

Mountain pine beetle-associated blue-stain fungi in lodgepole × jack pine hybrids near Grande Prairie, Alberta (Canada)

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Summary

The mountain pine beetle (MPB), the most serious pest of lodgepole pine in mountainous western Canada, spread northeastward into lodgepole × jack pine hybrids in the boreal forest of Alberta in 2006. The MPB vectors three species of blue-stain fungi, which contribute to the success of the beetles. These fungi were isolated from MPB larvae and galleries in several lodgepole × jack pine stands in the Grande Prairie region of northwestern Alberta in autumn 2006 and winter and spring 2007. Fungi were recovered from more than 95% of gallery systems. The three fungi were similarly prevalent but *Ophiostoma montium* was the most frequently isolated fungus at each sampling point, isolated from 72% to 90% of gallery systems compared with 63% to 78% for *Grosmannia clavigera*, and 61% to 86% for *Leptographium longiclavatum*. *Ophiostoma montium* and *G. clavigera* were isolated from more larvae than gallery samples, with the opposite true for *L. longiclavatum*. Most gallery systems contained multiple fungi with three fungi per gallery system being more common in autumn and winter and two fungi more common in the spring. The combination of *G. clavigera* and *L. longiclavatum* was less common among gallery systems with two fungi than either of the pairwise combinations containing *O. montium*. Fungal prevalence was the same above and below snow level. The prevalence of the three fungi did not differ significantly among stands sampled in the spring but stands with more *G. clavigera* tended to have less *L. longiclavatum*. The winter of 2006–2007 was colder than average throughout Alberta with temperatures below –30°C in November, January and February, and all three fungi were present after the cold winter while most larvae had died, suggesting that overwintering mortality of the fungi will not limit persistence and spread of MPB in the boreal forest.

1 Introduction

The mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins) is the most serious pest of lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelman) in western Canada (SAFRANYIK et al. 1974; CARROLL et al. 2003; ONO 2003). The MPB is polyphagous on almost all native and introduced pines throughout its range (AMMAN and COLE 1983). During the current outbreak, the MPB has killed millions of lodgepole pines in British Columbia, and it is estimated that 80% of the merchantable pine in that province will be dead by 2017 (BRITISH COLUMBIA MINISTRY OF FORESTS and RANGE 2008). In 2006, the MPB spread north-eastward into populations of lodgepole pine and lodgepole × jack pine hybrids in the boreal forest of Alberta, including an outbreak near Grande Prairie (ALBERTA SUSTAINABLE RESOURCE DEVELOPMENT 2008). The hybrid pine populations intergrade with populations of jack pine (*Pinus banksiana* Lambert), which extend throughout the boreal forest to the east coast of Canada. The spread of MPB eastward into jack pine forests could be ecologically, economically and socially disastrous for Canada (ONO 2003).

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Three blue-stain fungi, *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield [\equiv *Ophiostoma clavigerum* (Robinson-Jeffrey and Davidson) Harrington], *Ophiostoma montium* (Rumbold) von Arx and *Leptographium longiclavatum* Lee, Kim and Breuil, are associated with the MPB (e.g. RUMBOLD 1941; ROBINSON 1962; WHITNEY and FARRIS 1970; SIX 2003a; LEE et al. 2005). The precise roles of these fungi are uncertain, but their relationship with the MPB is considered mutualistic (SIX and PAINE 1998; SIX 2003b; ADAMS and SIX 2007; BLEIKER and SIX 2007; SIX and BENTZ 2007) because the beetles are unable to reproduce in the absence of the fungi (SIX and PAINE 1998). The fungi provide nutritional benefits throughout the life cycle of the beetle (SIX and PAINE 1998; ADAMS and SIX 2007; BLEIKER and SIX 2007) and may help the beetles overwhelm secondary host defences (RAFFA and BERRYMAN 1983) by reducing sap flow (MILLER et al. 1986; YAMAOKA et al. 1990). The fungi differ in temperature optima and tolerance (SOLHEIM and KROKENE 1998; LEE et al. 2005; SIX and BENTZ 2007; RICE et al. 2008) and MPB seem to benefit from carrying multiple fungi (SIX and BENTZ 2007), possibly by reducing their risk of being left without a fungal partner at extreme temperatures (ADAMS and SIX 2007; SIX and BENTZ 2007). In exchange, the beetles provide the fungi with reliable transportation between host trees and access to the phloem and sapwood tissues (SIX 2003b).

Several factors will determine the persistence and spread of the MPB in Canada's boreal forests, including the capacity of the MPB and associated fungi to colonize jack pine successfully. Artificial rearing experiments (SAFRANYIK and LINTON 1982; CERZKE 1995; D. W. Langor, unpublished data) and MPB-killed arboretum trees (FURNISS and SCHENK 1969) indicate that jack pine is a suitable host for the beetle. All three MPB-associated blue-stain fungi can colonize jack pine and appear to grow better in that species than in lodgepole pine (RICE et al. 2007a,b). Therefore, the eastward spread of MPB into jack pine forests is unlikely to be inhibited by host incompatibility.

