For. Path. 38 (2008) 113–123 doi: 10.1111/j.1439-0329.2007.00525.x

Mountain pine beetle-associated blue-stain fungi are differentially adapted to boreal temperatures

By A. V. Rice[1,3], M. N. Thormann[2] and D. W. Langor[1]

[1]Canadian Forest Service, Natural Resources Canada, Northern Forestry Centre, 5320-122 Street, Edmonton, AB T6H 3S5 Canada; [2]1925 Tomlinson Way, Edmonton, AB T6R 2R6 Canada; [3]E-mail: arice@nrcan.gc.ca (for correspondence)

Summary

Mountain pine beetles (MPB) are the most serious pest of lodgepole pine in Canada and are likely to invade boreal jack pine forests. MPB vector three blue-stain fungi, *Grosmannia clavigera*, *Ophiostoma montium* and *Leptographium longiclavatum*, which contribute to beetle success. Fungal survival at extreme boreal temperatures will contribute to their success in jack pine. Growth, sporulation and survival of the three fungi at −20 to 37°C were tested *in vitro*. Overwintering survival of *G. clavigera* and *O. montium* was assessed *in vivo*. All species grew at 5–30°C, with optimal growth at 20–25°C. *Grosmannia clavigera* and *L. longiclavatum* survived at −20°C, but *O. montium* died. Growth of *G. clavigera* and *L. longiclavatum* was inhibited at 30°C, but *O. montium* grew well. *Grosmannia clavigera* and *O. montium* overwintered in living pines. These results suggest that *G. clavigera* and *L. longiclavatum* were adapted to cold boreal winters but not hot summers, with the converse true for *O. montium*. Temperature tolerance varied among *G. clavigera* isolates. British Columbian and Californian isolates grew faster at 25°C than Albertan isolates. Isolates from Alberta and Idaho/Montana grew optimally at 20°C, while British Columbian and Californian isolates grew optimally at 25°C.

1 Introduction

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is currently the most serious pest of lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelmann) in western Canada (e.g. Safranyik et al. 1974; Carroll et al. 2003; Ono 2003). It also attacks other native and non-native pines found in the mountains of western Canada and USA (Amman and Cole 1983). In British Columbia (BC), the MPB has killed millions of lodgepole pines annually over the past several years (Huber and Borden 2001; Carroll et al. 2003). In 2005, more than 8.5 million hectares of lodgepole pine forests were affected (British Columbia Ministry of Forests and Range 2006) an increase of more than one million hectares since 2004 (British Columbia Ministry of Forests and Range 2005). The beetle is currently spreading eastward into Alberta (AB) (Ono 2003). Historically, there have been two recorded MPB outbreaks in AB (1940–1943, 1977–1985), both restricted to the southern regions of the province (Cerezke 1995; Ono 2003). In 1997, the MPB was observed in the Willmore Wilderness Area in west-central AB, a northerly increase in the beetle's range of more than 2° latitude since 1985 (Ono 2003). In BC, the species is distributed even farther north, including a large and expanding outbreak on the east side of the Rocky Mountains (Carroll et al. 2003; Ono 2003). Global warming is predicted to hasten this range expansion (Carroll et al. 2003).

In 2006, the beetle spread north-eastward into populations of lodgepole × jack pine hybrids, a spread of about 3° latitude northward and 5° longitude eastward since 1997.

Received: 16.2.2007; accepted: 20.6.2007; editor: T. Sieber

These hybrid populations intergrade with boreal populations of jack pine (*Pinus banksiana* Lambert), which extend across Canada to the east coast. The spread of MPB into boreal jack pine forests could be ecologically, economically and socially disastrous for Canada (Ono 2003). The ability of the MPB to attack and kill natural jack pine populations is unknown, but evidence suggests that this pine species is a suitable host for MPB. Artificial rearing experiments (Safranyik and Linton 1982; Cerezke 1995; Langor, unpublished data, 2006) indicated that MPB can survive and reproduce in cut sections of jack pine, and that survival and productivity were comparable to that within cut sections of lodgepole pine (Cerezke 1995). Furthermore, MPB attacked and killed a small number of 51-year-old jack pines in an arboretum in Idaho, although it is unknown whether the beetles successfully reproduced in these trees (Furniss and Schenk 1969).

