Chemotypic variations of lodgepole pine affect mountain pine beetle behaviour and growth of its symbiotic fungus

by

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Abstract

Plants generally show large chemotypic variations in susceptibility to phytophagous insects and pathogens. Plant chemical defenses, or secondary compounds, are important components of plant resistance to pest organisms. Among plants, coniferous trees produce complex oleoresins that contain toxic chemicals. Terpenes are the main components of oleoresins and mainly consist of volatile monoterpenes, semi-volatile sesquiterpenes, and non-volatile diterpene resin acids. Collectively, these terpenes play a critical complementary role in the defenses of conifers. Lodgepole pine is one of the most abundant and widespread conifer species in western North America. It shows strong constitutive and inducible defense responses to plant pathogens and herbivorous insects, particularly bark beetle species. The mountain pine beetle is one of the primary insect enemies of lodgepole pine. The mountain pine beetle is a native forest insect species in western North America, and during periodic outbreaks it can kill millions of pine trees. Yet, some individual trees survive from these high-density bark beetle attacks. Mechanisms driving the survival of these pine trees are not clear but are mostly likely related to their defense chemicals.

In this study, we utilized the natural variation in chemical defenses of lodgepole pine trees from progeny trials in northern Alberta. These were grouped in four clusters (chemotypes) based on the composition of mainly constitutive monoterpene concentrations using cluster analysis. We selected representative pine families in each cluster (total 11) and used their profiles in laboratory bioassays. Our goal was to determine whether chemotypes of lodgepole pine differentially affect performance of mountain pine beetle and its major fungal symbiont, *Grosmannia clavigera*. We hypothesized that the chemotypic variations in lodgepole pine families differentially influence host acceptance by mountain pine beetle and growth of *G. clavigera*.

We conducted two bioassays to assess the impact of chemotypes on the performance of mountain pine beetle and *G. clavigera*. For the mountain pine beetle assays, we used a diet that consisted of phloem and sapwood, mixed with agar and water. For *G. clavigera*, we prepared malt extract agar. In each bioassay, we either placed adult beetles or inoculated fungi on artificial diet amended with the monoterpene concentrations representing each of four pine clusters or 11 lodgepole pine families. We measured beetle egg gallery length and weight change as a proxy to host selection by beetles as well as fungal growth as a proxy to fungal responses to host chemistry. We found a significant effect of chemotypes on beetle egg gallery length and weight change. Three pine families were least suitable for beetle performance and two families were least suitable for the fungal growth. The families which showed the least suitability for beetle host acceptance had higher concentrations of limonene, γ-terpinene, 4-allylanisole, β-pinene, terpinolene, and cymene. The families with the lowest fungal growth had higher concentrations of α-pinene, 3-carene, camphene, myrcene, γ-terpinene, bornyl acetate, borneol, and total monoterpenes.

Overall, our study is the first to demonstrate how host phytochemistry differentially affects a bark beetle species and its fungal symbiont. Furthermore, this study reveals that performance of both mountain pine beetle and *G. clavigera* can be affected by host chemotypes. Pine families which show low suitability for both beetles and its symbiont can be potentially used in restoration of mountain pine beetle impacted forests in Alberta to promote beetle-resistant forests.

Preface

This thesis contains original work done by Aziz Ullah and has been written according to the guidelines for a thesis format of the Faculty of graduate Studies and Research at the University of Alberta. The concept and idea of this work originated from Aziz Ullah, his supervisor Dr. Nadir Erbilgin and his colleague Dr. Jennifer Klutsch of the University of Alberta. The whole thesis is composed of a single chapter.

This dissertation is dedicated to my beloved family

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Table of Contents

List of Tables

List of Figures

Introduction

Plants vary in their susceptibility to phytophagous insects and plant pathogens (Züst & Agrawal 2017). The presence of such variations in plants has long been known and has led to the development of resistant plant varieties against pests (Gerhold & Schreiner 1966; Wagner et al. 2002). These variations are critical for sessile plants as often, diverse physiological, morphological, and chemical responses improve their phenotypic plasticity and increase their survival probability in adverse conditions (Schlichting 1986; Agrawal & Fishbein 2006; Mithöfer & Boland 2012; Fürstenberg-Hägg et al. 2013). These abilities of plants are known to vary among different populations within a species (McKey 1979; Fajer et al. 1992) and can potentially contribute to further phenotypic variations that may result in speciation (Agrawal 2001).