Climate is also an important determinant of persistence and spread of MPB in boreal forests. The MPB and associated fungi must be able to survive cold winters to persist in the boreal region. Unseasonably cold temperatures caused marked declines in MPB populations in southern Alberta in 1984–1985 (see ONO 2003) and in northwestern Alberta in 2006–2007 (D. Lux, pers. comm., 2007) and 2007–2008 (A. V. Rice, unpublished data). The winter of 2006–2007 was colder and snowier than the most recent 30-year average across Alberta. At Grande Prairie, temperatures were below normal for every month except December and January (Fig. 1; ENVIRONMENT CANADA 2008) and plunged below -30°C for several days in late November and again in late January and early February, snow cover in the region was above normal for the entire winter (Fig. 2; ENVIRONMENT CANADA 2008). *In vitro* assessments (RICE et al. 2008) indicate that the three blue-stain fungi differ in cold tolerance: *G. clavigera* and *L. longiclavatum* survived at -20°C but *O. montium* died. To determine whether the interspecific differences in cold tolerance observed in the three MPB-associated fungi under laboratory conditions are evident under field conditions, fungi were isolated from MPB larvae and galleries during the autumn, winter and spring of 2006–2007 to determine whether these fungi persisted through the winter in MPB-attacked trees.

2 Materials and methods

Fungal species composition was assessed in MPB-infested lodgepole \times jack pine hybrid trees in several stands in the Grande Prairie (55.17°N , 118.80°W) region of northwestern Alberta. MPB attacked these stands in the summer of 2006, following a mass dispersal of beetles from infested lodgepole pine in adjacent areas of British Columbia (ALBERTA SUSTAINABLE RESOURCE DEVELOPMENT 2008). This marked the first reported time that MPB invaded lodgepole \times jack pine hybrids. MPB-infested trees were sampled for blue-stain fungi three times in 2006–2007: autumn (late October to early November 2006),

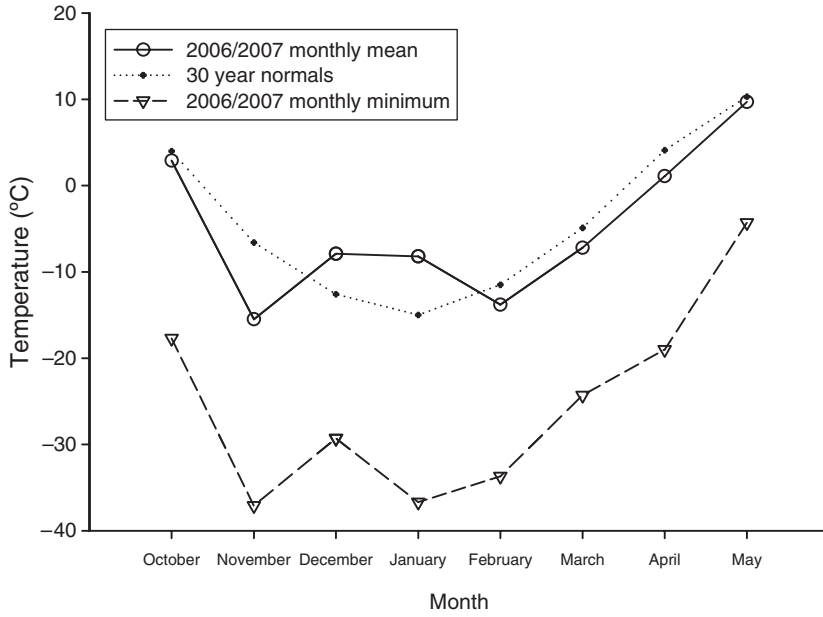


Fig. 1. Mean monthly and minimum temperatures recorded at Grande Prairie, AB, for October 2006–May 2007 and the 30-year normal mean monthly temperatures for October–May. Data from ENVIRONMENT CANADA (2008).