Three blue-stain ascomycetes, *Grosmannia clavigera* (Robinson-Jeffery and Davidson) Zipfel, de Beer and Wingfield [≡ *Ophiostoma clavigerum* (Robinson-Jeffery and Davidson) Harrrington], *Ophiostoma montium* (Rumbold) von Arx and *Leptographium longiclavatum* Lee, Kim and Breuil, are associated with the MPB (Rumbold 1941; Robinson 1962; Whitney and Farris 1970; Six 2003; Kim et al. 2005; Lee et al. 2005; Lim et al. 2005). The precise role of these fungi is not known, but they are thought to help the beetle overwhelm the defences of the host trees (Raffa and Berryman 1983) and provide necessary nutrition throughout the lifecycle of the beetles (Six and Paine 1998; Adams and Six 2007). The successful colonization of jack pine by the MPB-associated blue-stain fungi is considered to be one factor required for successful attack by MPB. All three fungi caused longer lesions on jack pine and lodgepole × jack pine hybrids than on lodgepole pine, indicating that fungal growth will not be a barrier to beetle colonization of these trees (Rice et al. 2007a).

The ability to grow and cause lesions in boreal pines is only one factor determining the suitability of these trees as hosts for the MPB and its associated fungi. The fungi and beetles must also survive and reproduce under boreal climatic conditions. Rice et al. (2007a,b) inoculated trees in late summer and harvested them in early autumn, thus, the fungi were not subjected to the seasonal temperature extremes that natural populations of the fungi would be required to survive. Therefore, it was necessary to determine the temperature tolerances of MPB-associated fungi as an indicator of potential pre-adaptation to life as boreal pathogens. Previous research indicated that two of the three fungi, *G. clavigera* and *O. montium*, differ in temperature tolerance. *Ophiostoma montium* grows faster at warmer temperatures than *G. clavigera*, which grows faster at cooler temperatures (Six and Paine 1997; Solheim and Krokene 1998). As a result, the two species are associated with the beetles during different parts of the growing season in the southern part of their range (Adams and Six 2007). In this study, we assessed the temperature tolerances of all three species *in vitro* and determined the ability of the two most common symbionts, *G. clavigera* and *O. montium*, to overwinter in inoculated trees. *Grosmannia clavigera* is more aggressive than *O. montium* on most hosts (Reid et al. 1967; Owen et al. 1987; Yamaoka et al. 1990, 1995; Solheim 1995; Solheim and Krokene 1998) and has often been considered a more important symbiont of MPB (e.g. Reid et al. 1967; Shrimpton 1973; Raffa and Berryman 1983; Owen et al. 1987; Yamaoka et al. 1990, 1995; Six and Paine 1998; Solheim and Krokene 1998). As such, we hypothesized that isolates of this species vary in their cold tolerance as a result of adaptation to temperature differences, with isolates from the northern part of the range growing faster at cooler temperatures than those from southern parts of the range. Thus, we compared growth rates and survivability of isolates from BC, AB, and the USA and of isolates from the 1980s and current outbreak in BC and AB.

2 Materials and methods

2.1 Fungal isolates

All isolates are deposited as live cultures at the Northern Forestry Centre Culture Collection (NOF). The three isolates each of *G. clavigera* and *O. montium* used in the field study were obtained from Dr. Colette Breuil (University of British Columbia) and were isolated originally by S. Lee from the sapwood of MPB-infested lodgepole pines in Kamloops, BC, and Banff National Park, AB. The isolates of *G. clavigera* used in the *in vitro* temperature tolerance study came from across the ranges of these fungi in Canada and the USA and were obtained from Drs. Colette Breuil and Diana Six (University of Montana), the University of Alberta Microfungus Collection and Herbarium (UAMH), and from MPB-infested lodgepole pine sapwood samples collected from Banff National Park in 2004 and 2005 and the Willmore Wilderness area in 2005. The isolates of *O. montium* and *L. longiclavatum* were isolated from MPB-infested lodgepole pine sapwood collected from Banff National Park in 2004 and 2005 and the Willmore Wilderness Area in 2005. The provenance of the isolates is shown in Table 1.