Herbivorous insects depend on plants to acquire their nutrient needs (Mitter et al. 1991; Price 1991; Rosenheim 1998; Novotny & Basset 2005). However, insects also have to face defensive chemistry of host plants (Ayres et al. 2000; Sterner & Elser 2002; Agrawal & Weber 2015; Raguso et al. 2015; Plassard 2018). Generally, insect herbivores prefer more nutritious plants with less defense capabilities over plants less nutritious but more defensive (Waring & Cobb 1992; Ayres et al. 2000; Agrawal & Weber 2015). Plants use a variety of ways to defend themselves from herbivores. Plant secondary compounds are important components of plant defenses to insect pests (Hanover 1971; Agrawal & Fishbein 2006; Whitehead & Bowers 2014; Raguso et al. 2015). Plant species rely on different secondary compounds to protect from enemies depending on the availability of resources, life history strategies, and plant-insect co-evolutionary interactions (Herms & Mattson 1992; Gershenzon 1994; Franceschi et al. 2005; Moreno et al. 2009; Whitehead & Bowers 2014; Kessler 2015; Raguso et al. 2015; Raffa et al. 2017; Jia et al. 2018). Primarily, defense compounds include nitrogen containing alkaloids, nonprotein amino

acids, amines, cyanogenic glycosides, and glucosinolates or carbon containing terpenes, flavonoids, polyacetylenes, and phenylproponoids (D'Auria & Gershenzon 2005). These compounds have vast diversity (Dixon 2003) and can have adverse effects against insect herbivores (Wink 1988, 2010; Evensen et al. 2000; Seybold et al. 2006; Lämke & Unsicker 2018). The effects include mortality, fecundity reduction, growth inhibition, and altered insect behavior (Dudareva et al. 2006; Arimura et al. 2009; War et al. 2012).

Plant chemical defenses can be constitutive (pre-existing) and induced (Edwards & Wratten 1985; Karban & Myers 1989; Dixon & Paiva 1995; Franceschi et al. 2005; War et al. 2012). Constitutive defenses are pre-formed in plants such as specialized cell walls, waxy epidermal cuticles, shells, trichomes, thorns, different bark types and secondary compounds. For example, pine tissues contain constitutive monoterpenes that provide immediate resistance to pest attacks (Franceschi et al. 2005). While inducible plant defenses are temporary chemical arbitrations initiated by a stress thereby making the plant less suitable for attack by insects or pathogens (Loughrin et al. 1994; Agrawal 1998; Wittstock & Gershenzon 2002; Durrant & Dong 2004; Kaplan et al. 2008). Most herbivorous insects, however, have evolved counter-resistance traits that may offset or minimize the damage by these plant defenses (Hilder & Boulter 1999; Bede et al. 2006; Despres et al. 2007; Pieterse & Dicke 2007; Reid & Purcell 2011).

Conifer-bark beetle (Coleoptera: Curculionidae, Scolytinae) interactions have received great attention over the last 50-60 years due to the economic and ecological significance of the species involved (Wood 1982; Christiansen et al. 1987; Raffa & Berryman 1987; Paine et al. 1997; Franceschi et al. 2005; Kurz et al. 2008). Oleoresins are widely considered the most important component of conifer defenses to bark beetles and contain some of the most toxic terpenoids including monoterpenes, sesquiterpenes, and diterpenes (Raffa & Berryman 1982, 1983; Croteau

et al. 1987; Raffa & Smalley 1995; Phillips & Croteau 1999; Martin et al. 2002; Raffa et al. 2005, 2017; Keeling & Bohmann 2006; Carmona et al. 2011; Chiu et al. 2017; Erbilgin 2019), and their roles in conifer defenses are well documented (Kolosova & Bohlmann 2012).

Conifer secondary chemistry is influenced by both genetic and environmental factors, and their interactions, and it shows a large inter- and intra-specific variation (Hanover 1971; Forrest 1981; Taft et al. 2015). In fact, some of the variation in secondary compounds within in conifers is often viewed as a result of co-evolutionary interactions with bark beetle enemies (Moore et al. 2014; Moreira et al. 2014). However, the amount of variation within a conifer species in the context of potential defenses against forest insects including bark beetles have been rarely investigated (Erbilgin et al. 2017; Erbilgin 2019).