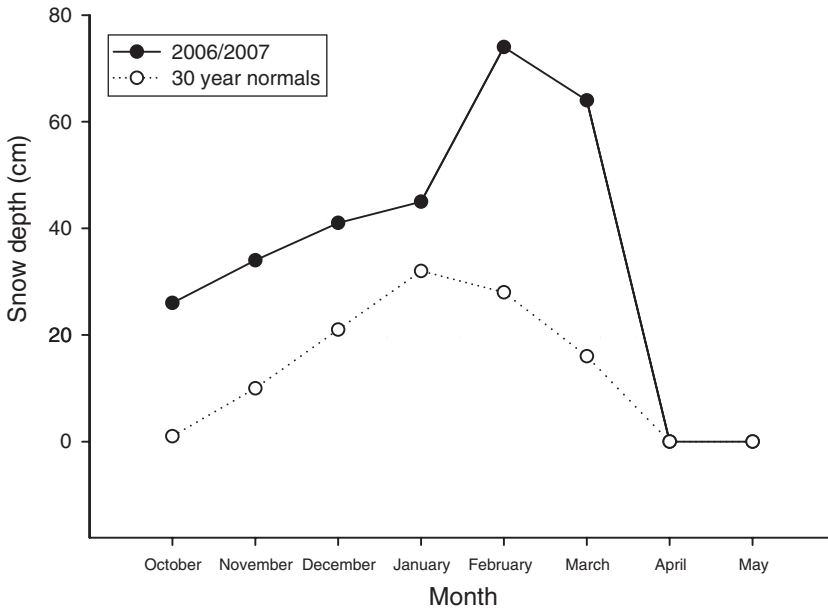


Fig. 2. Snow depth at Grande Prairie on the last day of each month from October 2006 to May 2007 and the 30-year normal. Data from ENVIRONMENT CANADA (2008).

winter (late January 2007) and spring (late March 2007) as part of existing MPB research studies. The sites, trees and samples from which the fungi were isolated differed for each sampling period. However, all samples were collected from the same geographic area, and the farthest distance between any two points was <50 km. Recent extensive surveys of MPB-associated blue-stain fungi throughout Alberta and eastern British Columbia (A. V. Rice, unpublished data) indicate relatively low variability in the relative occurrence of each fungal species within the region sampled in this study. Therefore, we have confidence that, although different stands and trees were sampled at each sampling point (autumn, winter, spring), the differences observed among the sampling points reflect temporal more so than geographic variation.

In the autumn period, samples consisting of bolts (about 30 cm long) were cut from the top, bottom and middle of the infested bole of each of nine trees. Bolts were transported to the laboratory and processed under aseptic conditions. Not all bolts contained MPB galleries. Two to three gallery systems (set of larval galleries originating from the same egg gallery) were sampled from each bolt that contained galleries (at least 120° apart around the circumference of the bolt). A total of 38 gallery systems were sampled, including 13 parental galleries without lateral larval galleries.

In late January ('winter'), samples were collected from 19 trees (above and below snow level). Four discs (10 cm in diameter and about 3 cm thick), containing intact phloem and xylem, were cut from each height (above and below snow) on each tree at right angles from one another using a 10-cm hole-saw and a chisel. Samples were cut from above the entrance holes of adult MPB to maximize gallery sampling, placed in zippered plastic bags and transported to the laboratory on ice. In spite of this step, not all discs contained larval galleries. Discs without larval galleries were discarded (B. Cooke, pers. comm., 2007). A single gallery system was sampled for each disc for a total of 82 gallery systems sampled including 19 that did not contain live or intact cold-killed larvae.

In late March ('spring'), samples were collected from 30 trees (10 trees from each of three stands) using the same protocol as the winter period. Four discs were collected at each of two heights on each tree (eight discs per tree): 0.5–1.0 and 1.3–1.6 m above the ground level. Only gallery systems with live or cold-killed larvae were sampled. In total, 54, 52 and 69 gallery systems were sampled from stands 1, 2 and 3 respectively.

When the bolts and discs arrived in the lab, they were stored at 3–5°C until processed (within 1 week). Outer bark was removed to expose adult and lateral larval galleries. Larvae were removed from the galleries using flame sterilized forceps and each larva was placed in a Petri plate containing 1.5% malt extract agar [MEA; 15 g traditional dark dry malt extract (Briess Malting, Chilton, WI, USA), 15 g Bacto agar (Becton, Dickinson and Company, Sparks, MD, USA), 1 l dH₂O]. Sapwood and/or phloem samples ('wood samples'; about 5 mm²) were removed from the margins of the larval galleries (adjacent to the larva) using flame-sterilized scalpels and forceps. The samples were surface-sterilized by flaming and plated on MEA. Fungi were subcultured and identified as they appeared. Blue-stain fungi were identified to morphological species based on cultural and microscopic morphology.

Only gallery systems from which fungi were isolated were used in the analyses. The prevalence of each fungus was presented as the percentage of gallery systems containing each species at each sampling time. Gallery systems were said to contain a species if it was isolated from larvae, wood samples or both. Results were analysed using a series of chi-square tests on the count data to compare: (i) the prevalence of the three fungi at each sampling period; (ii) the prevalence of each fungus at the three sampling points; (iii) the prevalence of each fungus on the two substrates (larvae, wood) at each sampling point; (iv) the prevalence of each fungus above and below snow in the winter samples; (v) the prevalence of each fungus in the three stands sampled in the spring; (vi) the number of gallery systems containing one, two or three fungi at each sampling point; and (vii) the

prevalence of each pairwise combination of species at each sampling point. A single test was used for each comparison at each sampling time.

