



The role of temperature variability in stabilizing the mountain pine beetle–fungus mutualism



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AUTHOR HIGHLIGHTS

- Develop a model for the growth of MPB associated fungi within a tree.
- Connect this model to an existing model for MPB development.
- Explore whether temperature variability acts to stabilize the MPB–fungus mutualism.
- Transitioning between thermal environments is most robust stabilizing mechanism.
- Spacing between fungal lesions also emerges as potential stabilizing mechanism.

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ABSTRACT

As global climate patterns continue to change and extreme weather events become increasingly common, it is likely that many ecological interactions will be affected. One such interaction is the multipartite symbiosis that exists between the mountain pine beetle and two species of fungi, *Grosmannia clavigera* and *Ophiostoma montium*. In this mutualism, the fungi provide nutrition to the beetle, while the fungi benefit by being dispersed to new host trees. Multi-partite mutualisms are predicted to be unstable due to strong direct competition among symbionts or natural selection for superior over inferior mutualists. However, this mutualism has remained stable over long periods of evolutionary time. In this paper, we developed a temperature-based model for the spread of fungi within a tree and connected it to an existing model for mountain pine beetle development. Using this integrated model for fungal growth, we explored the possibility that temperature variability is a stabilizing mechanism for the mountain pine beetle–fungi mutualism. Of the three types of temperature variability we tested: intra-year, inter-year and variability due to transitioning between different thermal habitats (thermal migration), we found that thermal migration was the most robust stabilizing mechanism. Additionally, we found that the MPB attack density or spacing between fungal lesions also had a significant effect on the stability of the system. High attack densities or close lesion spacings also tended to stabilize the system, regardless of temperature.

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1. Introduction

The mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) is a bark beetle native to western North America (Wood, 1982). This insect is a serious pest that also acts as a natural disturbance agent, helping to maintain forest ecosystem structure and function. Although MPB population eruptions have occurred for millennia, the size of current outbreaks is greater than those recorded in the past century (Taylor et al., 2007). The

distribution of lodgepole pine (*Pinus contorta*), a primary host for MPB, extends further north than the current MPB range suggesting that MPB is limited by climate. Within the past two decades, outbreak MPB populations have expanded north in British Columbia, Canada, and across the Rocky Mountains infesting a new host jack pine (*Pinus banksiana*) in eastern Alberta (Cullingham et al., 2011). Several factors are playing a role in MPB population success and expansion including warming temperatures and past management strategies that promoted landscape homogeneity (Raffa et al., 2008).

Considerable attention has been given to understand how temperature directly affects MPB development timing and cold temperature mortality, factors that influence geographic range expansion (Bentz et al., 2010; Safranyik et al., 2010; Hicke et al., 2006; Logan and Powell, 2001). However, little work has focused

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on how temperature affects the critical symbiosis that exists between the MPB and two fungal symbionts, *Grossmania clavigera* (*G. clavigera*) and *Ophiostoma montium* (*O. montium*). The symbiosis is critical for the survival of MPB (Six and Bentz, 2007), and thus, understanding how temperature affects the fungal partners is as important as understanding how temperature will directly affect the host beetle.

The symbiosis between MPB and fungi is a mutualism. The beetle provides benefit to the fungi through transport to new host trees. This occurs in specialized structures of the insect exoskeleton called mycangia which are located on the maxillary cardines of both males and females (Six and Paine, 1998). When new adults leave the tree they carry spores of the fungi in the mycangia which are then inoculated into the phloem of the next tree as beetles construct egg galleries. In turn, the fungi aid the beetles by providing critical nutrients for developing beetles (Six and Paine, 1998; Adams and Six, 2007; Bleiker and Six, 2007).

Nutritional benefits to MPB appear to be of two types. The fungi colonize both the phloem and sapwood of the tree. Mycelium growing in the sapwood picks up nitrogen and moves it into the phloem where the larvae feed and develop. Increases in phloem nitrogen due to the fungi can be substantial (40–50%) and likely account for observations that beetles developing with fungi have much higher survival rates and are significantly larger than those that develop without. The fungi also produce large amounts of ergosterol, a sterol that insects can convert to hormones that support molting and reproduction (Bentz and Six, 2006). MPB have been shown to require feeding on spores of fungi as new adults in order to produce eggs. This requirement may relate to ergosterol consumption (Six and Paine, 1998; Bentz and Six, 2006).

While the two fungi appear to impart the same types of benefits to the beetle, the degree of benefit varies (Six and Paine, 1998; Bleiker and Six, 2007; Goodman et al., 2012; Cook et al., 2010). Overall, *G. clavigera* appears to be superior in concentrating nitrogen (Goodman et al., 2012; Cook et al., 2010) and supporting brood development (Six and Paine, 1998; Bleiker and Six, 2007). Beetles developing with *G. clavigera* are larger and have higher survival rates (Six and Paine, 1998; Bleiker and Six, 2007). Larger size of beetles developing with *G. clavigera* may mean greater fecundity and overall fitness indicating that *G. clavigera* is the superior mutualist.

Like many mutualisms, the MPB–fungus system is predicted to be unstable. The two fungi use the same resource at the same time (a freshly killed tree) and ultimately must compete for host beetles when time comes for it to be dispersed. Therefore, they are expected to be in strong competition with one another, and that the stronger competitor should outcompete the weaker competitor over time. Likewise, natural selection would be expected to select for the superior mutualist with *G. clavigera* eventually moving to fixation with the beetle (Six, 2012). Nonetheless, the MPB–fungus mutualism has remained relatively stable over a long period of evolutionary time (Six and Bentz, 2007). This indicates that some sort of stabilizing mechanism exists. The stability of the mutualism may be tied to differential temperature tolerances of the two fungi interacting with a variable habitat. Optimal growth of *G. clavigera* occurs somewhere between 20 and 25 °C. *O. montium*, on the other hand, grows best at 25 °C (Rice et al., 2008). These differences have led to the hypothesis that the different temperature tolerances of the two fungi minimize direct competition by giving each an advantage at different times. It has also been hypothesized that having two fungal symbionts, each with different temperature optima, may allow the beetle to exist across a broad range of environmental conditions by ensuring that it is never without a symbiont (Six and Bentz, 2007). While the differential temperature tolerances of the two fungi may allow the beetle greater environmental amplitude, it may also be a major

mechanism supporting the existence of two symbionts in this mutualism.

We hypothesized that variability in temperature acts as a stabilizing mechanism for the mutualism. In particular, we considered three types of temperature variability: intra-year, inter-year and thermal migration. By intra-year variability, we mean day-to-day and seasonal fluctuations in temperature that allow an advantage in growth rate to switch back and forth between the fungi throughout the year. For example, in a typical year, warm summer temperatures allow *O. montium* to grow faster than *G. clavigera*. However, as fall temperatures cool, *G. clavigera* may gain the advantage until late spring or early summer, when *O. montium* regains the advantage.

Another form of variability we considered was inter-year variability, which includes differences in temperatures from year to year. Because some years are cooler and some warmer, this form of variability could result in some years where one species dominates and others where the other species gains the advantage. Over the long term, however, because the advantage passes back and forth, the relative prevalence of each may remain stable. The final form of temperature variability we considered was variability due to thermal migration. This variability represented varying thermal environments as fungi-carrying MPB moved between warm and cold environments on a landscape. This will be called the thermal migration hypothesis. Our theory was that *O. montium*, the warm-loving fungus, grows in prevalence during outbreak years at lower elevation environments and that *G. clavigera*, the cool-loving fungus, may gain in prevalence while the beetle occupies a cooler, high-elevation location. This would cause the relative proportion of each fungi to oscillate but remain essentially stable over time.