2.2 Field study

Pine trees were inoculated with three isolates each of *G. clavigera* (NOF 2894, 2895, 2896) and *O. montium* (NOF 2888, 2889, 2890) at three sites across central Alberta (one inoculation point per isolate per tree). Inoculation points were arranged in a single ring at breast height. For each inoculation point, a hole (5 mm diameter) was drilled through the bark and phloem to the sapwood. A mycelial plug (5 mm diameter) was placed in the hole, and the hole was sealed with a sterile wood dowel and parafilm. Three lodgepole pine trees were inoculated near the Berland River (53°45.413′N, 118°20.297′W), two lodgepole × jack pine hybrids were inoculated northeast of Blue Ridge (54°13.125′N, 115°16.297′W), and two jack pines were inoculated near the Logan River (55°20.620′N, 111°55.143′W). Trees were harvested in May 2006, 9 months after inoculation. 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 48 h of harvesting. Nine 5-mm^2-samples of sapwood were removed aseptically at random positions from the visible lesions surrounding each inoculation point, surface sterilized by flaming and plated onto 2% malt extract agar [MEA; 10.0 g Difco malt extract (Difco Laboratories, Detroit, MI, USA), 7.5 g Bacto agar (Becton, Dickinson and Co., Sparks, MD, USA), 500 ml d-H_2O] to recover the fungi. Recovered fungi were identified using morphological characteristics including cultural and microscopic morphology. Lateral spread of the fungi in the sapwood was minimal, and fungi were not recovered from beyond visible lesions (Rice et al. 2007b). Therefore, isolates recovered from lesion were assumed to be the strains inoculated at those points.

2.3 *In vitro* temperature tolerance

Isolates of *G. clavigera* collected from BC, AB and the USA during the 1980s, 1990s and 2000s were used in the temperature tolerance tests (Table 1). One isolate, received as *G. clavigera*, was re-identified as *L. longiclavatum*. Isolates of *L. longiclavatum* and *O. montium* from infected wood collected from Banff National Park and the Willmore Wilderness area in 2005 were also used (Table 1). Three replicates of each isolate were grown from mycelial plugs (5–7 mm diameter) placed in the centre of Petri plates (90 mm diameter) containing MEA and incubated at −20, 5, 15, 20, 25, 30, 35 and 37°C (±1.5°C).

Table 1. **Provenance information for isolates of *Grosmannia clavigera*, *Leptographium longiclavatum*, and *Ophiostoma montium* used in the *in vitro* temperature tolerance experiments**

Species	*NOF no.*	*Host tree*	*Location*	*Collection date*
G. clavigera	837[2]	Lodgepole pine	Westcastle, AB	1983
G. clavigera	841[2]	Lodgepole pine	Belly River, AB	1983
G. clavigera	842[2]	Lodgepole pine	Carbondale, AB	1983
G. clavigera	843[3]	Lodgepole pine	Riske Creek, BC	1982
G. clavigera	846[2]	Lodgepole pine	Beaver Mines, AB	1983
G. clavigera	1280[4]	Lodgepole pine	Mt. Terry Fox, BC	1987
G. clavigera	2891[3]	Lodgepole pine	BC	1982
G. clavigera	2893[5]	Lodgepole pine	Blairmore, AB	1983
G. clavigera	2894[6]	Lodgepole pine	Banff National Park, AB	2003
G. clavigera	2895[6]	Lodgepole pine	Banff National Park, AB	2003
G. clavigera	2896[6]	Lodgepole pine	Kamloops, BC	2001
G. clavigera	2897[6]	Lodgepole pine	Banff National Park, AB	2004
G. clavigera	2898[6]	Lodgepole pine	Banff National Park, AB	2004
G. clavigera	2899[6]	Lodgepole pine	Manning Park, BC	2003
G. clavigera	2900[6]	Lodgepole pine	Manning Park, BC	2003
G. clavigera	2901[6]	Lodgepole pine	Manning Park, BC	2003
G. clavigera	2902[6]	Lodgepole pine	Manning Park, BC	2003
G. clavigera	2939[7]	Ponderosa pine	Heartbar, CA	1993
G. clavigera	2940[7]	Ponderosa pine	Goumaz, CA	1993
G. clavigera	2941[7]	Ponderosa pine	Burney, CA	1993
G. clavigera	2942[7]	Jeffrey pine[1]	Indiana Summit, CA	1993
G. clavigera	2943[7]	Ponderosa pine	Arrowbear, CA	1993
G. clavigera	2944[7]	Sugar pine	Children's National Forest, CA	1994
G. clavigera	2945[7]	Lodgepole pine	Cold Creek, MT	2003
G. clavigera	2946[7]	Lodgepole pine	Twelvemile Creek, MT	2003
G. clavigera	2947[7]	Lodgepole pine	Hellroaring, ID	2003
G. clavigera	2948[7]	Lodgepole pine	Willmore Wilderness Area, AB	2005
G. clavigera	2949[7]	Lodgepole pine	Willmore Wilderness Area, AB	2005
L. longiclavatum	2892[5]	Lodgepole pine	Blairmore, AB	1983
L. longiclavatum	2954[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005
L. longiclavatum	2955[9]	Lodgepole pine	Banff National Park, AB	2005
L. longiclavatum	2956[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005
L. longiclavatum	2957[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005
O. montium	2888[6]	Lodgepole pine	Banff National Park, AB	2003
O. montium	2889[6]	Lodgepole pine	Banff National Park, AB	2003
O. montium	2890[6]	Lodgepole pine	Kamloops, BC	2003
O. montium	2950[9]	Lodgepole pine	Banff National Park, AB	2005
O. montium	2951[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005
O. montium	2952[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005
O. montium	2953[8]	Lodgepole pine	Willmore Wilderness Area, AB	2005