In North America, conifer trees are widely distributed (Critchfield 1980) and lodgepole pine (*Pinus contorta* Dougl. ex Loud.) is one of the most abundant and widespread species in western North America. Lodgepole pine exists in several regional forms, exhibiting geographic unit and heritable differences, and meriting recognition as four subspecies (spp.) (Critchfield 1980), which are: (1) Coastal region (shore pine) *P. contorta* Douglas ex Loudon spp. *contorta*, (2) Rocky mountains: *P. contorta* spp. *latifolia* (Engelm. Ex Wats.) stat. nov., 3) Mendocino white plains: *P. contorta* spp. *bolanderi* (Parl.) stat. nov., and 4) Sierra Neveda *P. contorta* spp. *murrayana* (Balf.) stat. nov. Among the four subspecies (Fig. 1-B), "*latifolia*" is the most abundant in North America (Critchfield 1985; Rehfeldt et al. 1999). The present distribution of *P. contorta* spp. *latifolia* extends from near latitude 64°N in the Yukon to latitude 37°N in southern Colorado (Ying & Liang 1994; Rehfeldt et al. 2001).

In western Canada, *P. contorta* spp. *latifolia* is the dominant lodgepole pine species which represents a key forest resource on over 26 million ha which is 22% of the total forest (Fig. 1) and

is primarily located in British Columbia and Alberta (Critchfield 1985). In Alberta, lodgepole pine grows on the eastern slope of the Rocky Mountains on four phytogeographic divisions (Montane, Subalpine, High Foothills, and Low Foothills) and some outlier areas (Tait et al. 1988). Due to its socio-economic importance, lodgepole pine is the second- most important reforestation species and covers around 22.3% of forests in Alberta (Safranyik at al. 2010). Lodgepole pine can be grown in wide range of soil types (Carlson et al. 1999). It also naturally shows strong constitutive and inducible defensive responses to bark beetles (Moreira et al. 2014; Burke et al. 2017; Klutsch et al. 2017b; Erbilgin 2019).

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins, MPB) is one of the primary native insect enemies of lodgepole pine (Wood 1982; Safranyik et al. 2010), and during periodic outbreaks, kills millions of pine trees (Raffa et al. 2008; Bentz et al. 2010; Safranyik at al. 2010). Beetles require living pine trees for successful reproduction. After emergence from natal trees, the pioneering female MPB flies and lands on a host tree and may either enter the host or fly away. However, if she enters the host, the beetle may still abandon the host if it finds it unsuitable after the initial feeding on the host phloem. Upon host acceptance, the female beetle competes with the host tree defenses, produces, and releases pheromones that attract both sexes of beetles and mates (Blomquist et al. 2010; Safranyik et al. 2010) (Fig. 2). Beetles oviposit eggs, emerging larvae feed on the phloem and spend the winter under bark and continue feeding in the spring until they transform into pupae in June and early July. In western North America, MPB is an obligate herbivore that has co-evolved with lodgepole pine (Raffa & Berryman 1983; Raffa et al. 2008, 2013).

Over the past four decades, climate change has increased MPB-suitable habitat by an estimated 75% (Bentz et al. 2010) which has amplified the beetle's access to pines at higher elevations and in more north-eastern locations, where only irregular exposures have previously

been recorded (Cudmore et al. 2010; Cullingham et al. 2011; Raffa et al. 2013). This aggressive bark beetle has caused significant impact to local lodgepole pine forests and trees on over millions of hectares have been affected during the last outbreak (Bentz et al. 2010; Safranyik at al. 2010). The beetles also vector mutualistic microorganisms (mostly fungi), some of which contribute to tree mortality while others benefit beetles as supplementary nutritional source (Ayres et al. 2000; Raffa et al. 2005; Bleiker & Six 2007; DiGuistini et al. 2007; Goodsman et al. 2012; Cale et al. 2017). The primary fungal symbionts of MPB are *Grosmannia clavigera* (Robinson-Jeffrey & Davidson), *Leptographium longiclavatum* Lee, Kim & Breuil, and *Ophiostoma montium* (Rumbold) von Arx (Whitney & Farris 1970; Paine et al. 1997; Lee et al. 2006). Among MPB fungal symbionts, *G. clavigera* is a widely spread fungus in Alberta (Roe et al. 2011). During host colonization, the beetles inoculate fungal symbionts into the tree and the resulting fungal colonization further reduces host tree resistance by impeding conduction, and thus assisting MPB in overcoming host tree defenses (Raffa & Berryman 1983; Ayres et al. 2000; Raffa et al. 2005; Bleiker & Six 2009). For instance, although limonene is known to be toxic to MPB (Chiu et al. 2017; Reid et al. 2017), *G. clavigera* uses it as a carbon source, which benefits the beetles indirectly (Wang et al. 2013; Cale et al. 2017).