In this paper we developed a temperature-driven model for fungal growth that includes effects of competition for space in a tree. This model was parameterized using growth data collected from *G. clavigera* and *O. montium* isolates from the mycangia of emerging MPB collected from Logan Canyon in northern Utah in 2010. To project fungal prevalence into the future, we combined the fungal growth model with an existing temperature-based model for MPB development (Powell and Logan, 2005) to determine the proportion of each fungi present in the pupal chambers as teneral adults prepare to leave the tree. Finally, using this integrated model, we simulated the outcomes for the mutualism for a variety of temperature scenarios (emphasizing intra-year, inter-year or thermal migration variability), and attempted to determine how these differences affected the relative prevalence of *G. clavigera* and *O. montium* in the mutualism. Using these models, our goal was to determine whether variations in temperature, and the differential rates of fungus growth at those temperatures, could explain the continued persistence of both *G. clavigera* and *O. montium* in the MPB–fungus mutualism.

2. Model development

2.1. MPB/fungus interactions

In summer, adult MPB emerge from the trees within which they developed and fly in search of new host trees. Females initiate attacks and release pheromones to attract other dispersing males and females. This results in a mass attack that often occurs over a period of a few days. In a successful attack, males and females excavate long J-shaped galleries in the phloem layer of the tree. Eggs are laid on the sides of the gallery and spores stored in the mycangia and on the body of the beetles are introduced into the phloem (Safranyik et al., 2007). From this point, the development of beetle brood and growth of the fungi into the tree occur at

a rate based on temperature (Safranyik et al., 2007). The fungal spores germinate and form hyphae that spread throughout the phloem and sapwood while MPB eggs hatch and the larvae progress through four stages while feeding on both phloem and fungal hyphae in larval galleries they construct (Safranyik et al., 2007). After larval development is complete, they construct pupal chambers where they pupate and then develop into new (teneral) adults. The new adults feed on spores that the fungi produce in a dense lining in the pupal chamber while packing their mycangia with spores (Safranyik et al., 2007; Six and Bentz, 2007). Brood adults then emerge from the tree in search of new hosts. This sequence typically takes 1 year depending on temperature although multiple years are required in cold environments.

To model the prevalence of *G. clavigera* and *O. montium* in relation to MPB development, we modeled the fungi as they spread outward (and captured space) from beetle galleries and computed the relative proportion of each fungus present in the tree as teneral adult beetles prepared to leave. This proportion was important as it directly influenced the proportion of each fungus being carried by the next generation of MPB. We used these models to describe the relationship between temperature and the rate of fungus growth, developed a model for fungal growth in a tree, and used this model in combination with an existing model for MPB development to project the mutualism into the future.

2.2. Fungal growth rate curves

Beetle-associated fungi grow by forming a mycelium (network of connected hyphae) that spreads radially outward at a rate governed by temperature. To model growth of the fungi, we developed a relationship between observed fungal growth rates and temperature that allowed us to calculate the area each fungus colonized based on a record of hourly temperatures.

2.2.1. Fungus growth data

Fungi were isolated from the mycangia of beetles emerging from lodgepole pine trees in Logan Canyon, UT in the summer of 2010 and 2011. These fungi were identified to species using morphology (Upadhyay, 1981). To determine the growth rates of each species as well as the variability within species, 3–4 replicates of 24 isolates of *G. clavigera* and 17 isolates of *O. montium* were grown in Petri dishes containing 2% malt extract agar (a growth medium) and held at constant temperatures of 5, 10, 15, 20, 25, 30, and 35 °C. These plates were monitored daily and the amount of area colonized by the fungi traced. "Image J" (Schneider et al., 2012) was used to measure the number of pixels (i.e., area) colonized each day by the fungi. The number of pixels was then converted to area.

Next, the 587 estimates of area colonized were converted to measurements of radius colonized and a line fitted to obtain a single radial growth rate for each Petri dish. Finally, maximum likelihood estimation was used to fit these radial growth rates to a distribution often used for modeling insect development (Logan, 1988; Regniere et al., 2012):

$$r(T) = B(e^{\alpha(T-T_0)} - 1) - B(e^{\alpha(T_m-T_0)} - 1)e^{(T-T_m)/b}, \quad (1)$$

assuming lognormal error. In this equation, $r(T)$ represents the rate of fungal growth per day in mm based on the temperature T in °C, B is a scaling factor that affects the maximum rate of fungus growth, α affects how quickly the growth rate increases at low temperatures, b is the thickness of the boundary layer between peak growth and the upper temperature threshold, and T_0 and T_m respectively represent the lower and upper temperature thresholds for the fungi in °C (i.e., the lowest and highest temperature where the fungus will continue to grow). These parameter values

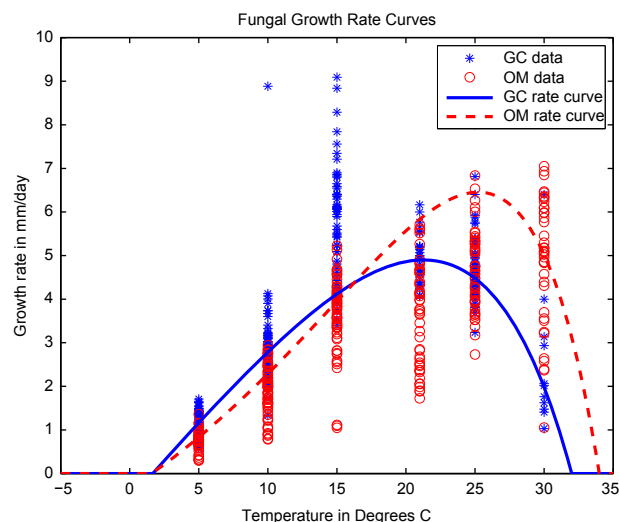


Fig. 1. Growth rate observations for *G. clavigera*, GC (*), and *O. montium*, OM (○), are shown with their parameterized growth rate curves. Note that *G. clavigera* (solid line) has a higher rate of growth when temperatures are below 16.3 °C, while *O. montium* (dashed line) has the higher rate of growth for temperatures above 16.3 °C and that its rate curve extends further to the right than *G. clavigera*. This confirms previous observations that *G. clavigera* is the “cool-loving” fungus while *O. montium* is the “warm-loving” fungus. Parameters for these rate curves are shown in Table 1.

Table 1

Parameters for the fungal rate curves (Eq. (1)). In these rate curves, α affects the rate of increase at low temperatures, b is the thickness of the boundary layer between peak growth and the upper temperature threshold, B is a scaling factor which affects the maximum rate of fungal growth, while T_0 and T_m are respectively the lower and upper temperature thresholds for the fungi in °C. These values were estimated using Maximum Likelihood estimation.

Fungus type	α	b	B	T_0 (°C)	T_m (°C)
<i>G. clavigera</i>	0.0041	8.0407	95.6120	0.9123	32
<i>O. montium</i>	0.0662	7.4949	3.8395	−0.0236	34

were obtained using a built-in MATLAB optimizer (the Nelder–Mead simplex algorithm, 'fminsearch') and the constraint that T_0 and T_m have biologically reasonable values ($0 < T_0 < 5$ and $T_m \approx 30$ °C). Fig. 1 shows the observed radial growth rates for *G. clavigera* and *O. montium* as well as their parameterized rate curves. The parameters for these curves are included in Table 1.