NOF, Northern Forestry Centre Culture Collection.
[1]Isolated from Jeffrey pine beetle (*Dendroctonus jeffreyi* Hopkins).
Collectors/Isolators:
[2]A. Tsuneda and P. J. Maruyama
[3]H. S. Whitney
[4]Y. Hiratsuka, P. J. Maruyama and Y. Yamaoka
[5]L. Sigler
[6]S. Lee
[7]D. Six
[8]E. Lee/A. Rice
[9]J. Park/A. Rice

To measure growth at 5, 15, 20 and 25°C, two perpendicular measurements of colony diameter (subtracting the diameter of the plug) were recorded daily until the largest colonies had filled the Petri plate. Growth rate was calculated by dividing the average diameter of each colony by the number of days incubated. Only the growth rates from the final day of incubation were used in the statistical analyses. The date at first sporulation, as noted by the presence of either asexually or sexually produced spores, was noted for each isolate at each temperature.

Growth and survival at temperatures of 30°C and higher were also assessed. Plates inoculated with mycelial plugs were incubated immediately at 30, 35 and 37°C to assess the ability of isolates to become established at these temperatures. Cultures were monitored daily for growth. If colonies failed to become established after 2 weeks, the cultures were moved to room temperature to determine whether the mycelium was still viable. The ability of established colonies to grow at 30°C and warmer was determined by placing colonies that had grown at room temperature until they were at least 10 mm diameter in incubators at 30, 35 and 37°C and measuring growth until the largest colonies had filled the Petri plates. Growth rates were calculated for 30°C as outlined above.

To determine survivability at −20°C, isolates were grown on Petri plates of MEA at room temperature for 7 days before being incubated at −20°C. Subcultures were taken from the frozen cultures after 1, 2, 3 and 4 weeks and 2 and 3 months. Growth and sporulation of the subcultures were recorded.

2.4 Data analyses

The growth rates of the three species were compared at each temperature using one-way analysis of variance (ANOVA). Isolates of *G. clavigera* were compared at each temperature on the basis of location and date of collection using a series of one-way ANOVAs and general linear models (GLMs) consisting of comparison of the main effects: geographical origin (location) and collection date (date). For the one-way ANOVAs, locations were BC, AB, California (CA) and Idaho/Montana (IM). Isolations from BC and AB occurred in the 1980s and 2000s, and these isolates were compared by the GLMs. Main effects were compared using Tukey adjustment of the least squared means. Statistics were performed using SAS (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Field study

Lesions were observed around all inoculation points. Isolates of both *G. clavigera* and *O. montium* were recovered from these lesions in both tree species and their hybrids. Isolates of *G. clavigera* were recovered from 20 of 21 inoculation points, only NOF 2895 failed to survive in one inoculation point on a hybrid pine. Isolates of *O. montium* were recovered from 18 of 21 inoculation points; NOF 2890 failed to survive in one lesion on a jack pine tree, and NOF 2889 failed to survive in two lesions, one on a jack pine and one on a lodgepole pine.

3.2 *In vitro* temperature tolerance

On average, *G. clavigera* and *L. longiclavatum* grew optimally at 20°C, and *O. montium* grew optimally at 25°C (Fig. 1). Optimal growth of individual isolates ranged from 8 to 17 mm day^{-1} and occurred at temperatures ranging from 15 to 25°C for isolates of *G. clavigera*, 20–25°C for isolates of *L. longiclavatum*, and 25°C for *O. montium* isolates.