In our investigations, we primarily focused on monoterpenes in the context of lodgepole pine defenses against MPB and its vectored fungus *G. clavigera*. Monoterpenes act as both physical and chemical barriers against MPB and its fungal associates (Yanchuk et al. 2008; Manning & Reid 2013; Chiu et al. 2017; Reid et al. 2017; Erbilgin 2019). For instance, Manning & Reid (2013) reported that monoterpenes show inhibitive effects on MPB gallery establishment and oviposition. In fact, Raffa & Berryman (1983) reported that resistant lodgepole pine trees produce high levels of α-pinene and limonene, which is further verified in field by Erbilgin et al.

(2017). However, monoterpenes could also have positive consequences on MPB and its vectored fungi (reviewed by Seybold et al. 2006). For example, female beetles metabolize the host monoterpene α-pinene to produce its aggregation pheromone, *trans*-verbenol (Pitman 1971; Miller & Lindgren 2000; Blomquist et al. 2010). Moreover, myrcene and terpinolene added to *trans*-verbenol and *exo*-brevicomin (male produced pheromone) showed more attractiveness to MPB (Billings et al. 1976; Miller & Lindgren 2000; Borden et al. 2008; Klutsch et al. 2017a).

Previous studies showed that lodgepole pine has large chemotypic variations in its range (Yanchuk et al. 2008; Ott et al. 2011; Moore et al. 2014; Erbilgin et al. 2017; Six et al. 2018). Such variation within a tree species generally reflects differences in environment and growing conditions (Moore et al. 2014). Chemotypic variations of lodgepole pine show biological consequences on MPB survival and outbreak (Erbilgin et al. 2017). Chemotypes are defined as chemically distinct phenotypes in a plant species, with differences in the composition of the secondary chemicals (Erbilgin 2019). For instance, Yanchuk et al. (2008) observed variations in lodgepole pine mortality from MPB in British Columbia and suggested that surviving trees likely have different chemical profiles than those killed by MPB during the outbreak.

In a recent review paper, Erbilgin (2019) provides substantial evidence of why particular chemical profiles (or chemotypes) of host trees matter in tree resistance to bark beetles. Briefly, concentrations of some individual monoterpenes matter for the bark beetles and their fungal symbionts but the overall secondary chemistry profile of trees seems to be more important than individual monoterpenes because individual monoterpenes play different roles in different stages of host colonization by bark beetles (Pureswaran & Borden 2005; Erbilgin et al. 2006, 2017). For instance, Erbilgin et al. (2017) investigated the mechanism underlying the ability of lodgepole trees to survive during MPB outbreaks in the field following observations that some pine trees

survived at the high density MPB attacks in western Alberta. They retrospectively deduced whether phytochemicals underlie survival of trees by comparing their chemistry to that of non-attacked trees in the same stands. They also compared MPB attack characteristics between resistant and beetle-killed trees. Beetle-killed trees had more beetle attacks and longer egg galleries than resistant trees, which also lacked the larval establishment found in beetle-killed trees. Resistant trees contained high amounts of toxic and attraction-inhibitive compounds and low amounts of pheromone-precursor and synergist compounds. Based on these results, they proposed a possible phytochemical mechanism to explain the roles of individual monoterpenes at different stages of MPB host colonization. The study concluded that the variation of chemotypic expression of local plant populations can have profound ecological consequences including survival during insect outbreaks. These results are further supported by investigations that these surviving trees also have different anatomical defense structures including resin ducts than those killed by MPB (Zhao & Erbilgin 2019; Zhao et al. 2019). Likewise, Six et al. (2018) found clear genetic differences between beetle-killed and surviving lodgepole pine trees in Montana (USA).