2.3. Fungal growth in cross section

Consider a horizontal section of a tree bole, which will be intersected by female MPB constructing egg galleries and depositing *G. clavigera* or *O. montium* spores from their mycangia as they burrow. Each intersection can be represented as a distinct fungal inoculation point distributed evenly around the circumference of the tree with some small additive error at a discrete location, that is, $x_i(0) = (i-1)\pi d/n + \epsilon$ where $i = 1, 2, \dots, n$, d is the diameter of the tree in mm, and ϵ is drawn from a normal distribution with mean zero (see Fig. 2). These locations were then assigned to be *G. clavigera* with probability p_{gc} or *O. montium* with probability $1-p_{gc}$. Because we restricted our model to a single dimension, we allowed the fungi to spread from these locations horizontally around the circumference of the cross section of the tree at a rate proportional to their radial growth rate (which is determined by temperature). To allow for the fact that fungal growth in a tree will likely be much slower than fungal growth in a Petri dish we also included a scaling factor, $\beta \in (0, 1]$, in the model (a description of

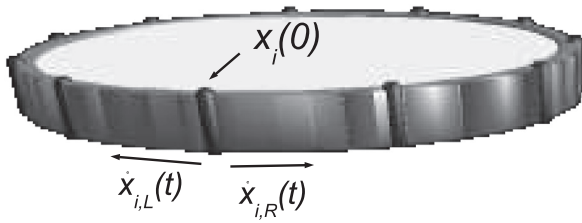


Fig. 2. A horizontal cross section of a tree bole that has been intersected by female MPB galleries (represented by dark striations). At each point, either *G. clavigera* or *O. montium* spreads horizontally outward at a rate based on the temperature at time t .

how β was estimated is included in Section 2.3.2). This allowed the movement in the left endpoint of the fungus lesion to be represented by

$$\dot{x}_{i,L}(t) = \begin{cases} -\beta r(T(t)) & \text{if } x_{i-1,R}(t) < x_{i,L}(t) \\ 0 & \text{if } x_{i-1,R}(t) \geq x_{i,L}(t) \quad (\text{i.e., if lesions overlap}), \end{cases} \quad (2)$$

at time t , and the growth of the right endpoint by

$$\dot{x}_{i,R}(t) = \begin{cases} \beta r(T(t)) & \text{if } x_{i,R}(t) < x_{i+1,L}(t) \\ 0 & \text{if } x_{i,R}(t) \geq x_{i+1,L}(t), \end{cases} \quad (3)$$

where $i = 1, 2, \dots, n$ and r represents either r_{gc} or r_{om} , the radial growth rate (which is a function of the temperature at time t) for *G. clavigera* or *O. montium*, depending on the type of fungus growing from lesion i .

In the model, competition by fungi for space within the tree is implemented by setting the fungal growth rates to zero when two fungal lesions meet. This is based on observations that only exploitative, not direct competition, occurs between the fungi (Bleiker and Six, 2007). This mechanism also accounts for the finite area available for fungal colonization within the tree; once available space between lesions is occupied, a fungus can no longer capture more space. Based on temperature, the model allows fungal growth to continue until the entire cross section is colonized or until a specified amount of time has gone by, such as the amount of time necessary for the MPB eggs to develop into teneral adults. After this the proportion of the two fungi present in the tree is calculated and used to update p_{gc} for the next generation of fungus lesions.

2.3.1. Temperature data

To project fungal growth and test our hypotheses, two long-term temperature datasets were used. One was collected in the Stanley Valley of the Sawtooth National Recreation Area (SNRA) in central Idaho and the other at Railroad Ridge (RRR), a high-elevation site located in the White Cloud Mountains in the Challis National Forest of central Idaho. The SNRA temperature dataset consists of hourly phloem temperatures from Julian day (JD) 200 of 1992 to JD 289 of 2004 collected at an elevation of approximately 2040 m (6700 ft). These were collected from the phloem of 12 different MPB-infested lodgepole pines (one per year) located throughout the SNRA and combined to form a continuous 12-year temperature record (Powell and Bentz, 2009). This dataset was used to test all three of our hypotheses. The RRR temperature dataset consists of hourly phloem temperatures recorded at the southern weather station established along RRR, a broad east/west running ridge that slopes from approximately 2900 m (9500 ft) to 3400 m (10 500 ft). This dataset was primarily used as the higher elevation site to test the thermal migration hypothesis. Simulations in this paper were performed using south bole aspect RRR phloem temperatures recorded from 1996 to 2004 (Logan and Powell, 2001).

2.3.2. Estimating the growth scaling parameter

The parameter β included in Eqs. (2) and (3) was used to scale the rate of fungus growth in a Petri dish to the much slower rate of fungal growth in a tree. A rough estimate for this parameter was obtained using the observation that beetle larvae will tunnel backwards to older sections of their larval gallery to eat fungus colonized phloem (Adams and Six, 2007). We assumed that the approximate total growth of a lesion could be estimated using the length of MPB larval galleries. For example, using 1994 temperatures from the SNRA dataset, the period from MPB oviposition to pupation took 198 days (using the MPB model which will be described in Section 2.4). During that time *G. clavigera* would have grown 28.84 cm on agar. To determine a rough estimate of the length the larvae would have colonized as they developed through their four larval instar stages, we measured the length of larval galleries on three pieces of stripped lodgepole bark in the USU Applied Entomology lab and observed larval gallery lengths between 3 and 5 cm. We used the mean of these measurements, 4 cm, which allowed us to calculate β as approximately $4/28.84 = 0.1387$. We note that this value of β serves only as a rough approximation, as different estimates could be obtained using a different year from the temperature record or if larval gallery lengths vary greatly from our observations. A more data-driven estimate for β is the subject of our next work which will utilize detailed information about MPB attacks and emergence, the proportion of each fungus entering a tree, and an hourly temperature record.

2.4. The MPB median developmental model

To connect the implications of fungal growth in a tree with the stability of the MPB–fungus mutualism, we used an existing model for MPB development. This model predicts the median emergence date of the MPB from each of their life stages (egg, larvae, pupae and teneral adult) (Powell and Logan, 2005). The model works by taking a year-long temperature record and calculating the corresponding median rates of development through each MPB life stage. Then, based on the median date of MPB attack on a tree, it determines the median date at which MPB transition between life stages. We used this model to determine the window of time during which teneral adult MPB feed on spore layers of the fungi that line the pupal chambers. This window was used to calculate the relative prevalence of *G. clavigera* and *O. montium* available to be carried to the next generation of host trees. Model details, including integration strategies and rate curve parameters for the eight phases of the MPB life cycle can be found in Powell and Logan (2005).

3. Model integration

Simulations to project fungal prevalence into the future were done by specifying host tree size, the average distance between fungal lesions (corresponding to the density of attacking MPB), the average proportion of each fungus being carried, the date the majority of MPB began attacking a particular tree (which acts as the date of inoculation for the fungi), and a year-long temperature record. Using these values, the MPB median developmental model described in Section 2.4 was used to calculate the teneral adult spore-feeding window. The fungal growth model from Section 2.3 was used to determine the relative proportion of *G. clavigera* and *O. montium* present in the tree at that time. The proportion of each fungus available during this window was averaged and used as the proportion carried by beetles to attack new host trees.

To ensure as much realism as possible, we used fungal lesion spacings which corresponded to observed MPB attack densities.

A previous study on MPB found that the minimum attack threshold (the lowest density of attacking MPB that will result in a successful MPB generation) for MPB in lodgepole pine is about 40 attacks/m², the optimal attack density is 62 attacks/m², and a high attack density is considered to be 80 attacks/m² (Raffa and Berryman, 1983). Given the fact that MPB parental galleries average 30 cm in length and that beetles prefer to attack trees 25 cm in diameter or larger (Safranyik et al., 2007), we found that 40–85 mm was a reasonable range to include for lesion spacings (where 54 mm corresponds to the optimal attack density) assuming that the MPB galleries are evenly dispersed across the tree bole. To reduce complexity of the simulations and minimize small sample size effects 1000 cross-sections were spliced together to form a vast mega-section. The number of fungal lesions varied depending on the spacing between lesions used.