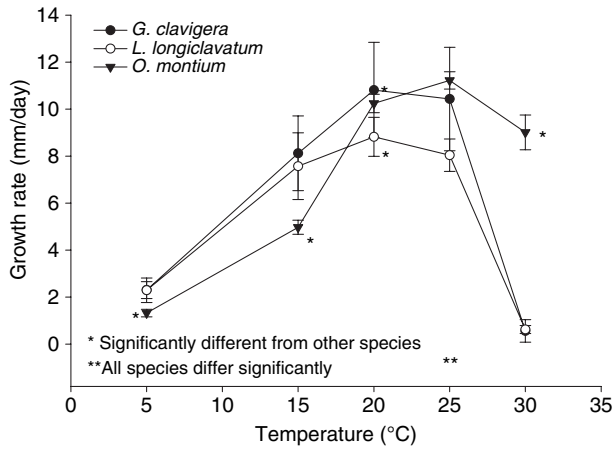

Fig. 1. Mean (±SE) growth rates of *Grosmannia clavigera*, *Leptographium longiclavatum*, and *Ophiostoma montium* at temperatures of 5–30°C

Growth of all species was inhibited at 5°C (Fig. 1). Growth of *G. clavigera* and *L. longiclavatum* was also inhibited at 30°C, but *O. montium* grew well at this temperature (Fig. 1). Isolates of *G. clavigera* and *L. longiclavatum* produced asexual spores at 5–25°C, but not at 30°C. In both species, sporulation was delayed at 5°C, occurring after 14–28 days of incubation. At the warmer temperatures, *L. longiclavatum* sporulated more quickly and more abundantly than *G. clavigera*. *Leptographium longiclavatum* sporulated at a similar rate at 15–25°C, with most isolates sporulating after 3 days of incubation. Isolates of *G. clavigera* began sporulating after 3–5 days of incubation at 20 and 25°C, but were delayed by 1–2 days at 15°C. None of the isolates of *O. montium* sporulated during the observed period at any of the temperatures tested.

Growth rates differed significantly among the species at 5, 15, 25 and 30°C, but not at 20°C. *Ophiostoma montium* grew more slowly at 5°C ($p \leq 0.0052$) and 15°C ($p \leq 0.0078$) than either *G. clavigera* or *L. longiclavatum* (Fig. 1). At 20°C, *L. longiclavatum* grew more slowly than either *G. clavigera* or *O. montium*, but the differences were significant ($p < 0.01$) only in pair-wise combinations (Fig. 1). All three species grew at different rates at 25°C ($p < 0.05$), with *L. longiclavatum* growing most slowly and *O. montium* most quickly (Fig. 1). At 30°C, *O. montium* grew much faster than *G. clavigera* or *L. longiclavatum* ($p < 0.0001$; Fig. 1), growing at almost 90% of its 20°C rate, while *G. clavigera* and *L. longiclavatum* grew at less than 10% of their 20°C rates.

The three species differed in their survival at −20°C. All isolates of *G. clavigera* survived 3 months at −20°C, but none of the *O. montium* isolates survived a week at this temperature. Isolates of *L. longiclavatum* varied in their tolerance of −20°C temperatures; NOF 2892 and 2956 each survived 1 month of freezing, and the other isolates survived 3 months. None of the isolates of any of the three species became established, grew, or survived at 35 or 37°C.

Isolates of *G. clavigera* from different locations differed in average growth rates at 5–20°C (Fig. 2), with isolates from IM growing faster at all three temperatures than isolates from the other three locations ($p < 0.05$). At 25°C, AB isolates grew more slowly than CA or BC isolates ($p < 0.05$). Growth rates did not differ at 30°C (Fig. 2). On average, isolates from BC and CA grew optimally at higher temperatures (25°C) than those from AB and IM (20°C) (Fig. 2).

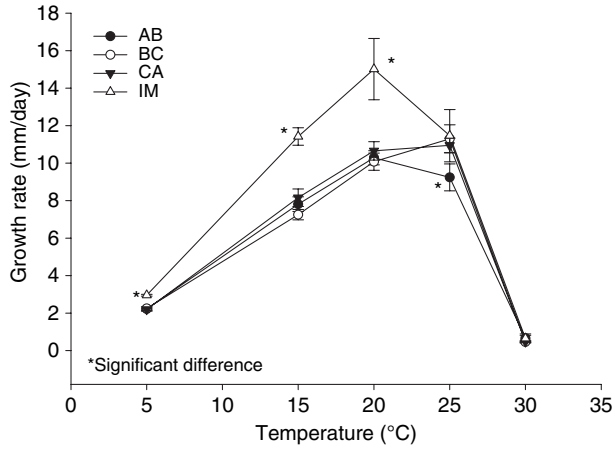

Fig. 2. Mean (±SE) growth rates of isolates of *Grosmannia clavigera* from Alberta (AB), British Columbia (BC), California (CA), and Idaho/Montana (IM) at temperatures of 5–30°C