3 Results

Blue-stain fungi were isolated from all 38 gallery systems sampled in the autumn, including wood samples from 36 (95%) and larvae from 23 (92%) gallery systems. In the winter samples, fungi were isolated from 79 (96%) gallery systems and 62 (98%) larval samples. These samples included 37 and 42 gallery systems from above and below the snow cover respectively. In the spring, fungi were isolated from 53 (98%), 51 (98%) and 69 (100%) gallery systems from stands 1, 2 and 3 respectively. Fungi were isolated from 46 wood samples (85%) and 51 larval samples (94%) in stand 1, 49 wood and larval samples (94%) in stand 2, and 68 wood and larval samples (99%) in stand 3.

The three fungi did not differ significantly in prevalence at any of the sampling points (Fig. 3a). However, *O. montium* was isolated from more gallery systems at each sampling period than the other two fungi. It was isolated from 90% of gallery systems that contained fungi in autumn compared with 66% for each of *G. clavigera* and *L. longiclavatum*. Among gallery systems with fungi that were sampled in the winter, *O. montium* was isolated from 90%, *L. longiclavatum* from 86% and *G. clavigera* from 78%. In the spring, *O. montium* was isolated from 72% of gallery systems that contained fungi, *G. clavigera* from 63% and *L. longiclavatum* from 61%. None of the three fungi differed significantly in prevalence among the sampling points ($p > 0.05$).

The fungi differed in prevalence on the two substrates (larvae and wood) (Fig. 3b), although significant differences among the fungal species were observed only on larvae and only in the spring ($p = 0.0013$) and when all sampling dates were pooled ($p < 0.0001$). Overall, *Ophiostoma montium* was isolated from more larval samples (72%) than either *L. longiclavatum* (43%) or *G. clavigera* (62%) while *L. longiclavatum* was isolated from more wood samples (63%) than *G. clavigera* (52%) or *O. montium* (61%). *Ophiostoma montium* was isolated more frequently from larval samples than from wood samples at all three sampling points, although the differences were only significant in the spring ($p = 0.019$). Although *G. clavigera* was isolated from a higher percentage of larval samples than wood samples at each sampling period, the differences were not significant ($p > 0.05$). The opposite pattern was observed for *L. longiclavatum*, which was isolated from significantly more wood samples than larvae in both the winter ($p = 0.0251$) and spring ($p = 0.0104$) samples and when all sampling dates were pooled ($p = 0.0017$). The percentage of wood samples occupied by both *L. longiclavatum* ($p = 0.0265$) and *O. montium* ($p = 0.0006$) differed among the three sampling points while no significant differences were observed among the sampling points in the prevalence of *G. clavigera* on wood or any of the three fungi from larvae ($p > 0.05$).

In winter, fungi were recovered from 37 galleries (29 with live or cold-killed larvae) sampled from above the snow cover and from 42 galleries (34 with live or cold-killed larvae) below the snow cover. There were no significant differences ($p > 0.05$) in the prevalence of any of the fungal species between gallery systems, larvae, or wood sampled from above and below the snow.

The three fungi differed in prevalence by up to 21% of gallery systems among the three stands sampled in March, although none of the differences were significant ($p > 0.05$). *Ophiostoma montium* was isolated from 64% to 80% of gallery systems (72% overall), *G. clavigera* was isolated from 57% to 72% (63% overall) and *L. longiclavatum* from 49% to 70% (61% overall). Differences in prevalence of the three species were not significant in any of the stands ($p > 0.05$). Spatial trends in relative prevalence of the three fungi were consistent for larval and wood samples: *L. longiclavatum* was more common in stand 3 and *O. montium* in stand 2 than in the other stands. Stands where *G. clavigera* was more

