ponderosae* Hopkins; (MPB)) have spread into lodgepole × jack pine hybrid (*Pinus contorta* Douglas × *Pinus banksiana* Lambert) forests in Alberta and are predicted to spread into jack pine forests. Their success in these forests is uncertain but will be influenced by multiple factors, including the ability of their associated blue-stain fungi to colonize the trees and the health of the encountered trees. Healthy and dwarf mistletoe infected pines at three sites across Alberta (one site per pine species) were inoculated with three isolates each of *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield and *Ophiostoma montium* (Rumbold) von Arx. Both fungi grew and caused lesions on all hosts, suggesting that MPB will not be limited by a lack of fungal growth. Both fungi caused longer lesions in jack and hybrid pines than in lodgepole pines, indicating that susceptibility varies among hosts and is greater in the novel systems than in the co-evolved one. *G. clavigera* caused longer lesions than *O. montium* in hybrids and lodgepole pines, while the two species caused similar-sized lesions on jack pine. Intraspecific variation was high in *G. clavigera*, with one isolate producing much shorter lesions than the other two. Dwarf mistletoe infestation had little effect on infection lesion length.

Key words: Jack pine, lesions, lodgepole pine, lodgepole × jack pine hybrids, mountain pine beetle, *Grosmannia clavigera*, *Ophiostoma montium*.

Résumé : Le dendroctone du pin (*Dendroctonus ponderosa* Hopkins; (MPB)) s'est étendu aux hybrides de pin lodgepole × pin gris (*Pinus contorta* × *Pinus banksiana*) dans les forêts d'Alberta, et on prédit qu'il s'étendra aux forêts de pin gris. Son succès dans ces forêts n'est pas certain, mais sera influencé par de multiples facteurs, incluant la capacité des champignons de bleuissures associés à coloniser les arbres, et de la santé des arbres rencontrés. Les auteurs ont inoculé des arbres sains et des arbres infectés par le gui nain, sur trois sites en Alberta (un site par espèce de pin), avec trois isolats de *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield, et trois isolats de l'*Ophiostoma montium* (Rumbold) von Arx. Les deux champignons se sont développés et ont produit des lésions sur tous les hôtes, ce qui suggère que le MPB ne sera pas limité par l'absence de croissance fongique. Les deux champignons causent des lésions plus longues chez le pin gris et les pins hybrides, ce qui indique que la susceptibilité varie selon les hôtes et est plus importante chez les nouveaux systèmes que ceux qui ont co-évolué. Les lésions du *G. clavigera* sont plus longues que celles de l'*O. montium*, chez les hybrides et le pin lodgepole, alors que sur le pin gris les lésions des deux champignons sont de même grandeur. La variation intraspécifique est grande chez le *G. clavigera*, un des isolats produisant des lésions beaucoup plus courtes que les deux autres. L'infection par le gui nain a peu d'effet sur la longueur des lésions.

Mots-clés : pin gris, lésions, pin lodgepole, hybrides pin lodgepole × pin gris, dendroctone du pin, *Grosmannia clavigera*, *Ophiostoma montium*.

[Traduit par la Rédaction]

Introduction

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is the most serious insect pest of many western Canadian pines, including lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelm.), whitebark pine (*P. albicaulis* Engelm.), and limber pine (*P. flexilis* James) (Safranyik et al. 1974; Yamaoka et al. 1990; Cerezke 1995; Solheim and Krokene 1998; Carroll et al. 2003; Lim et al.

2005). In British Columbia (BC), the MPB has killed millions of lodgepole pines annually over the past several years (e.g., Huber and Borden 2001; Kim et al. 2005). By 2004, more than 7×10^6 ha of lodgepole-pine forests were affected (British Columbia Forest Service 2005). Historically, two MPB outbreaks have been recorded in Alberta (1940–1943 and 1977–1985), both were restricted to southern Alberta (Cerezke 1995; Carroll et al. 2003; Ono 2003). It has been predicted that with global warming, more environments will be suitable for the beetle, and MPB will spread farther north and east, into Alberta and the Yukon (Carroll et al. 2003). This prediction has been borne out by recent observations of MPB in BC and Alberta. In 1997, the MPB was observed in lodgepole pine in the Wilmore Wilderness area, north of Jasper National Park in west-central Alberta, a northerly increase in its range of more than 2° latitude since 1985 (Ono

Received 7 February 2007. Published on the NRC Research Press Web site at canjbot.nrc.ca on 4 May 2007.

A.V. Rice,¹ M.N. Thormann, and D.W. Langor. Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada, 5320–122 Street., Edmonton, AB T6H 3S5, Canada.

¹Corresponding author (e-mail: arice@nrcan.gc.ca).

2003). In BC, the species is distributed even farther north than in Alberta, including a large and expanding outbreak on the east side of the Rocky Mountains in the Peace District (Ono 2003). In 2006, MPB spread into lodgepole × jack pine populations in northwestern Alberta. The further spread of MPB into boreal jack pine (*Pinus banksiana* Lambert) forests that spread eastward from Alberta to the Maritimes could be economically, socially, and ecologically disastrous for Canada (Ono 2003). Artificial rearing experiments (Safranyik and Linton 1982; Cerezke 1995) have indicated that MPB can survive and reproduce in cut sections of jack pine, and that survival and productivity may be comparable to that in lodgepole pine (Cerezke 1995). Furthermore, the MPB killed a small number of 51-year-old jack pine in an arboretum in Idaho, but it is unknown whether the beetles successfully reproduced in this host (Furniss and Schenk 1969).