In this MSc thesis, I used the diverse monoterpenoid compounds of lodgepole pine as a proxy to study the effect of lodgepole pine chemotypic variations on MPB and *G. clavigera*. We conducted bioassays in the laboratory due to the difficulty of testing tree resistance to bark beetles in the field (Erbilgin et al. 2006, 2017; Chiu et al. 2017; Reid et al. 2017; Erbilgin 2019). Our bioassays were designed to screen out the resistant and susceptible chemotypes and families of lodgepole pine against MPB and *G. clavigera*. In particular, we investigated how the constitutive monoterpene profiles of mature lodgepole pine trees affect the host acceptance (egg gallery construction and feeding) of MPB and growth of *G. clavigera*.

Thesis aims and objectives

In a related project, we developed secondary chemical profiles of 40 families of lodgepole pine in Alberta (Fig. S1). Using a cluster analysis, we found that lodgepole pine trees have four different chemotypes based on the individual monoterpene concentrations. In our investigations, we used these distinct chemical profiles to test the effect on insect performance. We hypothesize that the chemotypic variations in lodgepole pine families differentially influences host acceptance by MPB and growth of its fungal symbiont *G. clavigera*. We screened out the lodgepole pine families and chemotypes that had the most negative and positive consequences on the host acceptance by MPB. These resistant chemotypes could potentially be used for the development of MPB and *G. clavigera*-resistant lodgepole pine forests in the future. Furthermore, the outcome of this study will also be important to demonstrate how plant chemotypes impact plant-associated communities, such as insects and fungi (Whitham et al. 2008).

Materials and Methods

Progeny trial for lodgepole pine

In Alberta traditional tree improvement programs are under development since the 1970. Several strategies are involved for the development of these programs. Such as, starting from the selecting of wild species, finding parent trees from different sites for progeny trials, and at the end development of seed orchards and formation of breeding. Naturally, tree improvement involves long breeding cycles, but these programs designed to shorten the cycles. Lodgepole pine is one of Canada's primary commercial tree species. Furthermore, Alberta government and forest industry emphasizing their efforts on these programs and investing heavily for its betterment and

sustainability. As of 2014, thousands of hectares have been planted with improved pine seedlings (FGRMS 2009).

Region C Controlled Parentage Program

The C Controlled Parentage Program is the tree improvement program for lodgepole pine in the central boreal region of Alberta. The area of this breeding region covers between 53°59' – 55°17' N and around 114°42' – 117 °6' W with the distribution elevation range of 800 – 1200 m. The Blue Ridge Lumber, a division of West Fraser Mills Ltd. owned this region C tree improvement program, which was started in 198.

Region C progeny test series (G128) comprises 224 1st generation open pollinated families (seeds collected in the wild). In the spring of 1982, the tests were field planted in four test sites with 1+0 containerized seedlings. The following test design was used: Randomized control block design (with sets-in-reps), 5 replications, 4-tree row plots and with trees spaced at 2.5×2.5 m. Data were collected at least at age 9 and 30 for height, dbh (diameter at breast height), and western gall rust (*Endocronartium harknessii* JP Moore) infection. The site locations can be seen in Table S1 and Figure S2.

After chemical analysis of phloem tissue (see methods below), we selected one site (Judy Creek) to assess its impact on mountain pine beetle and its symbiont from a progeny trial in northern Alberta (Table S1). The Judy Creek site had on average a moderate concentration of total monoterpenes (5,783.20 ng/mg fresh weight, FW) compared to the other sites (site average range = 4330.11 ng/mg FW – 6294.94 ng/mg FW). Therefore, our bioassays would represent the average monoterpene concentrations found in the progeny test population.

Lodgepole pine phloem collection and chemical analysis

We sampled the lodgepole pine trees from a progeny trial in northern Alberta (54°24' N, 115°34' W; elevation 1097 m) for defense chemistry in July 2017. The site was composed of 35 yr-old pines planted in row plots of four trees from 232 families and replicated five times. To make sure the range of variability among pine families was sampled, we selected 40 families and 10 trees per family (n=373 total due to some missing trees).

We sampled phloem at 1.4 m height up on the north-facing side of stems of trees using a cork borer (1.9 cm in diam.). The samples were immersed immediately in liquid nitrogen, transported on dry ice, and stored at -40°C until processing. After removing the outer bark, we ground the phloem tissue to a fine powder with a mortar and pestle in liquid nitrogen, extracted, and analyzed compounds using established methods (Klutsch et al. 2016; Erbilgin et al. 2017). Briefly, 100 mg of fresh phloem tissue was extracted in 0.5 ml hexane with internal standard of 0.004% pentadecane. Each sample was vortexed with solvent for 30 s, left at room temperature in a sonic bath for 10 min, and centrifuged at 16,100 rcf at 0°C for 15 min.