3.1. Hypothesis evaluation

To determine whether temperature variability is allowing both fungi to persist in the mutualism we constructed several vectors of hourly temperature data which included intra-year, inter-year or thermal migration variability and used these to project fungal prevalence into the future. For simplicity, we began each simulation with the relative proportion of each fungus at 50% and examined how different thermal scenarios eroded or enhanced mutualism stability.

Temperature sets to test intra-year variability were constructed by dividing the SNRA temperature dataset into 1-year increments and repeating them for a specified number of years. This allowed us to determine whether variability present in a single year's temperature record was enough to maintain both fungi in the mutualism. Inter-year variability was tested by projecting fungal prevalence into the future using our two observed long-term temperature datasets as well as datasets constructed by randomly permuting year-long chunks of observed temperature data from the SNRA or RRR. The final form of variability we considered was variation caused by portions of the MPB population periodically transitioning between different thermal environments. In this case, we selected the number of years the MPB would be at a low elevation or warm environment, n_1 , and the number of years they would be at a high elevation or cool environment, n_2 , and then randomly selected n_1 years from the Sawtooth dataset and n_2 years from the RRR dataset and repeated this process for the number of cycles we wished to observe. See Table 2 for more explanation about how these datasets were constructed.

4. Results

4.1. Intra-year variability

When projecting fungal prevalence through time, we found that for any given year, one fungus would nearly always gain a slight advantage over the other. Due to the stochastic nature (lesion location and type) of the fungal growth model, this advantage could potentially switch back and forth between the two fungi if the simulation was repeated, particularly if the

Table 2
Method of constructing simulated temperature datasets to test our hypotheses. The hypothesis name is listed on the left while the right column describes how year-long chunks of observed temperatures were used to construct corresponding temperature datasets.

Intra-year	year k , year k , year k , ...
Inter-year	1992, 1997, 1995, ...
Thermal migration	SNRA ₁₉₉₉ , SNRA ₁₉₉₇ , ... SNRA ₁₉₉₄ , RRR ₁₉₉₆ , RRR ₂₀₀₀ , ... RRR ₁₉₉₇ , ...

advantage gained by one fungus was quite small. However, when projected over a long time period, we found that the stability of the mutualism was strongly dependent on how closely the fungal lesions were spaced (i.e., density of MPB attacks). When lesions were close (40 mm and below, indicating a very high density of attacking MPB), intra-year variability allowed both fungi to maintain a strong presence in the mutualism in most of the thermal scenarios we tested (Figs. 3 and 4, plots A and B). As lesion spacing increased, we found that the advantage ceased to pass back and forth as often, nearly always allowing one fungus to continue to gain an advantage (Figs. 3 and 4, plots C–J). This would eventually cause the relative prevalence of the other fungus to move toward zero. As lesion spacing increased, the rate at which one fungus began to dominate the mutualism also tended to increase. Exceptions to this with SNRA data occurred in 1994 and 2003 (Fig. 3 plot J) and RRR data in 2000 (Fig. 4 plots E–J). These will be discussed further below.

Several simulations were performed repeating a single-year temperature series to test whether intra-year variability could stabilize the mutualism. In each case, the simulation began on JD 205 with *G. clavigera* and *O. montium* initially present in the mutualism at a relative proportion of 50%. Projecting their relative proportions forward using a repeated string of yearly temperatures from the SNRA or RRR, we found that when fungal lesions were spaced 40 mm apart (equivalent to a very high MPB attack density, ~90 beetles/m²), the mutualism remained stable (Figs. 3 and 4, plot A). However, as lesion spacings increased, stability decreased (Figs. 3 and 4, plots C–J). *O. montium* repeatedly gained an advantage over *G. clavigera* causing the relative proportion of *G. clavigera* to decrease over time. Further increases in lesion spacing allowed *O. montium* to benefit more each year, and *G. clavigera* was lost from the mutualism after a few hundred years. Interestingly, for SNRA temperatures in 1994 and 2003, the behavior of the system changed for very large lesion spacings (80–85 mm): 1994 temperatures began to favor *G. clavigera* and 2003 temperatures less strongly favored *O. montium*. Relative to the SNRA dataset, 1994 and 2003 were remarkable only in that 1994 was cooler in late summer/early fall and 2003 had a longer winter, with cooler temperatures beginning in September and lasting through February (versus October–January). A similar result occurred for RRR simulations in that for large lesion spacing, 2000 temperatures began to favor *O. montium* (Table 3).

4.2. Inter-year variability

Simulations testing effects of inter-year variability on the mutualism produced similar results. When projecting fungal prevalence using the observed temperature records from the SNRA and RRR, we found that closely spaced fungal lesions (40 mm) allowed the relative proportion of each fungus to stay very near 50% while increases in lesion spacing allowed one fungus to gain an advantage over the other. Generally *O. montium*, the warm fungus, would prevail using SNRA temperatures (Fig. 5, plot A) while *G. clavigera* benefited slightly more often than *O. montium* (approximately 55% of the time) when using the RRR temperatures (Fig. 5, plot B), although the change in relative prevalence was often quite small (less than 1%). In repeating these simulations, we found substantial variability. However, the overall trend indicated that *G. clavigera* will be lost from the mutualism at the low elevation site, while *O. montium* will be lost, although more slowly, at the higher elevation site.

When simulated over a longer timescale using a simulated dataset (200 years, Fig. 6), we found that this trend continued. Small spacing between lesions (40–45 mm) corresponding to high densities of attacking MPB would result in long-term stability. However, as the density of MPB attacks decreased, and spacing