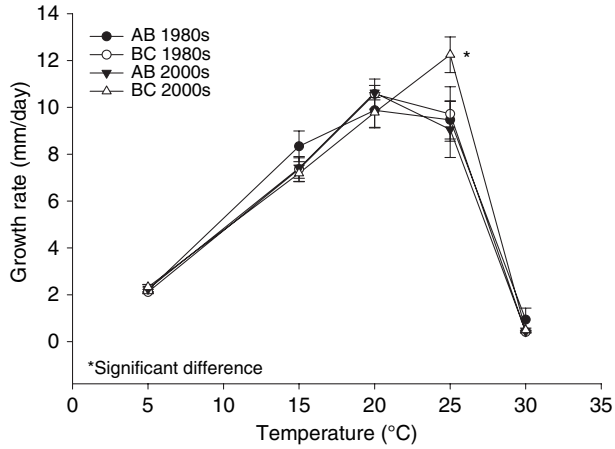

Fig. 3. Mean (±SE) growth rates of *Grosmannia clavigera* isolates collected from Alberta (AB) and British Columbia (BC) during the current (2000s) and previous (1980s) outbreaks at temperatures of 5–30°C

Isolates collected from AB in the current outbreak differed neither in growth rates from those collected during the previous (1980s) outbreak at any temperature, nor did the AB isolates differ from those collected in BC in the 1980s (Fig. 3). Isolates of *G. clavigera* collected from BC in the 2000s outbreak grew faster at 25°C than isolates collected from BC in the 1980s and isolates collected in AB during either the 1980s or current outbreak (Fig. 3), although only the differences with the AB isolates were significant ($p < 0.05$).

4 Discussion

MPB-associated blue-stain fungi differ in temperature tolerances (Six and Paine 1997; Solheim and Krokene 1998; Lee et al. 2005; Adams and Six 2007). These differences are

potentially important determinants of the spatial and temporal distribution of these fungi and may also influence the behaviour of the beetles (Adams and Six 2007). Existing temperature tolerance studies, this study included, have been conducted on isolates grown on Petri plates in the laboratory rather than on live trees, thereby potentially limiting the applicability of results to natural conditions. Notably, the ability of fungi to survive freezing in wood may be greater than their ability to survive the same temperatures on agar. Thus, while survival on frozen agar most likely indicates survival in wood, the converse may not correlate as well. However, the results of Adams and Six (2007) indicated that the temperature tolerances observed in the laboratory correlated well with patterns of fungal distribution observed in the field, suggesting that the *in vitro* results are applicable.

Ophiostoma montium grows better at higher temperatures than *G. clavigera* (Six and Paine 1997; Solheim and Krokene 1998). We found that the differences between the two species were most pronounced at the extreme temperatures (≤ 15°C and 30°C), with very little difference between the two species at 20–25°C. Previous research suggested that *L. longiclavatum* grows optimally at 25°C, and that its growth at 25°C is about half that of *G. clavigera* (Lee et al. 2005). However, we found that only one isolate grew optimally at 25°C, with the other four growing optimally at 20°C. We also found that while, on average, *L. longiclavatum* grew more slowly than *G. clavigera* at 20–25°C, the growth rates of the *L. longiclavatum* isolates were well within the range observed for *G. clavigera* isolates. At lower temperatures (5–15°C), the growth of most isolates of *L. longiclavatum* was similar to that of most *G. clavigera* isolates. The slight difference in temperature profiles for *L. longiclavatum* and *G. clavigera* may explain some of the observed spatial trends of *L. longiclavatum*. It has been reported at extremely low frequencies (<1% of galleries) from the southern portions of the MPB range (Lee et al. 2005), where average temperatures are higher, and its lower growth rate may prevent it from competing effectively with *G. clavigera*. We have recovered it at relatively high frequency (<50% of galleries) from the Willmore Wilderness Area near the north-eastern extreme of the MPB range, where average temperatures are 5–10°C cooler than in southern BC (Environment Canada 2007). In fact, *L. longiclavatum* was recovered more frequently than *G. clavigera* from the Willmore material, suggesting that when the growth rates are equal, other factors, such as preferential feeding by beetles, may influence the preponderance of the two species.