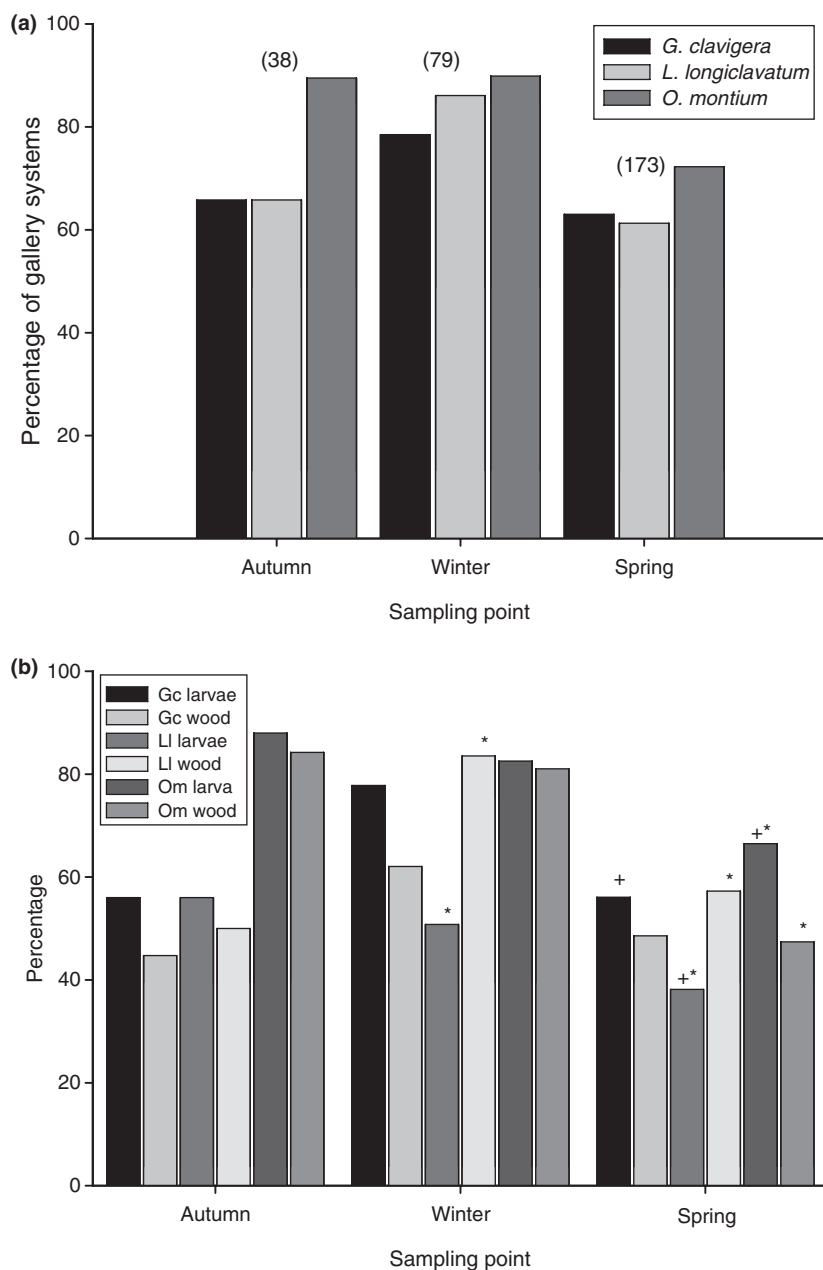


Fig. 3. Percentage of samples from which each of *Grosmannia clavigera*, *Leptographium longiclavatum* and *Ophiostoma montium* was isolated: (a) Gallery system [larvae, sapwood/phloem ('wood') from gallery walls or both]. Observed differences were not significant ($p > 0.05$). (b) Percentage of larvae and wood samples from which each fungus was isolated. Asterisks indicate significant differences among substrates while '+' indicates significant differences among fungal species.

common generally had less *L. longiclavatum* associated with gallery systems. *Grosmannia clavigera* and *O. montium* were isolated from more larval samples than wood samples in each stand with the converse true for *L. longiclavatum*, although the only significant differences were for *O. montium* in stand 1 ($p = 0.0356$).

Most gallery systems (75–93%) contained more than one fungal species (Fig. 4). In autumn and winter, it was more common to have three fungal species per gallery system (Fig. 4), although the difference was significant only in the winter ($p < 0.0001$), while in the spring it was more common to have two fungal species per gallery system ($p < 0.0001$). The proportions of gallery systems with one, two and three species differed among the sampling dates ($p < 0.05$). The proportion of gallery systems with three fungi was the lowest in the spring ($p < 0.0001$) whereas the proportions with one ($p = 0.0244$) and two ($p = 0.0250$) species were the highest in the spring (Fig. 4). The percentages of samples occupied by one, two or three fungi was consistent among substrates at each timing point ($p > 0.05$) but differed among sampling dates. There was no significant difference in the proportion of wood or larval samples occupied by two fungi, and it ranged from 40% to 50% at all sampling points and was generally the most common situation. Three fungi per larval or wood sample was more common in the winter (30–40%) than in the spring (6–10%) while the opposite was true for one fungal species per sample (12–18% in winter and 40–43% in spring) ($p < 0.001$). In autumn, there were no significant differences in the number of fungal species isolated from either wood or larval samples ($p > 0.05$).

Among gallery systems containing two fungi, not all pairwise combinations occurred with the same frequency (Fig. 5). The combination of *G. clavigera* and *L. longiclavatum* was the least common at all sampling points, although the difference was significant only in