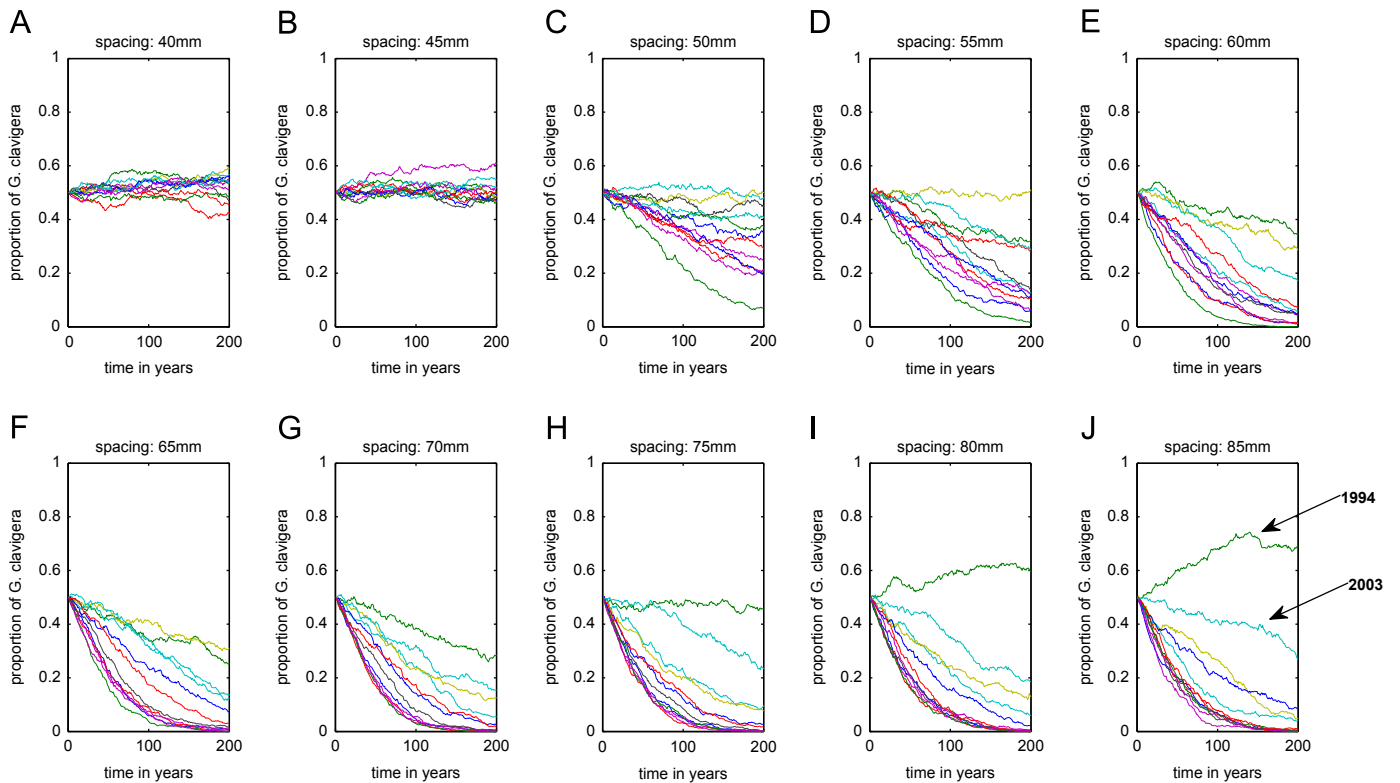


Fig. 3. Testing the intra-year variability hypothesis and the effect of lesion spacing on fungal prevalence using SNRA temperature data. Each subplot shows 12 curves (corresponding to the 12 years of temperature observations from the SNRA dataset, south bole) representing the predicted prevalence of *G. clavigera* after iterating the model 200 times using a single year's temperatures. We note that when lesion spacing was small (40–45 mm, A and B, corresponding to very high attack density), both *O. montium* and *G. clavigera* remained present in similar proportions for the entire 200 years. As fungal spacing increased, however, more and more years resulted in *G. clavigera* being lost from the mutualism (C–J). Interestingly, further increases in spacing allowed 1994 temperatures to favor *G. clavigera* and lessened the advantage *O. montium* which was able to obtain for 2003 temperatures (J).

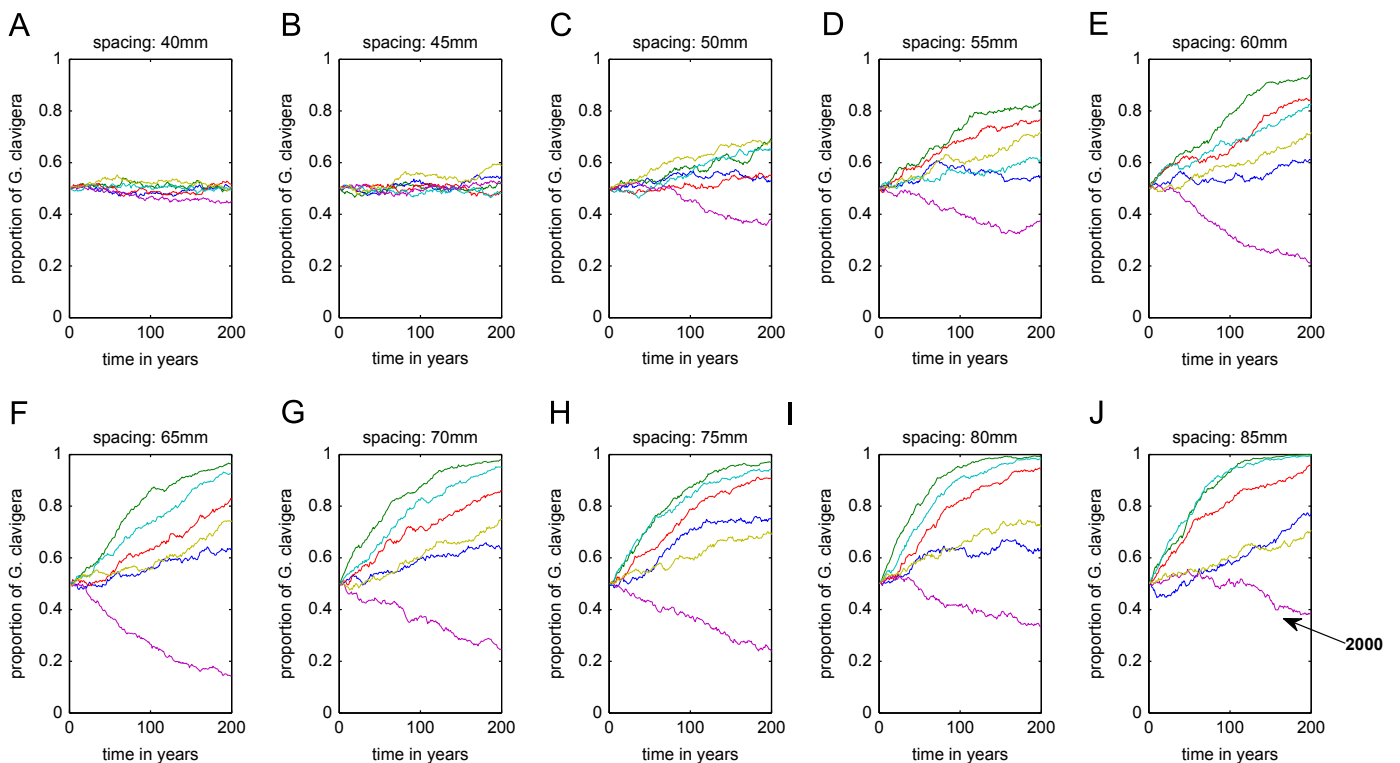


Fig. 4. Testing the intra-year variability hypothesis and the effect of lesion spacing on fungal prevalence using RRR temperature data. Each subplot shows 6 curves (corresponding to the 6 years of temperature observations from the RRR dataset, 1996–2001, south bole) representing the predicted prevalence of *G. clavigera* after iterating the model 200 times using a single year's temperatures. As with the SNRA temperatures, when spacing between lesions was small (40–45 mm, A and B) *O. montium* and *G. clavigera* were able to remain present in similar proportions for the entire 200 years. As fungal spacing increased, the coolness of the climate favored *G. clavigera* and *O. montium* began to be lost from the mutualism (D–J). An exception occurred for temperatures from 2000, a warm year, in which larger lesion spacings allowed *O. montium* to gain in prevalence and *G. clavigera* to diminish.

between lesions increased, *O. montium* would continue to gain an advantage in the SNRA, slowly forcing *G. clavigera* out of the mutualism while *G. clavigera* would continue to gain an advantage at RRR.

4.3. Migration between different thermal environments

When testing the thermal migration hypothesis, we found that the ultimate outcome of the system was sensitive to the number of years (or MPB generations) spent in each habitat but that for certain lesion spacings the outcome became less sensitive to these time periods. In general the relative proportion of *G. clavigera* in the mutualism tended to decrease when MPB occupied the warmer, low-elevation location and increase when MPB occupied the cooler, high-elevation location. Provided the ratio between the low and high elevation periods was appropriate, the overall relative prevalence of the two fungi would oscillate up and down, and the mutualism would not ultimately destabilize except at the most sparse lesion spacings (Fig. 7, plot A). If the period spent at low elevation became too long, however, then we would see a trend favoring *O. montium* (Fig. 7, plot B). Conversely, if the period spent at high altitude was too long, then the trend would favor *G. clavigera*. The prevalence of each fungus was less sensitive to MPB attack density in these simulations than in those for MPB remaining in a single thermal environment.

5. Discussion/conclusions

In this study, we developed a temperature-driven model for fungal growth in a tree which includes effects of competition for space. This model was parametrized using fungal growth data and combined with an existing model for MPB development. Using

Table 3
Approximate correlation between lesion spacing in mm and MPB attack density in beetles/m² (assuming that female galleries are dispersed evenly across the tree bole).