The different temperature tolerances between *O. montium* and *G. clavigera* are likely important determinants of the temporal distribution of these species in southern parts of their range, where temperatures range from −10°C to above 30°C. *Grosmannia clavigera* predominated in the spring and early summer in populations in Montana and was largely replaced by *O. montium* later in the season when temperatures increased (Adams and Six 2007). This pattern is likely to occur in other parts of the range where temperatures are similar. In northern parts of the range and in the boreal region where winter temperatures are lower, the temperature tolerances of the different species may determine the spatial as well as temporal distribution of the fungi. For example, if the inability of *O. montium* to survive −20°C carries over from agar to trees, it suggests that this species will have difficulty surviving cold boreal winters when temperatures routinely drop below −20°C for more than a week at a time. These difficulties will be most pronounced in regions or years when snow cover is reduced. The beetles and fungi overwinter in the trunks of attacked trees, and heavy snow cover provides insulation that improves the odds for beetle and fungal survival. Although *O. montium* survived the mild winter of 2005–2006 (temperatures 3–8°C above normal from November to January and about normal for February–March, and rarely below −20°C; Environment Canada 2007) in pines in northern AB, its survival in colder winters is not guaranteed. If *O. montium* is unable to survive boreal winters, it would have to be re-introduced each summer and may eventually be excluded from boreal MPB populations. Summer temperatures in the boreal region can

exceed 30°C for short periods, which might kill *G. clavigera* and *L. longiclavatum*, at least in trees exposed to direct sunlight. If the heat did not kill the beetles directly, it might still interrupt their lifecycle by killing the fungi. If *O. montium* had been eliminated from the population because of its failure to survive cold winters, a particularly hot summer might interrupt the lifecycle of the beetle by killing-off its blue-stain fungi at an important feeding time.

We hypothesized that isolates of *G. clavigera* from different parts of the MPB range would have different temperature tolerances, with isolates from the northern part of the range adapted to cooler temperatures and isolates from southern areas adapted to warmer temperatures. Although variations were observed, many were not well correlated with latitude; however, there was some evidence of possible adaptation. Isolates from both BC and CA, where temperatures are warmer than in AB, grew faster than AB isolates at 25°C. In addition, most AB isolates grew optimally at 20°C, with most of the rest growing at about the same rate at 20 and 25°C. The only isolate that grew optimally at 15°C was from AB. Meanwhile, all but one BC isolate and two CA isolates grew optimally at 25°C. Notably, the isolates from IM, where *G. clavigera* is largely replaced by *O. montium* at higher temperatures (Adams and Six 2007), grew faster at 5–20°C than isolates from any other location but levelled off to growth rates similar to the other isolates at 25–30°C. This suggests that these isolates may have evolved to maximize their growth at lower temperatures to fill a particular niche.

It was possible that time since collection might have influenced the temperature tolerance of these fungi. However, we did not find significant overall differences between the growth rates of isolates collected in the 1980s and 2000s in BC and AB that would suggest that time since collection has influenced this aspect of their biology. We did observe some differences among isolates from the 1980s and 2000s, but these are more likely to indicate adaptation to different temperature regimes than effects of remaining in culture collections. BC isolates collected in the 2000s grew significantly faster at 25°C than their AB counterparts and slightly faster than BC isolates collected in the 1980s. However, there were no differences at other temperatures, as would be expected if time since collection was the determining factor.

All three fungi can grow in boreal jack pine and produce lesions in the sapwood (Rice et al. 2007a, b), but that does not guarantee their success in the boreal region. The three species of blue-stain fungi associated with the MPB seem to be differentially adapted to boreal temperatures. *Grosmannia clavigera* and *L. longiclavatum* are better adapted to cold temperatures than *O. montium* and are thus more likely to survive cold boreal winters. As such, they are more likely to become established in boreal and northern MPB populations, whereas cold winters could limit or exclude *O. montium*. *Grosmannia clavigera* and *L. longiclavatum* are not well adapted to hot summer temperatures, with extremely reduced growth and survival at 30°C. Thus, hot summer temperatures could interrupt the growth of these fungi. Assuming that *O. montium* had already been excluded from the population, this could result in a period when none of the blue-stain fungi were active or in the death of the fungi. MPBs apparently need to feed on fungi to breed (Six and Paine 1998; Adams and Six 2007). If a period of the year when temperatures were not appropriate for any of their fungi corresponded with an important feeding period, the lifecycle of the beetle could be interrupted. These results suggest that the boreal environment with hot summer temperatures and cold winter temperatures could provide a challenge for the fungi and the beetles that depend on them. This challenge could be ameliorated, however, if global warming causes winter temperatures to increase to the point where *O. montium* can overwinter, as was the case in 2005–2006, when mortality in *O. montium* was less than 15%. With the advancement of MPB into boreal regions of AB in 2006, we will be in a position to test these hypotheses *in vivo* in the near future.