The blue-stain ascomycetes *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield (\equiv *Ophiostoma clavigerum* (Robinson-Jeffrey and Davidson) Harrington) and *O. montium* (Rumbold) von Arx are symbiotically associated with the MPB (e.g., Tsuneda and Hiratsuka 1984; Yamaoka et al. 1990, 1995; Solheim 1995; Solheim and Krokene 1998; Six 2003a; Kim et al. 2005; Lim et al. 2005). Interactions between bark beetles and blue-stain fungi are widespread, and the nature of these interactions is complex and context dependent (e.g., Klepzig and Wilkens 1997; Paine et al. 1997; Klepzig et al. 2001a, b; Six 2003b; Kopper et al. 2004; Six and Klepzig 2004; Harrington 2005). In the case of MPB and its associated fungi, a beneficial relationship has been demonstrated between MPB and *G. clavigera* (Raffa and Berryman 1983; Six and Paine 1998). The relationship between MPB and *O. montium* is less understood but may range from beneficial to mildly antagonistic, with some studies indicating that *O. montium* can serve as a food source, but other studies indicating a neutral or negative effect on the beetle (Six and Paine 1998; Adams and Six 2007). *Grosmannia clavigera* may help the beetle overwhelm the host tree defences (Raffa and Berryman 1983) and can improve brood production and emergence of the beetle, presumably by serving as a food source (Six and Paine 1998). In fact, in the only published study comparing beetle success in the presence and absence of the fungi, the beetles were unable to reproduce in the absence of the fungi (Six and Paine 1998). Although the fungi alone can kill host trees, it has been suggested that the combined action of the fungi and the beetles is responsible for the rapid death of the hosts, since fungal-induced mortality occurs over a much longer time scale than mortality following natural beetle infestation (e.g., Mathre 1964; Reid et al. 1967; Basham 1970; Strobel and Sugawara 1986; Owen et al. 1987; Yamaoka et al. 1990, 1995; Solheim and Krokene 1998; Kim et al. 2005). Inoculation studies on multiple host tree species indicate that *G. clavigera* is more virulent than *O. montium* (e.g., Reid et al. 1967; Shrimpton 1973; Owen et al. 1987; Yamaoka et al. 1990, 1995; Solheim and Krokene 1998), although *O. montium* can also kill experimentally inoculated trees and is associated with most MPB populations (Mathre 1964; Basham 1970; Strobel and Sugawara 1986). The suitability of jack pine as a host for these fungi is unknown, but since fungal colonization ap-

pears to be required for successful beetle attack, this knowledge may help assess the susceptibility of living jack pine to infestation by MPB.

Other pests could influence the susceptibility of pine stands to MPB attack. Dwarf mistletoe (*Arceuthobium americanum* Nuttall ex Engelmann) is one of the most damaging pests of lodgepole and jack pines in western Canada (Brandt et al. 2005). Interactions between dwarf mistletoe and MPB have been suggested for lodgepole pine (Hawksworth et al. 1983); however, empirical evidence is equivocal, with some studies suggesting that mistletoe-infected trees are more susceptible to MPB attack, others suggesting that they are less susceptible, and some studies suggesting no relationship (see Hawksworth et al. 1983 for a discussion).

We inoculated lodgepole, jack, and hybrid pine trees at three sites across central Alberta with isolates of the two MPB-associated blue-stain species to test the hypotheses that both fungi can successfully colonize all tree species and that *G. clavigera* is more virulent than *O. montium*. We assessed intraspecific variation in relative virulence using three isolates of each fungal species from different parts of their Canadian range. We also examined the relative susceptibility of mistletoe-infected and healthy trees to the two fungi.

Materials and methods

Fungal isolates

Three isolates each of *G. clavigera* and *O. montium* were used in this study. The fungi were obtained from Colette Breuil at the University of British Columbia and were originally isolated by S. Lee from the sapwood of lodgepole pine trees infested with MPB. All isolates are deposited as live cultures at the Northern Forestry Centre Culture Collection (NOF). One isolate of each species (*G. clavigera* NOF 2896 (= Kw 1407) and *O. montium* NOF 2890 (= Kw 413)) originated from Kamloops, BC, in 2001, and the remaining two isolates of each species (*G. clavigera* NOF 2894 (= B5) and NOF 2895 (= B20), *O. montium* NOF 2888 (= B15) and NOF 2889 (= B19)) originated from Banff, Alberta, in 2003. The two Banff isolates of each species represent distinct populations as revealed through DNA sequence analyses (C. Breuil, personal communication, 2005).

Study sites

Three sites across central Alberta were chosen for tree inoculations. The pine forests at each site were mature (>50 years), and dwarf mistletoe infection was present on some trees. Inoculated trees were at least 20 cm in diameter at breast height. Trees were considered infected if brooms were visible in the crown branches and uninfected if there were no signs of infection (brooms or dwarf mistletoe plants) at the time of inoculation. Crown branches were also examined after the trees were harvested. Heavily infested trees were selected, because the most readily observable effect would likely be on heavily infested trees. Five healthy and five mistletoe-infected lodgepole pine trees were inoculated near the Berland River between Hinton and Grande Cache (53°45.413'N, 118°20.297'W). Five healthy and three mistletoe-infected hybrid pines were inoculated at a site northeast of Blue Ridge (54°3.317'N, 115°18.298'W).

Five healthy and four mistletoe-infected jack pine trees were inoculated near the Logan River northwest of Lac La Biche (55°20.620'N, 111°55.143'W). Inoculation of all three 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, Michigan), 15 g agar (Fisher Scientific, Fair Lawn, New Jersey), 1 L dH₂O) 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 media; both were plugged with dowels. The eight holes (six strains of fungi and two controls) were at least 5 cm apart in a ring encircling the tree at breast height. Parafilm[®] strips (American National Can, Neenah, Wisconsin) were wrapped around the trees at the inoculation sites to reduce contamination, and plastic sheets (about 50 cm wide) were wrapped around the trees and tied with nylon ropes to protect the inoculations from desiccation and contamination. Trees were inoculated in late August, corresponding with the time when most beetles complete host colonization in Alberta (Langor 1989). Trees were harvested 6 weeks after inoculation in October 2005. 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 72 h of harvesting, and the lengths of the lesions were measured at each inoculation point. Lesion length measures the vertical effect of the fungi on the host and has been used previously as an indicator of relative virulence (e.g., Solheim and Krokene 1998; Masuya et al. 2003; Lieutier et al. 2004), and has been shown to be positively correlated with the ability of pathogenic fungi to cause tree death (Lieutier et al. 2004). Samples of sapwood were removed aseptically from the inoculation points, lesions, and from beyond visible lesions and were surface sterilized and plated onto MEA. Fungi were recovered and identified using morphological characters, including colony and conidial morphology.