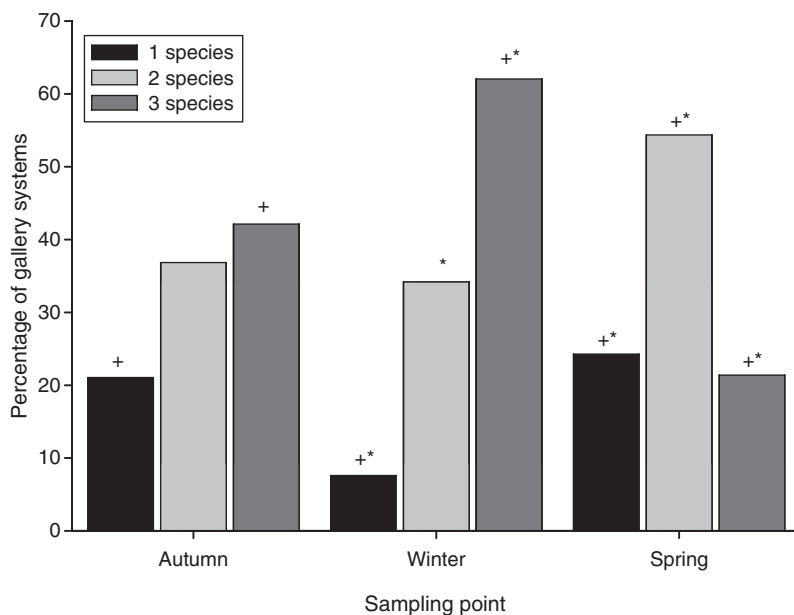


Fig. 4. Percentage of gallery systems (larvae, sapwood/phloem ['wood'] or both) from which one, two or all three species of mountain pine beetle-associated blue stain fungi were isolated. Asterisks indicate statistically significant differences within sampling points while '+' indicates significant differences among sampling points.

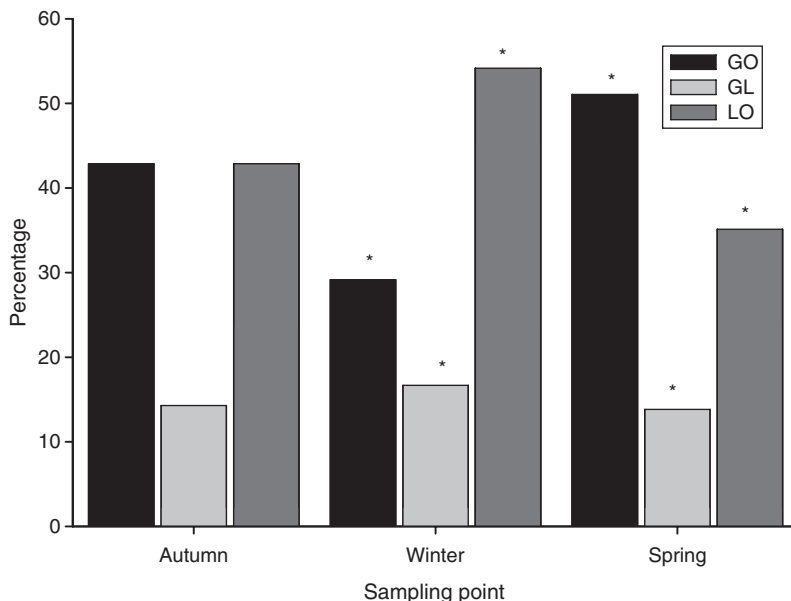


Fig. 5. Percentage of gallery systems [larvae, sapwood/phloem ('wood') or both] with two fungi from which each pairwise combination of blue stain species was isolated. Combinations are *Grosmannia clavigera* and *Ophiostoma montium* (GO), *G. clavigera* and *Leptographium longiclavatum* (GL), and *L. longiclavatum* and *O. montium* (LO). Asterisks indicate statistically significant changes within sampling points and no significant differences were observed among sampling points.

the spring ($p < 0.0001$). The other two species combinations were equally common in the autumn but the combination of *L. longiclavatum* and *O. montium* was more common in the winter and the combination of *G. clavigera* and *O. montium* was more common in the spring. Only the combination of *G. clavigera* and *O. montium* differed significantly in prevalence among the three sampling points ($p = 0.0121$). The combination of *G. clavigera* and *O. montium* was the most common on larvae in the winter ($p = 0.0005$) and spring ($p < 0.0001$), but the three combinations did not differ significantly in prevalence on larvae in the autumn ($p > 0.05$). There was no significant difference in the prevalence of any combination on larvae among the sampling points ($p > 0.05$). On wood samples, the combination of *L. longiclavatum* and *O. montium* was most common in the winter ($p = 0.0020$) while there was no significant difference in the autumn or spring ($p > 0.05$). Only the combination of *L. longiclavatum* and *O. montium* differed in prevalence from wood samples among the sampling points ($p = 0.0404$) and it was the highest in the winter. The combination of *G. clavigera* and *O. montium* was higher on larvae than wood in both winter ($p = 0.0023$) and spring ($p = 0.0097$).

