



Commentary

Home on the (expanding) range: evaluating the effectiveness of a novel host's induced defenses against the mountain pine beetle–fungal complex

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The mountain pine beetle (MPB; *Dendroctonus ponderosae*) is one of the most destructive forest pests, responsible for the death of billions of coniferous trees from Mexico to Alaska (Bentz et al. 2009). Behavioral plasticity helps the beetle sustain endemic levels until the right conditions are met, thus releasing constraints on population growth and resulting in a population eruption (Raffa et al. 2008). These large-scale outbreak events have resulted in significant impacts on ecosystem function and negative effects on local and regional economies (Ayles and Lombardero 2000, Kurz et al. 2008, Edburg et al. 2012). While the mechanisms contributing to bark beetle outbreaks are complex (Raffa et al. 2008, Bleiker et al. 2014), predicted increases in mean annual global temperatures will influence insect population success and expansion both directly via changes in development (Parmesan 2006, Jamieson et al. 2012) and indirectly via altered host defenses (Lusebrink et al. 2016, Erbilgin et al. 2017, Jamieson et al. 2017). The role that plant secondary compounds play during an insect host expansion, however, is unclear, especially for insects that rely on associated symbionts, as is the case for the MPB and its associated blue stain fungi.

Phloem-feeding bark beetles are intimately linked to the defensive chemicals of their hosts, particularly monoterpenes (C₁₀). Monoterpenes play a critical role in beetle behavior, physiology, reproduction and survival by serving as precursors to aggregation pheromones, synergists of pheromones, as well as lethal defenses depending on the composition and vapor concentrations (Seybold et al. 2006). During outbreaks, MPB attack en masse to surpass both physical and constitutive chemical

defenses of healthy well-defended trees, as well as the induced changes in the quantity and quality of a host's terpene and phenolic chemistry (Franceschi et al. 2005). This means that a rapid induced chemical response can mean life or death for both the tree and MPB (Raffa et al. 2008, Boone et al. 2011, Keefover-Ring et al. 2016). Thus, understanding how MPB attack induces changes in defensive chemistry provides important insights into successful beetle colonization and the propagation of mass attacks (Raffa et al. 2005).

Just as individual monoterpenes can have varying effects on MPB, they can also differentially affect the fungal symbionts of MPB. Some host monoterpenes can impede the growth of fungi, which MPB relies on for nutrition, development and survival (Bleiker and Six 2007). Fungi not only provide a nutrient rich food source for bark beetles (Bentz and Six 2006, Adams and Six 2007, Bleiker and Six 2007, Cook et al. 2010, Goodsmann et al. 2012), but can aid in overcoming tree defense (Klepzig and Six 2004, Hammerbacher et al. 2013) and metabolizing toxic monoterpenes (Wang et al. 2013, 2014). While the failure of the fungi would result in the demise of the beetle, the effect of rapidly induced monoterpene production on fungal function during colonization of novel hosts and its effect on the success and spread of the MPB into new habitats remains to be elucidated.

In Canada, the MPB has expanded its range from lodgepole-dominated forests through a lodgepole-jack pine (*Pinus banksiana* Lambert) hybrid zone into jack pine forests (Cullingham et al. 2011). The ecological and economical impacts of this range expansion have the potential to be disastrous (Ono 2003).

One possibility is that this insect–pathogen complex has capitalized on the naïveté of novel host plants and will continue to exploit their lack of co-evolved defenses relative to their traditional host (Mooney and Cleland 2001). Hybrid zones would then serve as effective bridges between potential hosts during range expansions, especially considering that lodgepole and jack pines share similar secondary defense compounds necessary for pheromone production (Lusebrink et al. 2013, Erbilgin et al. 2014). Jack pine has also proven to be a suitable host to all three MPB-associated blue stain fungi, which grow better in jack pine relative to lodgepole (Rice et al. 2007). It is now not a question of whether jack pine can serve as a host, but rather what interspecific factors are driving the infestation and how might tree defenses influence potential outbreak events?