Statistical analyses

Differences in fungal lesion lengths were examined by two analyses of variance (ANOVA). The first analysis used a general linear model consisting of a three-way comparison of the main effects: tree species, presence or absence of dwarf mistletoe, and treatment. Treatments were *G. clavigera*, *O. montium*, agar plug, and the empty control. The second analysis, evaluating each specific fungal isolate instead of the pooled “fungal species”, was conducted in a similar manner. 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 ($N =$

216, $W = 0.97$) for fungal species pooled and for each fungal isolate separately ($N = 216$, $W = 0.96$). Two sets of analyses were performed; all lesion lengths were included in the first set, but agar controls were eliminated from the second set. All analyses were performed using SAS (SAS Institute Inc., Cary, North Carolina).

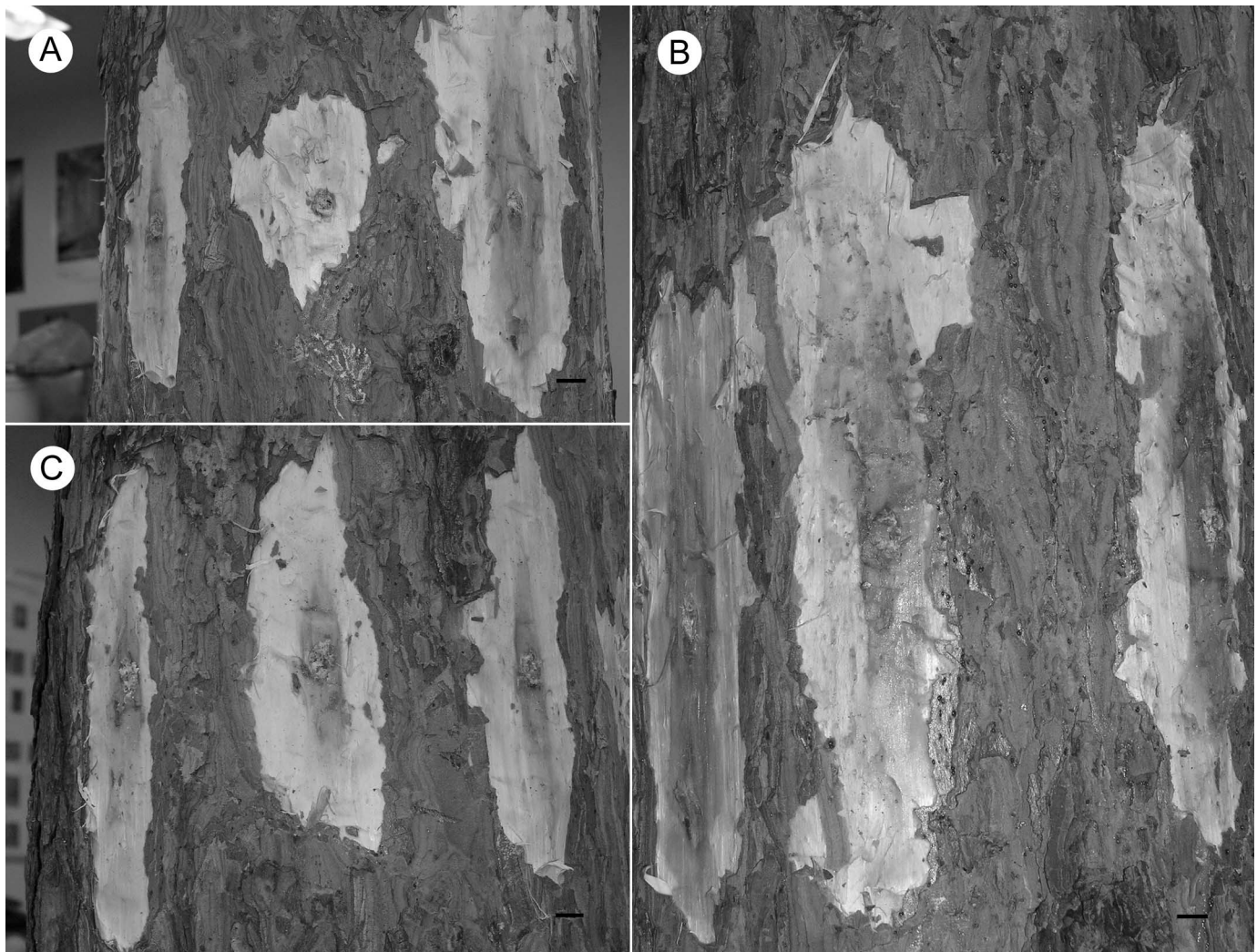
Results

After 6 weeks, necrotic lesions were visible on the sapwood (Fig. 1) around most of the inoculation points. Both species were recovered from their respective inoculation points and lesions but not from beyond the visible lesions. *Grosmannia clavigera* was recovered from lesions around 11 of the agar controls but not from the empty holes or from the *O. montium* inoculations. Lesions ranged from absent on most empty control points to more than 200 mm long. Among the controls, lesion lengths were longer around those inoculated with an agar plug than those that did not receive any kind of inoculum ($p < 0.0001$). A large (75 mm) lesion was observed around a single empty control hole. In this instance, an unidentified sterile white fungus was recovered from the lesion and inoculation point. On average, lesions produced by both fungi were longer than those caused by drilling holes alone (Fig. 2A; $p < 0.0001$) but were not always longer than agar controls. The inclusion of agar controls in the statistical analyses did not significantly affect the results, and only the results from the first set of analyses are shown (agar controls included).