Spacing (mm)	Approximate number of beetles/m ²
40	85
50	67
60	56
70	48
80	42

this integrated model, we projected fungal prevalence through time using observed and constructed temperature datasets which allowed us to test whether variability in temperature was capable of stabilizing the MPB–fungus mutualism. We found that of the three hypotheses tested, thermal migration was the most robust stabilizing mechanism for the MPB–fungus mutualism independent of lesion spacing. This type of variability allowed *G. clavigera* and *O. montium* to remain in the mutualism indefinitely given appropriate rates of migration. Our simulations did not indicate that either intra- or inter-year variability alone were capable of stabilizing the mutualism. We acknowledge that inter-year variability could produce similar results as thermal migration, although temperature records used in our model analyses did not include years with sufficiently cool and warm temperatures.

Additionally, we were surprised to find that spacing between lesions (or the density of attacking MPB) influenced stability of the mutualism. All three sources of variability we tested appeared to be plausible stabilizing mechanisms when MPB attack densities were at or above the optimal attack density of 62 beetles/m² (corresponding to a lesion spacing of 54 mm or closer). As spacing between lesions increased (corresponding to lower MPB attack densities) thermal migration variability emerged as the only temperature mechanism capable of stabilizing the system. This raises the question, what about lesion spacing acts to stabilize the mutualism?

5.1. How does lesion spacing stabilize fungal prevalence?

To explore the dynamics of lesion spacing, we used the fungal growth rate curves from Section 2.2 to plot the competition between two lesions, each of the different fungal species. Using these rate curves, we plotted the cumulative length of phloem, each fungus could colonize in an average year (SNRA 1995) to visualize differences between the overall growth of the fungi (when infinite space was available to colonize). Next we plotted the cumulative length that *G. clavigera* could colonize coming from one direction and the cumulative length *O. montium* could colonize coming from the opposite direction and varied the initial distance between them (representing different lesion spacings) (Fig. 8). Displayed this way the point at which the curves cross indicates the time at which the space between two lesions is totally colonized and how much was colonized by each species.

The cumulative growth of the two fungi was quite similar and could be divided into three phases: rapid growth in fall, slow or zero growth in winter, and rapid growth again in spring. Qualitatively, the biggest difference between these curves occurred at the

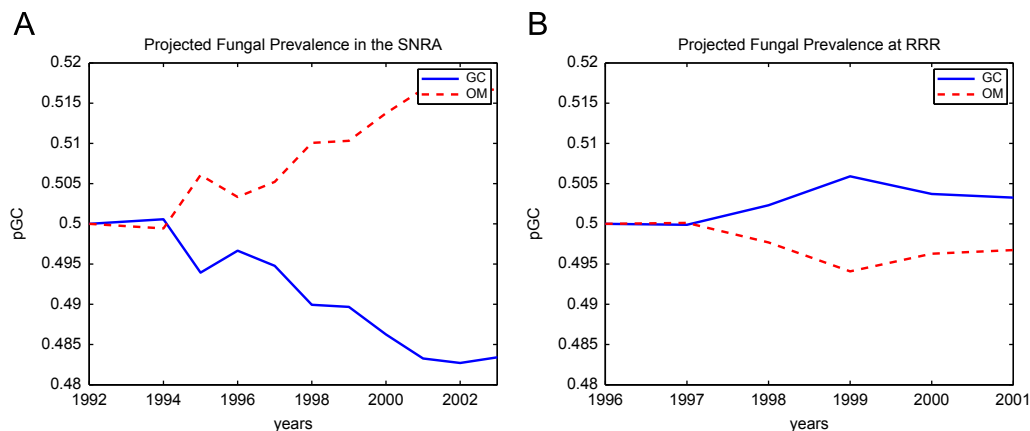


Fig. 5. Testing the inter-year variability hypothesis using observed temperature datasets. One realization of the projected proportion of *G. clavigera* (GC, solid line) and *O. montium* (OM, dashed line) present in the mutualism over time in (A) the SNRA and (B) the higher elevation site, RRR, using a lesion spacing of 54 mm. These plots suggest that over time, regardless of inter-year variability, *G. clavigera* may be lost from the mutualism in temperature regimes similar to lodgepole pine stands in the SNRA, while *O. montium* could be lost from the system in higher elevation whitebark pine stands, albeit more slowly.

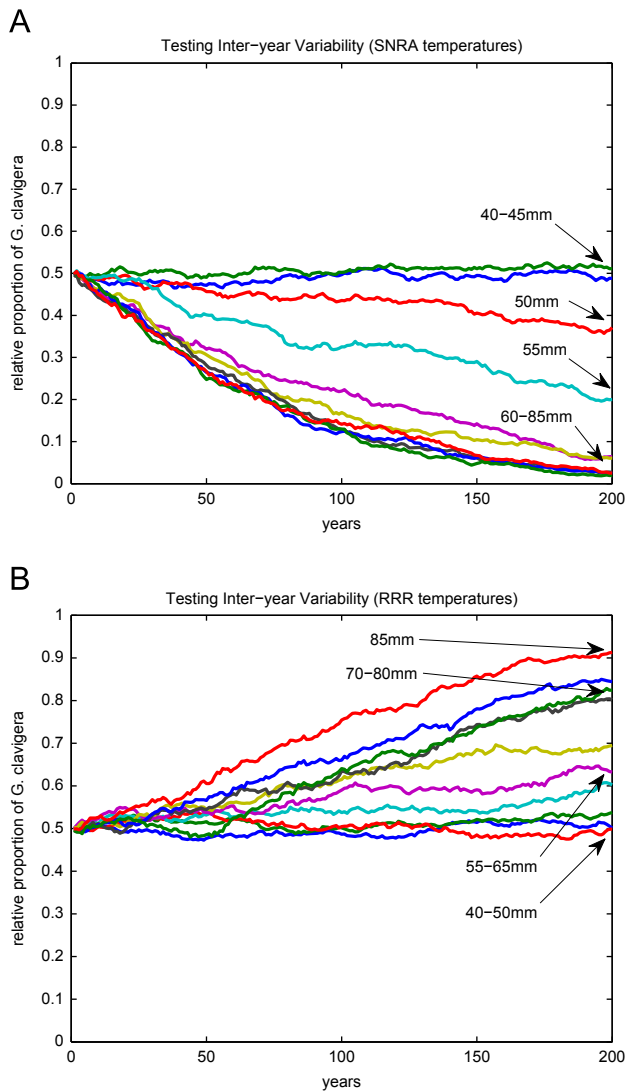


Fig. 6. Testing the inter-year variability hypothesis. The projected proportion of *G. clavigera* present in the mutualism over time is shown for the (A) SNRA and (B) RRR with lesion spacings ranging from 40 to 85 mm using a hypothetical dataset created by sampling random year-long chunks from the observed temperature sets. Note that low lesion spacings (40–45 mm, indicating high densities of attacking MPB) have the potential to stabilize the system for a long period of time while large lesion spacings (i.e., fewer attacking MPB) can cause *G. clavigera* to be lost from the mutualism in the SNRA, and *O. montium* to be lost from the mutualism at RRR.

transition between phases (~60–100 days after attack and ~270–320 days after attack). In these regions, the average growth rate of the fungi was quite different which could have a large impact on where the fungi intersect (Fig. 8 plot B).