4 Discussion

Blue-stain fungi were isolated from almost all gallery systems sampled at each sampling point. *Ophiostoma montium* was the most frequently isolated species but all three species were common, recovered from at least 60% of gallery systems at each sampling point and no statistically significant differences in overall prevalence were observed among the three species. No statistically significant differences in the prevalence of any of the fungi were

observed among the sampling dates but two interesting trends emerged. *Ophiostoma montium* was isolated from a smaller percentage of the gallery systems sampled in the spring than in the autumn and winter while *G. clavigera* and *L. longiclavatum* were isolated from a higher percentage of gallery systems sampled in the winter than in the spring. Overall, each fungus was isolated from at least 40% of sampled larvae and at least 50% of phloem/sapwood samples taken from the walls of the galleries. The fungi differed in overall prevalence from larval samples, with *O. montium* being isolated the most frequently and *L. longiclavatum* the least. This pattern was observed at each of the three sampling points, but was only significant in the spring samples and when all samples were pooled. No significant differences were observed in the prevalence of the three fungi from wood samples, although *G. clavigera* was isolated slightly less frequently than the other two species at each sampling point. In autumn, all three fungi were recovered from more larvae than wood samples, although this difference was not statistically significant for any of the three species. This trend was repeated at the other sampling dates for *O. montium* and *G. clavigera* but was statistically significant only for *O. montium* in the spring samples. Conversely, *L. longiclavatum* was recovered from significantly more wood samples than larvae in both the winter and spring samples.

The prevalence of *L. longiclavatum* in our samples is similar to that observed in other areas of northwestern Alberta (RICE et al. 2008; A. V. Rice, unpublished data) but in marked contrast with that observed in the USA and British Columbia (LEE et al. 2005; ADAMS and SIX 2007; SIX and BENTZ 2007; BLEIKER and SIX 2008). In the northern USA, *L. longiclavatum* has been isolated from MPB mycangia (LEE et al. 2005) but is absent to rare in many beetle populations and is considered an incidental associate of the beetle (SIX and BENTZ 2007; BLEIKER and SIX 2008; D. Six, pers. comm.). In British Columbia, *L. longiclavatum* may be more common than in the USA but still much less prevalent than *G. clavigera* and *O. montium* (LEE et al. 2005; C. Breuil, pers. comm.) but it is still isolated rarely from the surface of the MPB (LEE et al. 2005). Temperature has been proposed as a determinant of the temporal distribution of MPB-associated fungi (ADAMS and SIX 2007; SIX and BENTZ 2007) and RICE et al. (2008) suggested that it could also influence spatial distribution and be responsible for the apparent north-south divide in prevalence on *L. longiclavatum*. It is also possible that *L. longiclavatum* is only associated with a small percentage of adult MPB in northern MPB populations but that is able to grow faster at cool temperatures allows it to colonize more phloem than *G. clavigera* and *O. montium* in northern environments. This hypothesis would explain the higher prevalence of *L. longiclavatum* on gallery samples than larvae observed in the winter and spring samples but it does not explain all of our results. If this hypothesis was true, we would expect *L. longiclavatum* to be rare on live adults and larvae. Autumn was the only sampling period where all of the sampled larvae were alive and live adults were available for sampling. Autumn was the only sampling point at which *L. longiclavatum* was more prevalent in larvae than wood samples and was recovered from the same percentage of larvae as *G. clavigera*. Eight live adults were sampled in the autumn (data not shown) and *L. longiclavatum* was isolated from six of them while *O. montium* was isolated from four and *G. clavigera* from three. This suggests that *L. longiclavatum* is associated with a greater percentage of MPB in northern Alberta populations than in southern populations.

The role of *L. longiclavatum* in association with MPB has not been tested but its phylogenetic (LEE et al. 2005), morphological (LEE et al. 2005) and physiological (LEE et al. 2006; RICE et al. 2007b, 2008) similarities with *G. clavigera* suggest that it could occupy a similar niche and that the relationship between MPB and *L. longiclavatum* could be functionally similar to that between *G. clavigera* and MPB. The relationship between MPB and *G. clavigera* has been shown in a number of studies to be mutually beneficial (SIX and PAINE 1998; SIX 2003b; ADAMS and SIX 2007; BLEIKER and SIX 2007; SIX and BENTZ 2007). The similarity between *L. longiclavatum* and *G. clavigera* also suggests a higher

degree of niche overlap between these two species than that hypothesized for *G. clavigera* and *O. montium* (BLEIKER and SIX 2007; SIX and BENTZ 2007). Given the apparent similarity of *L. longiclavatum* to *G. clavigera*, it is probable that carrying both *L. longiclavatum* and *O. montium* conveys similar benefit to carrying *G. clavigera* and *O. montium*, but that carrying only *G. clavigera* and *L. longiclavatum* is less beneficial. This hypothesis is supported by the scarcity of *G. clavigera* and *L. longiclavatum* in pairwise combination, the similar prevalence of *G. clavigera/O. montium* and *L. longiclavatum/O. montium* combinations, as well as the inverse relationship in abundance of *G. clavigera* and *L. longiclavatum* among stands sampled in the spring. The predominance of galleries containing all three fungi in the autumn and winter suggests a further advantage to the MPB of carrying all three fungi, indicating that *G. clavigera* and *L. longiclavatum* may provide complimentary benefits to the beetle similar to those hypothesized for *G. clavigera* and *O. montium* (BLEIKER and SIX 2007, 2008).