Mean lesion lengths differed among the three tree species ($p \leq 0.0009$), the four treatments ($p < 0.0001$), and the eight “isolates” (three each of *O. clavigera* and *O. montium* and the two controls) ($p < 0.0001$) (Fig. 2). Lesion length on each tree species was affected significantly by the treatment ($p = 0.0031$) and the “isolate” ($p = 0.0386$). Mistletoe did not have a significant effect on lesion length, alone ($p \geq 0.4149$) or in combination with tree species ($p \geq 0.8816$), treatment ($p = 0.1106$), or isolate ($p = 0.3996$) (Table 1).

On average, *G. clavigera* caused longer lesions (107 ± 5 mm) than *O. montium* (79 ± 4 mm) when lesion lengths on all three hosts were pooled ($p < 0.0001$) (Fig. 2A). The differences were statistically significant only on hybrid pines ($p < 0.0001$), where the lesion lengths differed by more than 50 mm. On lodgepole pine, lesions caused by *G. clavigera* (82 ± 4 mm) were longer than those caused by *O. montium* (63 ± 3 mm), but this difference was not statistically significant ($p = 0.5502$). On jack pine, the two species caused similar-sized lesions ($p = 0.9999$) that averaged about 110 ± 7 mm in length. Isolates of *G. clavigera* varied significantly ($p < 0.0001$) in their abilities to cause lesions, with NOF 2895 inducing shorter lesions (80 ± 8 mm) than NOF 2896 (116 ± 10 mm) and NOF 2894 (124 ± 8 mm) (Fig. 2B). Differences among the isolates were not statistically significant on lodgepole ($p \geq 0.8879$) or jack ($p \geq 0.8358$) pines. On hybrid pines, lesions induced by NOF 2895 were significantly shorter than those induced by NOF 2894 ($p = 0.0236$), but did not differ significantly from those induced by NOF 2896 ($p = 0.6675$). The other two isolates (NOF 2894 and 2896) did not differ significantly from each other ($p = 0.9822$). Lesions caused by the three isolates of *O. montium* did not differ significantly from each other ($p \geq$

Fig. 1. Lesions observed on the sapwood of a jack pine tree. (A) Lesions caused by inoculation with (left to right) *Ophiostoma montium*, drilling alone, and *Grosmannia clavigera*. (B) Lesions caused by three isolates of *G. clavigera*. (C) Lesions caused by three isolates of *O. montium*. Scale bars = 10 mm.



0.9580) and averaged 100–120 mm in length on jack pine and 55–75 mm on the other two tree species.

Lesion lengths differed among the host species (Fig. 2C). On average, lesions were longest on jack pine (96 ± 6 mm), followed by hybrids (85 ± 7 mm), with the shortest lesions on lodgepole pine (64 ± 3 mm). The differences between jack and hybrids were not statistically significant ($p \geq 0.1622$), whereas differences between lodgepole pine and each of the other two species were significant ($p \leq 0.0454$).

The two fungal species differed in the relative length of the lesions they induced on each of the host trees. *Grosmannia clavigera* produced its longest lesions on hybrid pine (128 ± 12 mm), followed closely by jack pine (115 ± 9 mm), with the shortest lesions on lodgepole pine (82 ± 4 mm). Only the differences between lodgepole pine and the other two species were significant ($p \leq 0.0335$). *Ophiostoma montium* induced much longer lesions on jack pine (108 ± 8 mm) than on either lodgepole pine (63 ± 3 mm) or hybrid pine (68 ± 5 mm). Only the differences between jack pine and the other two species were statistically significant ($p \leq 0.0115$).

Discussion

Two blue-stain fungi associated with MPB, *G. clavigera* and *O. montium*, grew and caused lesions in the phloem and sapwood of jack pine, lodgepole pine, and their hybrids in northern Alberta. Whereas lodgepole pine is the most common host of the beetle in western Canada and is known to be susceptible to both fungi (e.g., Strobel and Sugawara 1986; Yamaoka et al. 1990, 1995; Solheim and Krokene 1998), the lesions on jack pine were significantly longer, suggesting that it is potentially more susceptible to these fungi. The introgression of the jack pine genome into lodgepole pine populations (i.e., hybrids) increases the potential susceptibility of the hybrids to these fungi, especially *G. clavigera*, compared with pure lodgepole pine stands. The successful growth of the fungi on jack and hybrid pines suggests that MPB success on these trees will not be limited by the capacity of associated fungi to colonize the host.

Differences in susceptibility to the fungi among the host species suggest that the dynamics of the MPB–host–fungus relationship will not be identical on each of the host species. The effect of fungal virulence on MPB success is unknown,

but two competing hypotheses are possible. One hypothesis suggests that benefit to the beetle is proportional to fungal virulence (i.e., more virulent fungi provide more benefit), because virulent fungi aid in overwhelming host defences. If this hypothesis is true, then the high susceptibility of jack pine to both *G. clavigera* and *O. montium* indicates that this pine species is at particular risk from MPB and its associated fungi. A converse hypothesis suggests that benefit to the beetle is inversely proportional to fungal virulence (i.e., less virulent fungi provide more benefit), either because virulent fungi compete with the beetle for tree resources or because of direct antagonism. In this case, the consequences of greater susceptibility of jack pine to *G. clavigera* and *O. montium* compared with lodgepole pine are more complex. If fungal growth and virulence reduces beetle output on a susceptible tree and damage caused by the beetles is greater than that caused by the fungi, increased susceptibility to the fungi could provide protection from beetle damage and thus benefit the tree. It is probable that both hypotheses are true, with virulent fungi benefiting the beetles in early stages of colonization but then inhibiting them after establishment (e.g., Paine et al. 1997; Klepzig et al. 2001a, 2001b; Kopper et al. 2004). Additionally, both hypotheses ignore the potential nutritional roles of the fungi. In any case, the greater susceptibility of jack and hybrid pines to MPB-associated fungi will likely alter the balance of the beetle–tree–fungus interactions that have co-evolved on lodgepole pine and other traditional hosts.