It is unclear how induced secondary compounds in naïve host pines affect MPB-vectored fungal function. As a consequence, there is a major gap in our understanding of primary drivers of the beetle–fungal complex expansion. In this issue of *Tree Physiology*, Cale et al. (2017) begin to address this discrepancy by coupling fungal inoculations of lodgepole and jack pine in the field with a series of laboratory bioassays to assess how induced defenses affect the growth and reproduction of three strains of MPB-associated blue stain fungi. The most aggressive MPB symbiont, *Grosmannia clavigera*, was inoculated at several densities representing various attack pressures during host colonization. Beyond a critical threshold of attack a tree's defenses can become exhausted and overwhelmed, ultimately leading to tree mortality (Raffa and Berryman 1983). Interestingly, monoterpene profiles did not differ in either lodgepole or jack pine as a function of attack density, with the exception of myrcene in jack pine. Myrcene, a pheromone synergist, increased 500% from the lowest to the highest inoculation density in jack pine, but was still produced in lower amounts relative to lodgepole. Attack density had no effect on α -pinene, a precursor to the synthesis of the aggregation pheromone *trans*-verbenol, but total induced α -pinene was 3000% higher in jack pine than lodgepole supporting previous work demonstrating that higher levels of α -pinene in the novel host facilitates greater beetle colonization via pheromone production (Erbilgin et al. 2014). The authors note that combined with high levels of α -pinene, greater induction of myrcene at higher attack densities (Figure 1) could synergize aggregate cues thus promoting greater colonization in jack pine. Furthermore, this study confirms that jack pine does not rapidly produce significant levels of limonene following attack relative to lodgepole (Figure 1). Limonene is known to be toxic to bark beetles and characteristic of beetle resistant trees (Raffa and Berryman 1983, Raffa et al. 2005, Boone et al. 2011, Manning and Reid 2013), thus this may prove to be a dangerous limitation to jack pine's resistance strategy.

Once MPB do successfully attack a tree and subsequently inoculate their host with one or more fungal species, the next step is to determine how tree chemistry affects the growth and

reproduction of the fungus. Cale et al. (2017) address this issue with a set of bioassays where growth and reproduction of three main MPB-vectored fungi are measured when grown on media amended with either low or high levels of the three most important host monoterpenes: myrcene, limonene and α -pinene. The two levels reflected the low and high induction levels observed in response to simulated MPB attack of each monoterpene separately for lodgepole and jack pine, representing the extremes that fungi would encounter. While both levels of all three monoterpenes affected growth, reproduction or both of all three fungal species, the magnitude and directionality of these responses varied (Figure 1). Overall, results from the fungal bioassays indicate that jack pine's susceptibility to MPB-vectored fungi is similar to that of lodgepole pine. Given this and the apparent superior defensive chemistry of lodgepole leads Cale et al. (2017) to conclude that MPB will likely expand its range into jack pine forests.

The work by Cale et al. (2017) lays the groundwork for understanding the role of rapidly induced defenses from a novel host in potentially facilitating the spread of MPB beyond its historical range, which seems inevitable with a warming climate. The results of this study highlight the multifaceted nature of induced monoterpenes. For example, chemical responses of individual compounds that benefit the bark beetle via aggregate pheromone production can have concomitant detrimental species-specific effects on fungal growth and/or reproduction (e.g., α -pinene). Given the exclusive reliance of MPB on its symbiotic fungi, it is critical to understand how induced terpenes affect fungal function during colonization and throughout the invasion sequence to better predict MPB success and rates of potential expansion. Cale et al. (2017) provide convincing evidence that interspecific variation in the magnitude and composition of the induced response will likely promote MPB expansion into jack pine stands, but future studies should also focus on identifying the role of intraspecific variability in induced defenses within jack pine populations. Mountain pine beetle has been shown to preferentially attack particular host genotypes (Yanchuk et al. 2008), namely faster-growing families. Recent work, however, suggests that herbivore selection for fast- and slow-growing genotypes can change based on growth stage (juvenile versus adult) and along the course of the outbreak (de la Mata et al. 2017). Like growth, induced defenses are also under genetic control and growth–defense trade-offs, with defense including resin secondary chemistry and not only resin duct characteristics, can have important implications for beetle choice and success. Thus a better understanding of the relationship between growth and chemical defense within populations and the effectiveness of the induced response against the MPB–fungal complex will offer critical insights regarding susceptibility and mechanisms driving the spatiotemporal patterns of outbreaks.