When lesions were tightly spaced, the fungi intersected early in the fall when their growth was roughly linear and average temperatures were near the temperature at which *G. clavigera* and *O. montium* grow at the same rate (where the fungal rate curves in Fig. 1 intersect, ~16.3 °C based on our parameterization). For example, in the SNRA in 1995 the mean temperature for the first 60 days after attack (JD 205 to JD 265) was 15.23 °C. This helped to stabilize the mutualism since most of the available space was colonized in the fall when the fungal growth rates were nearly indistinguishable, leaving less potential for winter and spring temperatures to affect the system. As the space between lesions increased, the fungi intersected later in the year, closer to one of the transitions between phases where the realized growth characteristics of the fungi were most different. This generally

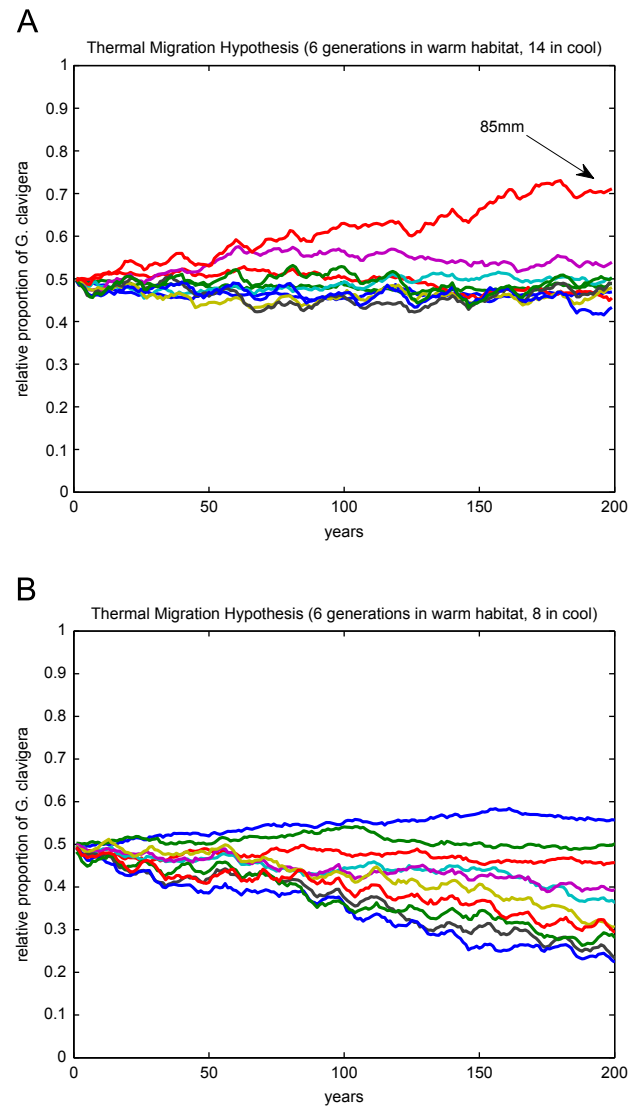


Fig. 7. Testing the thermal migration hypothesis as a portion of the MPB population periodically transition between two distinct thermal habitats. We allowed the MPB to alternate between 6 generations in the SNRA (perhaps in an outbreak phase) and 14 generations (~24 years) in a cooler location, RRR (A) and 6 generations in the SNRA and 8 at RRR (~40 years) (B). From these plots, we see that appropriate patterns of transitioning between thermal environments can maintain both fungi in the mutualism for all but the most distant lesion spacings while different ratios of transitioning may lead to destabilization.

resulted in one fungus claiming slightly more space than the other. Simulations are based on a constant date of attack each year, although in reality attack date will vary annually as temperature influences insect development and emergence timing. Changing date of attack could significantly influence fungal interactions and future work will include using observed MPB and fungal parameters observed at field plots to evaluate and improve the model. Model improvements will also include a coupled competition model for the total area colonized by each fungus under the bark and variability in fungal growth rates.

For the majority of years, temperatures in the SNRA favored *O. montium* (Fig. 3) and we saw the relative proportion of *G. clavigera* decrease over time for lesion spacings above 45 mm. One anomaly, however, was that large lesion spacings altered the outcome in fungal prevalence in 1994 and 2003, causing 1994 temperatures to favor *G. clavigera* and reducing the advantage *O. montium* could obtain in 2003 (Fig. 3, plot J). To explore this further in the context of lesion spacing, we looked at the overall cumulative growth of

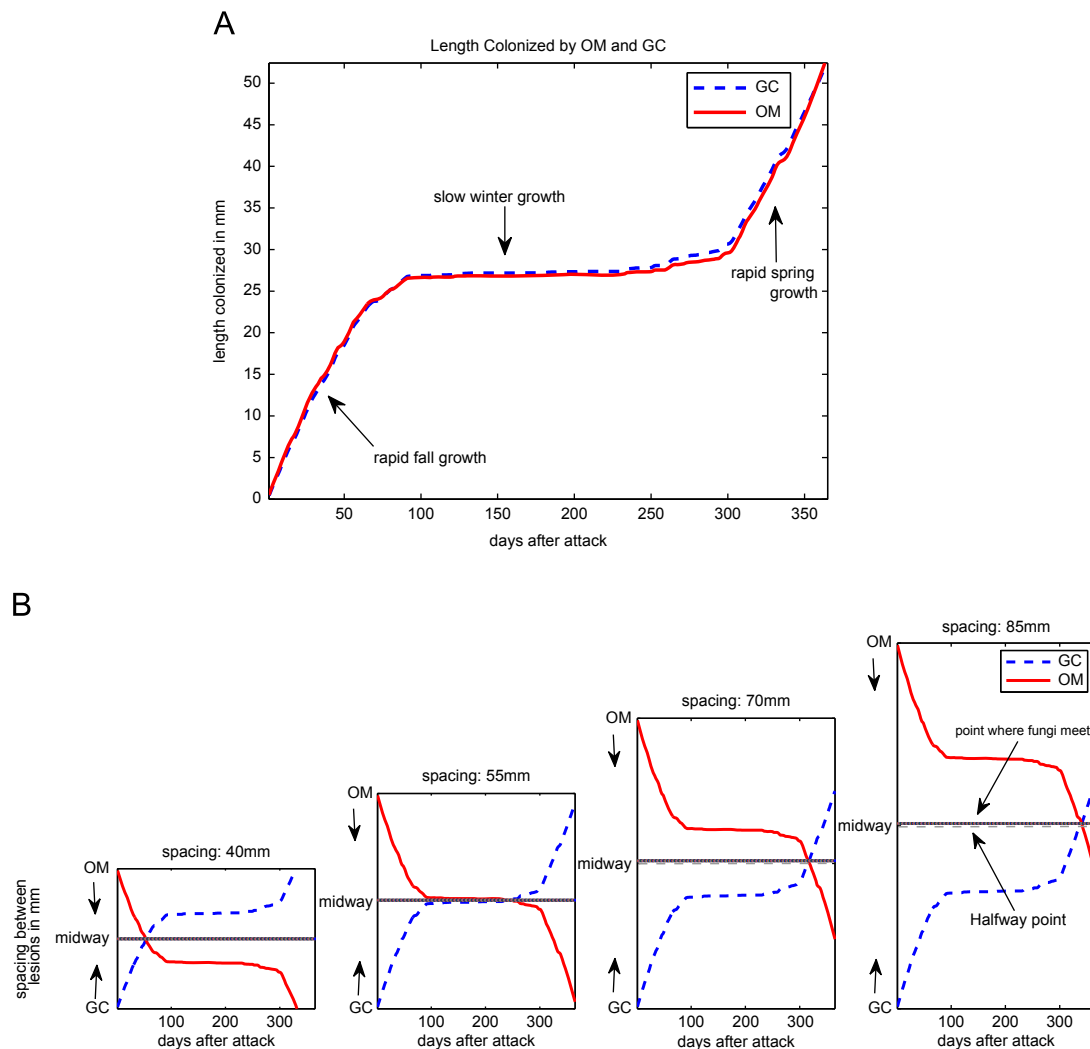


Fig. 8. Effect of spacing on the proportion of a tree each fungus is able to colonize. Cumulative growth of *G. clavigera* and *O. montium* (A), and the dynamics of two lesions with different fungal type initially 40, 55, 70, and 85 mm apart (B). We allowed *O. montium*, OM (solid line), to grow from one direction while *G. clavigera*, GC (dashed line), grows from the other. Note, the point of intersection of the fungi (horizontal black line) is affected by the initial space between lesions. When the space is small (40 mm, on left), the fungi intersect during their initial rapid growth phase (when their growth is nearly identical). Each colonizes in approximately 50% of the space available (regardless of winter and spring temperatures) and the mutualism remains stable. As space between lesions increases the fungi intersect in late fall or early spring where the fungi display the most different behavior. For this particular year, wider space between lesions allowed *G. clavigera* to gain a small advantage. This mechanism allows one fungus, depending on temperature, to colonize more space and gain dominance in the mutualism, leading to a destabilization over time. Phloem lengths colonized in this plot were calculated using SNRA 1995 temperatures, an attack date of JD 205 and $\beta = .1387$.