The lodgepole pine stand inoculated in this study had never experienced MPB infestation prior to this study, yet these trees were still less susceptible to the fungi than the tested jack and hybrid pines. This observation suggests that there is some innate resistance to the fungi in lodgepole pine. It also suggests that this resistance has not evolved in the closely phylogenetically related jack pine, and that the resistance may be reduced by hybridization.

Previous inoculation studies on co-evolved hosts have indicated that *G. clavigera* is consistently more virulent than *O. montium* (Reid et al. 1967; Owen et al. 1987; Yamaoka et al. 1990; Solheim and Krokene 1998), although these differences were not significant in shore pine (*Pinus contorta* var. *contorta* Douglas) (Solheim and Krokene 1998). While we observed this pattern in lodgepole pine, the co-evolved host, and the hybrid hosts, we did not find significant differences between the fungi on the novel host, jack pine. These observations lend further support to the idea that interactions among the beetle, host, and fungi will likely be altered on jack pine compared with lodgepole pine, and that patterns observed on co-evolved hosts may not be generally applicable. These results highlight the need for additional studies comparing beetle, fungal, and host dynamics on all affected tree species.

Most previous inoculation studies using these fungi (Reid et al. 1967; Shrimpton 1973; Strobel and Sugawara 1986; Yamaoka et al. 1990, 1995) have used a single isolate of each fungal species and, thus, intraspecific variability in virulence could not be estimated or assessed. Such variability is common in related fungi (e.g., Lieutier et al. 2004), and is likely common in MPB-associated species given their broad geographical and host ranges. The three isolates of *G. clavigera* used in our study varied significantly in viru-

lence, with one isolate (NOF 2895, from Banff National Park) inducing consistently shorter lesions on all host species than the other two isolates (one from Banff and one from Kelowna, BC). This pattern contrasts with that observed by Solheim and Krokene (1998), who found that the two isolates of *G. clavigera* used in their study did not differ significantly in their effects on host trees. Like Solheim and Krokene (1998), we found little intraspecific variation in the virulence of *O. montium*. Owen et al. (1987) used multiple isolates of both *G. clavigera* and *O. montium* in their inoculations of ponderosa pine seedlings but do not report intraspecific variation. It could be suggested that some of the observed variation in virulence is due to the cultures having been stored in collections for different amounts of time. Virulence usually decreases with storage time and thus, we would expect the older collections to be the least virulent ones, but this was not the case. This discrepancy suggests that the variation in virulence is real and is not an artefact of the experimental design. The small number of isolates used in both studies undoubtedly underestimates the true amount of intraspecific variation in virulence in these species, and a broader selection of isolates needs to be tested, including isolates from beetle populations that are most likely to spread into the boreal forest. This observed intraspecific variation highlights the difficulties inherent in formulating generalized models of behaviour, as models developed using one fungal isolate may not be applicable to situations involving other isolates of the same species. Variation in host resistance also likely exists within and between pine stands, and this variation must be quantified before host susceptibility can be generalized across the entire range of jack pine.

In contrast with the differences in response to fungal inoculation observed among the tree species, the responses to empty control holes were similar among the tree species; drilling caused few or no lesions on any tree species. Contamination of the agar plugs with *G. clavigera* at the time of inoculation was a problem. Some *G. clavigera* conidia survived the sterilization of the tools used to inoculate the trees and were able to become established on the agar plugs, and thus infect the tree, but were unable to become established in the empty holes or in the presence of other fungi. This assertion is supported by the fact that *G. clavigera* was not recovered from around the empty controls or *O. montium* inoculations. More effective sterilization procedures should be used for future inoculation studies. The fact that the presumably much lower-density inoculum inadvertently introduced to the agar control sites produced lesions as long as some holes that received much larger amounts of inoculum further indicates the high potency of *G. clavigera*.

The low-density inoculations used in this study may not accurately assess host response to the high-density inoculations that would arise from a beetle mass attack, but they likely represent a more accurate model of the early stages of beetle introductions into the forests, whereby a few beetles may initially attack a single tree. They also enable investigation of an individual host tree's response to colonization by different species and different isolates of the same species that would be much more difficult to elucidate from higher inoculum-density studies.

The current infestation of many of Alberta's boreal pine

Fig. 2. Lengths of lesions caused by inoculation with *G. clavigera*, *O. montium*, agar plugs, and drilling alone (control). Central points indicate means, the ticks on the error bars indicate standard errors, and maximum and minimum observations. Different letters indicate that means are significantly different. (A) Length of lesions caused by *G. clavigera*, *O. montium*, and controls. Data for the three tree species and individual isolates of each fungus are pooled ($n = 27$ for each control and $n = 81$ for each fungus). (B) Lengths of lesions caused by the fungal isolates and controls. Data for the three tree species are pooled ($n = 27$). (C) Lengths of lesions on the three pine species. Data for the two fungal species and controls are pooled ($n = 80$ for lodgepole pine, $n = 72$ for jack pine, and $n = 64$ for hybrid pine).