Stand-specific differences in prevalence of *O. montium* could explain the lower prevalence of *O. montium* in spring compared with autumn and winter but it could also reflect the mortality of *O. montium* over the winter months. BLEIKER and SIX (2008) found that the percentage of phloem colonized by *O. montium* declined over the year following attack and attributed the reduction to fungal death. Given the inability of *O. montium* to survive *in vitro* freezing at -20°C for even short periods of time (RICE et al. 2008), a decrease in prevalence was expected. Instead *O. montium* remained relatively prevalent after several cold periods with temperatures below -35°C for several days (90% in winter and 72% in spring). The discrepancy between expected and realized survival could be explained by the unreliability of *in vitro* temperature tolerance as a model for *in situ* survival or by intraspecific variability in cold tolerance. Support for the latter explanation is evidenced by the survival on agar at -20°C of several strains of *O. montium* isolated in March (A. V. Rice, unpublished data). Less cold-tolerant strains, such as those studied by RICE et al. (2008) could have been killed by the cold temperatures, explaining any differences that were not explained by stand-specific effects. These results suggest that cold winters will not kill off all strains of *O. montium* but could rapidly select for cold-hardy strains. RICE et al. (2008) hypothesized that extreme boreal temperatures might provide a barrier to MPB persistence by leaving the MPB without a fungal partner if winters were too cold for *O. montium* and summers too hot for the other species. Our results show that this scenario is unlikely given that cold-tolerant *O. montium* strains persisted in most gallery systems and these isolates can grow at 30°C (A. V. Rice, unpublished data), indicating that some strains of *O. montium* have the potential to survive the full range of temperatures experienced in the boreal region.

Stand-specific effects could account for most of the differences in prevalence of *G. clavigera* and *L. longiclavatum* among the sampling periods, given the variability in prevalence of these fungi among stands sampled in the spring. However, stand-specific effects might not be the only explanation. An inverse relationship was observed between *G. clavigera* and *L. longiclavatum* among stands sampled in the spring, with stands in which *L. longiclavatum* was more prevalent having less *G. clavigera*. As such, we would expect higher prevalence of one species but not both. BLEIKER and SIX (2008) reported an increase in the percentage of phloem samples colonized by *G. clavigera* over the winter, which they attribute to the ability of *G. clavigera* to cooler temperatures and colonize more phloem than *O. montium* (BLEIKER and SIX 2008). Since *G. clavigera* and *L. longiclavatum* have similar cold tolerances, both of these species would likely be able to colonize more phloem and sapwood when daytime temperatures exceeded 0°C , which they did in December and January (ENVIRONMENT CANADA 2008). *Leptographium longiclavatum* grows faster than *G. clavigera* at temperatures $<15^{\circ}\text{C}$ (RICE et al. 2008), enabling it to colonize even more resources than *G. clavigera*, and explaining why it has an even higher prevalence than *G. clavigera* in the winter samples.

More than one fungus was recovered from most of the gallery systems sampled at each sampling point. In both autumn and winter, three fungi per gallery system were most common and one fungus least common, although the difference was significant only in the winter. In spring, two fungi per gallery system were most common and three fungi per gallery system least common. The apparent decrease in the percentage of gallery systems with all three fungi could be due to stand-specific differences but more likely represents a true reduction in live fungi within trees. A similar decrease was observed by BLEIKER and SIX (2008) in the number of galleries occupied by both *G. clavigera* and *O. montium* a year after MPB attack. BLEIKER and SIX (2008) suggest that this reduction is likely due to changes in tree chemistry and could have implications for beetle development and nutrition. In our samples, the reduction in the number of gallery systems containing all three fungi seems to be a consequence of reduced prevalence of *O. montium* in gallery samples and *L. longiclavatum* in larval samples, suggesting that factors other than tree chemistry could play a role. If a reduction in live fungi can be expected over the course of a year, starting with three fungi may increase the likelihood that at least one fungus is still present in late spring when the beetle is completing development.

Snow cover is considered an important determinant of MPB survival in cold winters as heavy snow provides insulation that improves the odds of beetle survival. Unlike larval survival in the winter samples, which was higher in the gallery systems sampled from under the snow than from those sampled above the snow (B. Cooke, unpublished data), fungal prevalence did not differ between above- and below-snow gallery systems. In the Grande Prairie region, larval mortality was >90% over the winter of 2006–2007 (B. Cooke, unpublished data) while any reduction in the fungal prevalence was slight. Given that the winter of 2006–2007 was colder than the recent average, all three MPB-associated blue-stain fungi are likely to survive most boreal winters and beetle cold tolerance, rather than fungal cold tolerance, is more likely to provide a barrier to MPB persistence and spread throughout the boreal forest.

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