each fungi during 1994 and 2003 (Fig. 9) to see how their growth matched up over these years, and in particular, how the two fungi transitioned between slow winter growth and rapid spring growth (which would affect their point of intersection between lesions with very large space between them).

We found that although 1994 was a cooler year the growth of each fungus was similar during the first 60 days following the MPB attack (Fig. 9, plot A). As the year went on, there were brief periods which favored *O. montium*, but overall *G. clavigera* held the advantage. This meant that with finite space available, the two fungi could either meet early in the year when the fungi had nearly identical growth, slightly later, perhaps early in the transition between fall and winter phases when *O. montium* had a brief advantage, or later, when *G. clavigera* had a clear advantage. This explained how in simulations for intra-year variability the curve representing 1994 was stable for small lesion spacings (Fig. 3, plots A and B), began to favor *O. montium* as lesion spacings increased, and how it switched and began favoring *G. clavigera* for the largest spacings (Fig. 3, plot J). The year 2003 also produced anomalous results in that the relative proportion of *G. clavigera* was somewhat

higher after 200 years for large lesions spacings rather than small ones (Fig. 3). The mechanism behind this also became clear upon inspection of the cumulative length each fungus would have colonized (Fig. 9, plot B). Both fungi grew quite similarly with the exception of the transition between slow winter growth and fast spring growth which would have altered the outcome of the mutualism for large lesion spacings. Based on this analysis, it seems that for current temperatures lesion spacing is quite a robust stabilizing mechanism, which begs the question: will it maintain stability in an altered temperature regime?

Because global temperatures are projected to warm in the coming decades, we added a warming trend of 1 and 2 °C per 10 years and repeated the projections testing the thermal migration hypothesis (Fig. 10). In doing so, we found that the closest lesion spacings (40–45 mm) continued to stabilize the system. When temperature became too warm (i.e., after several decades), MPB development slowed as temperature exceeded the optimal developmental thresholds of larvae and pupae, resulting in substantially delayed teneral adult emergence. We halted simulations once the warming trend caused teneral adult emergence to be

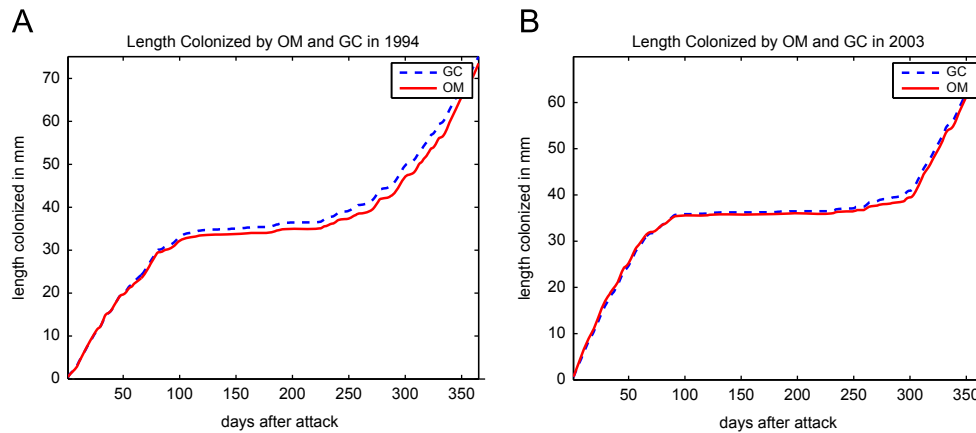


Fig. 9. Exploring the effect of lesion spacing on fungus prevalence for two anomalous years, 1994 (A) and 2003 (B). The relative position of the cumulative growth curves for *G. clavigera*, GC (dashed line), and *O. montium*, OM (solid line), and how they transition between rapid fall growth, slow winter growth and rapid spring growth are able to explain how differences in lesion spacings could affect the outcome of the mutualism.

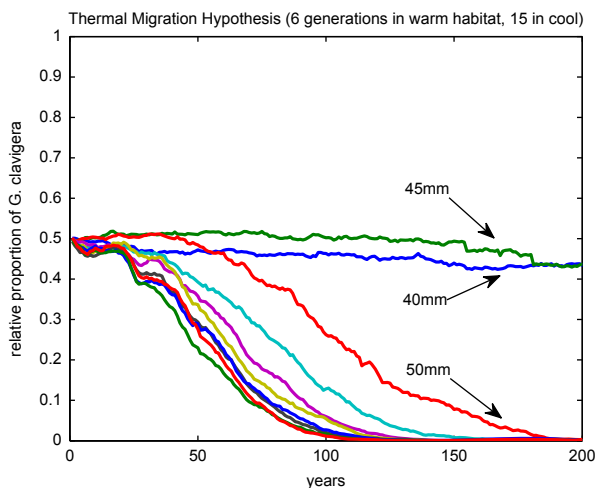


Fig. 10. Result of thermal migration simulation after adding a 1 °C per 10 year warming trend. Close lesion spacing (40–45 mm) continues to stabilize the system. At higher lesion spacings (55–85 mm), however, the continued warming caused *G. clavigera* to lose its advantage in the cooler habitat causing it to be lost from the mutualism over time.

delayed beyond the next year. For larger lesion spacings, the warming trend caused *G. clavigera* to lose its advantage in the cooler habitat, removing the possibility for it to make up for losses in prevalence unless MPB attack density remained extremely high. This means that over time, *G. clavigera* will likely be forced from the MPB–fungus mutualism unless there is a stabilizing mechanism distinct from temperature or one that can override the effects of temperature.

Our results indicated that of the three forms of temperature variability tested, the thermal migration hypothesis was capable, on its own, of stabilizing the system over the long term. Inter-year variability could potentially act in the same way, provided there are sufficiently cool and warm years, though none of the observed years in our dataset from the SNRA were cool enough to allow *G. clavigera* to increase significantly in prevalence and the RRR years were not warm enough for *O. montium* to increase in prevalence. We also acknowledge that MPB attack density does not remain constant and it is possible that changes in attack density along with changes in temperature could act to stabilize the system. In particular, attack density increases during population eruptions and the cycle of endemic to outbreak population phases this insect is known for may also help to stabilize the

system. Nonetheless, if climate change results in *G. clavigera* being forced out of the mutualism, at least in portions of the MPB range, there could be important ramifications for MPB and pine trees. For one, *G. clavigera* is thought to be nutritionally superior to *O. montium* and supports greater beetle fitness (Six and Paine, 1998). Completely replacing *G. clavigera* with *O. montium* could result in lower fecundity and survival of MPB, affecting its ability to respond to conditions that would normally support an outbreak. It may also diminish environmental buffering by reducing the symbiont community associated with the beetle to one species with a reduced environmental amplitude (Six and Bentz, 2007).

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