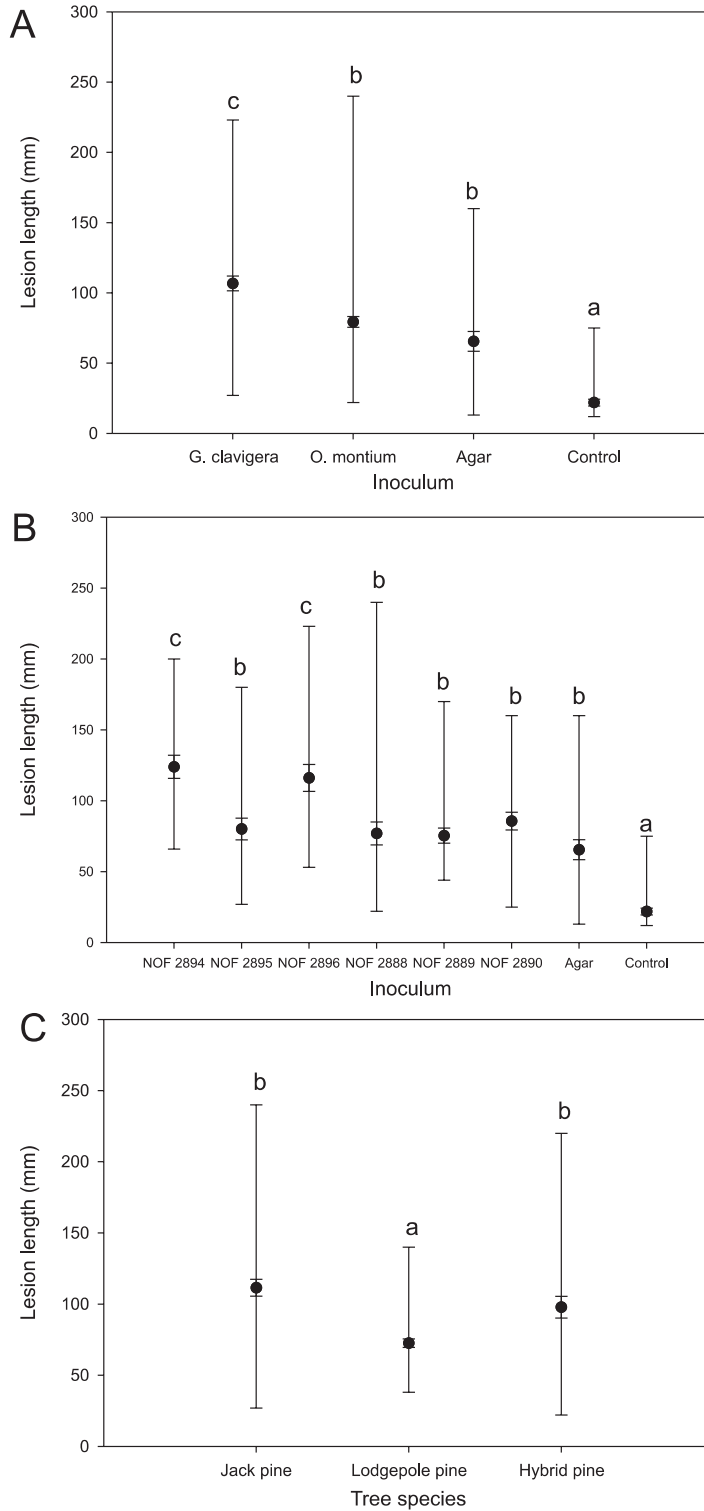


Table 1. Results of analysis of variance (ANOVA) of lesion lengths for different treatments.

Factor	df	ANOVA 1		ANOVA 2	
		F value	p value	F value	p value
Tree species	2	7.32	0.0009	16.72	<0.0001
Mistletoe	1	0.00	0.9908	0.67	0.4149
Tree sp. × mistletoe	2	0.13	0.8816	0.03	0.9744
Treatment	3	41.26	<0.0001	–	–
Tree sp. × treatment	6	3.42	0.0031	–	–
Mistletoe × treatment	3	2.03	0.1106	–	–
Tree sp. × mistletoe × treatment	6	0.57	0.7554	–	–
Isolates	7	–	–	22.41	<0.0001
Tree sp. × isolates	14	–	–	1.82	0.0386
Mistletoe × isolates	7	–	–	1.05	0.3996
Tree sp. × mistletoe × isolates	14	–	–	0.29	0.9947

Note: ANOVA 1 used tree species, mistletoe infection, and fungal species, and ANOVA 2 used tree species, mistletoe infection, and fungal isolates.

forests with dwarf mistletoe was seen as a possible predisposition for infection by mountain pine beetles and their associated fungi. We did not find significant differences in susceptibility of mistletoe-infected and uninfected trees of the three species, nor did we find significant differences in the virulence of individual fungal species or isolates on mistletoe-infected and uninfected trees. These results suggest that the effect of mistletoe infection on the success of MPB-associated fungi is likely limited. This observation is supported by the variable results of previous studies on lodgepole pine, some of which found positive correlations between mistletoe and MPB infestations and some of which found negative correlations (see Hawksworth et al. 1983). Notably, the extent of mistletoe infection in each stand and in each of the infected trees was not quantified. More work is needed to track the effects of varying levels of mistletoe infection on colonization by both the beetles and their fungi. Other forest pathogens may also predispose pines to MPB and its associated fungal pathogens. Additional research should relate fungal colonization and MPB attack with infection by other pathogens, including root diseases, which have been shown previously to influence bark beetle attack (see Paine and Baker 1993 for a review).