Acknowledgements

Colette Breuil and Diana Six provided isolates. Jane Park and Erica Lee provided bark and wood samples for fungal isolation. James Hammond, Colin Myrholm, Daryl Williams, Melissa Day, and Lionel and Howard Peterson provided technical assistance. James Brandt assisted with site selection. Funding was provided by Alberta Sustainable Resource Development and the Mountain Pine Beetle Initiative.

References

Adams, A. S.; Six, D. L., 2007: Temporal variation in mycophagy and prevalence of fungi associated with developmental stages of *Dendroctonus ponderosae* (Coleoptera: Curculonidae). Environ. Entomol. **36,** 64–72.

Amman, G. D.; Cole, W. E., 1983: Mountain pine beetle dynamics in lodgepole pine forests Part II: Population dynamics. USDA Forest Service General Technical Report INT-145.

British Columbia Ministry of Forests and Range, 2005: 2004 Summary of forest health conditions in British Columbia. Available from: http://www.for.gov.bc.ca/hfp/health/overview/2004.htm (accessed January 2006).

British Columbia Ministry of Forests and Range, 2006: 2005 Summary of forest health conditions in British Columbia. Available from: http://www.for.gov.bc.ca/hfp/health/overview/2005.htm (accessed October 2006).

Carroll, A. L.; Taylor, S. W.; Régnière, J.; 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, Kelowna, BC, October 30–31, 2003. Ed. by Shore, T. L.; Brooks, J. E.; Stone, J. E. Victoria, BC: 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.

Environment Canada, 2007: Canadian climate normals or averages 1971–2000. Available from: http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html (accessed February 2007).

Furniss, M. M.; 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.

Huber, D. P. W.; 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.; 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.

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

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

Ono, H., 2003: The mountain pine beetle: scope of the problem and key issues in Alberta. in: Mountain Pine Beetle Symposium: Challenges and Solutions, Kelowna, BC, October 30–31, 2003. Ed. by Shore, T. L.; Brooks, J. E.; Stone, J. E. Victoria, BC:Can. For. Serv. Pac. For. Cent. Inf. Rep. BC-X-399, pp. 62–66.

Owen, D. R.; Lindahl Jr, K. Q.; Wood, D. L.; Parmeter Jr, J. R., 1987: Pathogenicity of fungi isolated from *Dendroctonus valens, D. brevicomis*, and *D. ponderosae* to Ponderosa pine seedlings. Phytopathology **77,** 631–636.

Raffa, K. F.; 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.; Watson, J. A., 1967: Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Bot. **45,** 1115–1126.

Rice, A. V.; Thormann, M. N.; Langor, D. L., 2007a: Virulence of and interactions among mountain pine beetle-associated blue-stain fungi on two pine species and their hybrids in Alberta. Can. J. Bot. **85,** 316–323.

Rice, A. V.; Thormann, M. N.; Langor, D. L., 2007b: Mountain pine beetle-associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole × jack pine hybrids in Alberta. Can. J. Bot. **85,** 307–315.

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

Rumbold, C. T., 1941: A sapstaining fungus *Ceratostomella montium* n. sp. and some yeast associated with two species of *Dendroctonus*. J. Agric. Res. **62,** 589–601.

Safranyik, L.; Linton, D. A., 1982: Survival and development of mountain pine beetle broods in jack pine bolts from Ontario. Can. For. Serv. Res. Note **2,** 17–18.

Safranyik, L.; Shrimpton, D. M.; Whitney, H. S., 1974: Management of lodgepole pine to reduce losses from the mountain pine beetle. Victoria, BC: Can. For. Serv. 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., 2003: A comparison of mycangial and phoretic fungi of individual mountain pine beetles. Can. J. For. Res. **33,** 1331–1334.

Six, D. L.; Paine, T. D., 1997: *Ophiostoma clavigerum* is the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi*. Mycologia **89,** 858–866.

Six, D. L.; Paine, T. D., 1998: Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus 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.; 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.

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

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