A clear next step is to assess potential synergisms between different induced chemical defense compounds and how this

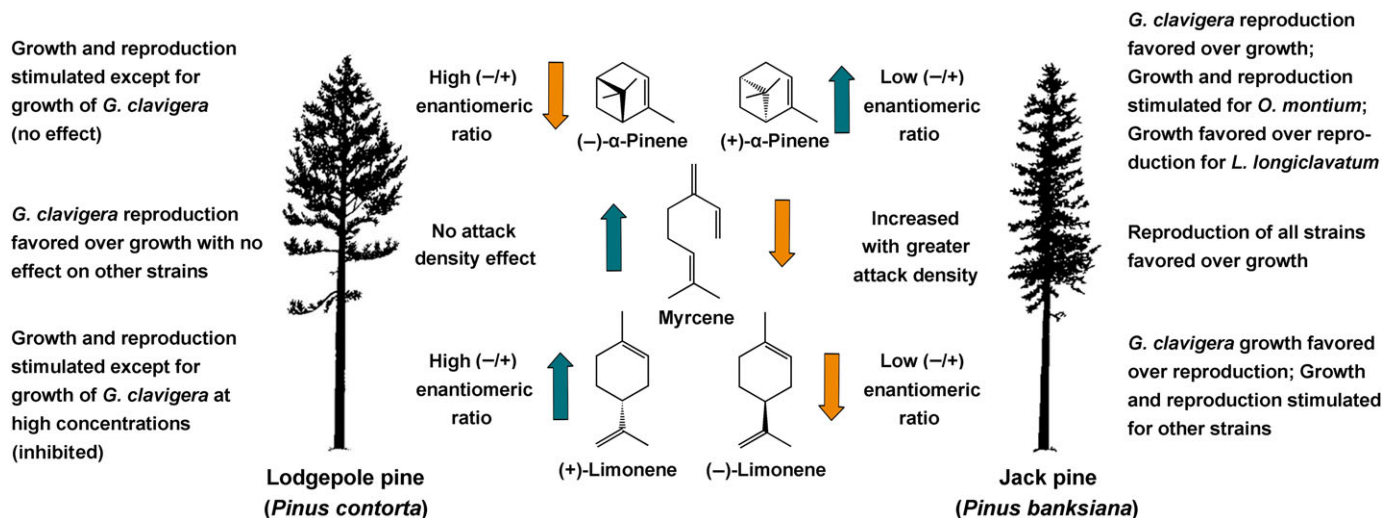


Figure 1. A conceptual model showing the directionality of the induced responses of α -pinene, myrcene and limonene (arrows) for lodgepole pine (left) and jack pine (right) relative to one another following simulated MPB–fungi attack. Text next to the arrows provides information regarding induced changes in the enantiomeric ratios for α -pinene and limonene and the attack density effect for myrcene. General trends for the growth and reproduction of three strains of blue stain fungus associated with MPB (*G. clavigera*, *O. montium* and *L. longiclavatum*) are provided next to each tree species and in line with each compound as reported by Cale et al. (2017) following media amendments with high or low concentrations of each compound.

might enhance resistance or susceptibility to various symbionts. It would also be worth comparing other facets of lodgepole and jack pine's complete defense syndromes, including primary metabolites and other secondary chemistry that may confer differential resistance to MPB and its fungal symbionts (Raffa et al. 2017). Only with this knowledge will we be able to fully assess whether the induced response in jack pine is complementary in its effectiveness against insects and fungi or if trade-offs may be present and subsequently influence the efficacy of both pests (Raffa et al. 2017). While *G. clavigera* is considered the primary invader in lodgepole (Solheim 1995) and was subsequently used to inoculate trees in this study, it is unclear if one of the other two fungal symbionts associated with MPB, *Leptographium longiclavatum* and *Ophistoma montium*, may become more prevalent in jack pine. Thus, it is worth investigating how jack pine responds to the different associated fungi and how the induced responses affect all three MPB-associated blue stain fungi. Finally, successful range expansion of the MPB–fungi complex depends on the beetle and fungi's reproductive rates, density dependencies and dispersal dynamics, all of which are affected by host tree secondary chemistry as well as the interspecific interactions they mediate. Life history trade-offs are likely during range expansion due to these new selection pressures (Burton et al. 2010), and the Cale et al. study sets the stage to address these feedbacks as we seek to predict the dynamics of this complex system.

Conflict of interest

None declared.

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