Recently, a new blue-stain fungus (*Leptographium longiclavatum* Lee, Kim and Breuil) was described from MPB in BC, where it was recovered at a low frequency (Lee et al. 2005). The role of this fungus in the beetle's lifecycle is unknown, as is its virulence to MPB host trees (Lee et al. 2005), but it has been suggested that it is ecologically similar to *G. claviger* and *O. montium*. We have recovered this species from MPB galleries from Banff National Park and the Wilmore Wilderness area (A.V. Rice, unpublished observation, 2005), where it occurred with a higher frequency than *G. claviger*. Its virulence to Alberta's boreal pine species should be assessed and compared with that of *G. claviger* and *O. montium*, given that it occurs at a high frequency in the MPB population in closest proximity to the boreal forests.

Our study has highlighted some ways to better direct future work. First, single stands were selected for each tree species, and it is impossible to gauge the strength of site-specific effects. In the current model, these effects are included in the term that also includes tree species. Given

that these tree species do not grow in the same locations, the effects of site can never be completely removed from those of tree species. However, future studies involving trees at multiple sites, representing a range of boreal conditions, would determine the potential importance of these effects and the general applicability of the results. Second, trees were inoculated in the summer and harvested in early fall when temperatures were moderate. The success of the fungi and the beetles in the trees is dependent on their abilities to overwinter in cold boreal conditions. The overwintering survivability of *G. claviger* and *O. montium* in jack and hybrid pines and in northern lodgepole pine stands is not known. Third, virulence is not the only fungal characteristic likely to influence their effect on the beetles. Other factors, such as nutritional quality and biochemical activity (including the production of pheromones and other volatiles), are likely to be important (Six and Klepzig 2004) and will probably vary among individual isolates and species of fungi and on different hosts. These characteristics should be explored, and the functional interactions among the beetles, fungi, and hosts should be examined under a variety of conditions. Fourth, the success of the fungi in jack pine trees does not guarantee the success of the beetles. Beetle success will depend on various factors, including phloem thickness (Amman 1972; Langor 1989), which may not influence the fungi. The potential of MPB to select, attack, and breed in jack pines must be determined. Ultimately, the success of the beetles in live jack pine and their threat to Canada's boreal pine forests can be determined only by the unfortunate invasion of this species into boreal forests.

Acknowledgements

Colette Breuil provided the isolates of *G. claviger* and *O. montium*. James Hammond, Daryl Williams, Michael Michaelian, and Lionel and Howard Petersen provided technical assistance. James Brandt assisted with site selection. Jan Volney and Ken Mallet provided comments on a draft of this manuscript. Funding was provided by Alberta Sustainable Resource Development and the Mountain Pine Beetle Initiative.

References

Adams, A.S., and Six, D.L. 2007. Temporal variation in myco-

- phagy and prevalence of fungi associated with developmental stages of *Dendroctonus ponderosae* (Coleoptera: Curculionidae). *Environ. Entomol.* **36**: 64–72.
- Amman, G.D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *J. Econ. Entomol.* **65**: 138–140.
- Basham, H.G. 1970. Wilt of loblolly pine inoculated with blue-stain fungi of the genus *Ceratocystis*. *Phytopathology*, **60**: 750–754.
- Brandt, J.P., Hiratsuka, Y., and Pluth, D.J. 2005. Germination, penetration, and infection by *Arceuthobium americanum* on *Pinus banksiana*. *Can. J. For. Res.* **35**: 1914–1930. doi:10.1139/x05-113.
- British Columbia Forest Service. 2005. 2004 Summary of Forest Health Conditions in British Columbia. Available from www.for.gov.bc.ca/hfp/health/overview/2004.htm [accessed January 2006].
- Carroll, A.L., Taylor, S.W., Régnière, J., and Safranyik, L. 2003. Effects of climate change on range expansion by the mountain pine beetle in British Columbia. In *Mountain Pine Beetle Symposium: Challenges and Solutions*. 30 and 31 October 2003, Kelowna, British Columbia. Edited by T.L. Shore, J.E. Brooks, and J.E. Stone. *Can. For. Serv. Pac. For. Cent. Inf. Rep. BC-X-399*. pp. 223–232.
- Cerezke, H.F. 1995. Egg gallery, brood production, and adult characteristics of mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in three pine hosts. *Can. Entomol.* **127**: 955–965.
- Furniss, M.M., and Schenk, J.A. 1969. Sustained natural infestation by the mountain pine beetle in seven new *Pinus* and *Picea* hosts. *J. Econ. Entomol.* **62**: 518–519.
- Harrington, T.C. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. In *Insect-fungal associations ecology and evolution*. Edited by F.E. Vega and M. Blackwell. Oxford University Press, New York. pp. 257–291.
- Hawksworth, F.G., Lister, C.K., and Cahill, D.B. 1983. Phloem thickness in lodgepole pine: its relationship to dwarf mistletoe and mountain pine beetle (Coleoptera: Scolytidae). *Environ. Entomol.* **12**: 1447–1448.
- Huber, D.P.W., and Borden, J.H. 2001. Protection of lodgepole pines from mass attack by mountain pine beetle, *Dendroctonus ponderosae*, with nonhost angiosperm volatiles and verbenone. *Entomol. Exp. Appl.* **92**: 131–141.
- Kim, J.-J., Allen, E.A., Humble, L.M., and Breuil, C. 2005. Ophiostomatoid and basidiomycetous fungi associated with green, red, and grey lodgepole pines after mountain pine beetle (*Dendroctonus ponderosae*) infestation. *Can. J. For. Res.* **35**: 274–284. doi:10.1139/x04-178.
- Klepzig, K.D., and Wilkens, R.T. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. *Appl. Environ. Microbiol.* **63**: 621–627. PMID:16535518.
- Klepzig, K.D., Moser, J.C., Lombardero, M.J., Hofstetter, R.W., and Ayres, M.P. 2001a. Symbiosis and competition: complex interactions among beetles, fungi and mites. *Symbiosis*, **30**: 83–96.
- Klepzig, K.D., Moser, J.C., Lombardero, M.J., Ayres, M.P., Hofstetter, R.W., and Walkinshaw, C.J. 2001b. Mutualism and antagonism: ecological interactions among bark beetles, mites, and fungi. In *Biotic interactions in plant-pathogen associations*. Edited by M.J. Jeger and N.J. Spence. CAB International, Cambridge, Mass. pp. 237–267.
- Kopper, B.J., Klepzig, K.D., and Raffa, K.F. 2004. Components of antagonism and mutualism in *Ips pini*-fungal interactions: relationship to a life history of colonizing highly stressed and dead trees. *Environ. Entomol.* **33**: 28–34.
- Langor, D.W. 1989. Host effects on the phenology, development, and mortality of field populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *Can. Entomol.* **121**: 149–157.
- Lee, S., Kim, J.-J., and Breuil, C. 2005. *Leptographium longiclavatum* sp. nov., a new species associated with the mountain pine beetle, *Dendroctonus ponderosae*. *Mycol. Res.* **109**: 1162–1170. doi:10.1017/S0953756205003588. PMID:16279410.
- Lieutier, F., Yart, A., Ye, H., Sauvard, D., and Gallois, V. 2004. Variations in growth and virulence of *Leptographium wingfieldii* Morelet, a fungus associated with the bark beetle *Tomicus piniperda* L. *Ann. For. Sci.* **61**: 45–53. doi:10.1051/forest:2003083.
- Lim, Y.W., Kim, J.-J., Lu, M., and Breuil, C. 2005. Determining fungal diversity on *Dendroctonus ponderosae* and *Ips pini* affecting lodgepole pine using cultural and molecular methods. *Fungal Divers.* **19**: 79–94.
- Masuya, H., Kaneko, S., and Yamaoka, Y. 2003. Comparative virulence of blue-stain fungi isolated from Japanese red pine. *J. For. Res. (Harbin)*, **8**: 83–88.
- Mathre, D.E. 1964. Survey of *Ceratocystis* spp. associated with bark beetles in California. *Contrib. Boyce Thompson Inst.* **22**: 353–362.
- Ono, H. 2003. The mountain pine beetle: scope of the problem and key issues in Alberta. In *Mountain Pine Beetle Symposium: Challenges and Solutions*. 30 and 31 October 2003, Kelowna, British Columbia. Edited by T.L. Shore, J.E. Brooks, and J.E. Stone. *Can. For. Serv. Pac. For. Cent. Inf. Rep. BC-X-399*. pp. 62–66.
- Owen, D.R., Lindahl, K.Q., Jr., Wood, D.L., and Parmeter, J.R., Jr. 1987. Pathogenicity of fungi isolated from *Dendroctonus valens*, *D. brevicornis*, and *D. ponderosae* to *Ponderosa* pine seedlings. *Phytopathology*, **77**: 631–636.
- Paine, T.D., and Baker, F.A. 1993. Abiotic and biotic predisposition. In *Beetle-pathogen interactions in conifer forests*. Edited by T.D. Schowalter and G.M. Filip. Academic Press Inc., San Diego, Calif. pp. 61–79.
- Paine, T.D., Raffa, K.F., and Harrington, T.C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annu. Rev. Entomol.* **42**: 179–206. doi:10.1146/annurev.ento.42.1.179. PMID:15012312.
- Raffa, K.F., and Berryman, A.A. 1983. Physiological aspects of lodgepole pine wound response to a fungal symbiont of the mountain pine beetle *Dendroctonus ponderosae*. *Can. Entomol.* **115**: 723–734.
- 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**: 1115–1126.
- Safranyik, L., and Linton, D.A. 1982. Survival and development of mountain pine beetle broods in jack pine bolts from Ontario. *Can. For. Serv. Res. Note* **2**. pp. 17–18.
- Safranyik, L., Shrimpton, D.M., and Whitney, H.S. 1974. Management of lodgepole pine to reduce losses from the mountain pine beetle. *Can. For. Serv. For. Tech. Rep. No. 1*.
- Shrimpton, D.M. 1973. Age- and size-related response of lodgepole pine to inoculation with *Europhium clavigerum*. *Can. J. Bot.* **51**: 1155–1160.
- Six, D.L. 2003a. A comparison of mycangial and phoretic fungi of individual mountain pine beetles. *Can. J. For. Res.* **33**: 1331–1334. doi:10.1139/x03-047.
- Six, D.L. 2003b. Bark beetle-fungus symbioses. In *Insect symbiosis. Contemporary topics in entomology series*. Edited by T.A. Miller. CRC Press, Boca Raton, Fla. pp. 97–114.
- Six, D.L., and Klepzig, K.D. 2004. *Dendroctonus* bark beetles as model systems for studies in symbiosis. *Symbiosis*, **37**: 207–232.
- Six, D.L., and Paine, T.D. 1998. Effects of mycangial fungi and host tree species on progeny survival and emergence of *Den-*

- droctonus ponderosae* (Coleoptera: Scolytidae). Environ. Entomol. **27**: 1393–1401.
- 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 mountain pine beetle associated blue-stain fungi, *Ophiostoma clavigerum* and *Ophiostoma montium*. Can. J. Bot. **76**: 561–566. doi:10.1139/cjb-76-4-561.
- Strobel, G.A., and Sugawara, F. 1986. The pathogenicity of *Ceratocystis montia* to lodgepole pine. Can. J. Bot. **64**: 113–116.
- Tsuneda, A., and Hiratsuka, Y. 1984. Sympodial and annelidic condiation in *Ceratocystis clavigera*. Can. J. Bot. **62**: 2618–2624.
- Yamaoka, Y., Swanson, R.H., and Hiratsuka, Y. 1990. Inoculation of lodgepole pine with four blue-stain fungi associated with mountain pine beetle, monitored by a heat pulse velocity (HPV) instrument. Can. J. For. Res. **20**: 31–36.
- 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.