To identify chemical compounds, mainly monoterpenes we ran a sub-sample of extracts on a Gas Chromatograph/Mass Spectrometer (GC/MS, Agilent 7890A/5062C, Agilent Tech., Santa Clara, CA, USA). After identification with authentic standards, we used a GC/Flame Ionization Detector (GC/FID, Agilent 7890B) to run all the samples. The method used for both GC/MS and GC/FID was as follows: Sample extract (1 µl) was injected with a split injection (10:1) into the GC equipped with an HP-Innowax column (I.D. 0.25 mm, length 30 m) (Agilent Tech.) with helium carrier gas flow at 1.1 ml min^{-1}, and a temperature of 40°C for 1 min, increased to 55°C by 30°C min^{-1} and held for 0.5 min, increased to 122°C by 8°C min^{-1} and held for 2 min, increased to 200°C by 10°C min^{-1}, and then to 260°C by 20°C min^{-1} and held for 1 min. To identify

and quantify individual compounds, we used authentic standards: borneol (Chemical Purity: 99%), α-terpinene (95%), γ-terpinene (97%), α-terpineol (90%) (Sigma-Aldrich, St. Louis, MO, USA), 3-carene (98%), terpinolene (90%), α-pinene (98%), β-pinene (98%), limonene (99%), myrcene (90%), camphene (90%), *p*-cymene (99%), 4-allylanisole (98%) (Fluka, Sigma-Aldrich, Buchs, Switzerland), bornyl acetate (97%), (SAFC Supply Solutions, St. Louis, MO, USA), and β-phellandrene (77%) (Glidco Inc., Jacksonville, FL, USA). The units were based on fresh weight.

Determination of chemical clusters

To define chemotypes in the 40 pine families, we used natural log transformation for monoterpene concentrations, to meet assumptions of normality and homogeneity of variance. We then calculated the best linear unbiased predictors (BLUPs) using a mixed linear model with block and presence of western gall rust infection as a fixed effect and a half-sib pedigree as a random effect (ASReml-R ver. 4.1 in R ver. 3.4.0).

We used the standardized family estimated breeding values to perform a partitioning around medeiod clustering analysis, which minimizes the sum of dissimilarities (fpc package ver. 2.1-11.1). We estimated the optimum number of clusters using the average silhouette width. We used a Duda-Hart test to determine whether there was more than one cluster (P=0.05). From this cluster analysis, we identified four clusters composed of pine family estimated breeding values (Fig. S1A).

To determine the individual concentrations of chemical compounds to use in bioassays, we used the average sum of monoterpene concentrations for each family and identified the 5th, 50th, and 95th percentiles of each of four clusters (Fig. S1B; Table S2). Having three representative

families per cluster allowed us to test the range of concentrations within each cluster. Cluster 4 contained only two families (Fig. S1A) and thus we only have two representative families for this particular cluster. We used the family mean for each compound in the selected percentiles in fungal and MPB bioassays as described below, except for β-phellanderene. The market value of β-phellanderene was so high, we were unable to use this compound in our main bioassays. However, we used β-phellanderene for separate dose response studies in order to observe its effect on MPB and its symbionts.

Testing the effects of host pine monoterpenes on MPB host acceptance

To test the effects of particular pine families or chemotypes, we used a bioassay that consists of media (9:1 ratio of phloem:sapwood mixed with agar and water) amended with host chemicals in Petri dishes (60 mm diam. × 15 mm height) (Fisher Scientific, Toronto, ON, CAN.). We obtained phloem and sapwood (xylem) samples from healthy lodgepole pine trees, and freeze-dried them for 72 h. The samples were then ground using TissueLyser II (Qiagen, Montreal, QC, CAN), sieved through a 0.5 mm mesh, and autoclaved at 105°C for 20 min before use. This sampling process likely removed majority of volatile and semi-volatile compounds including monoterpenes and sesquiterpenes from the tissues and retained non-volatile compounds like acids and phenolics compounds (Wallin & Raffa 2000). We prepared the MPB diet stock solution separately for each of the 11 pine families (see details below). For each stock solution, we dissolved 2 g of Bacto-agar (Fisher Scientific) in 60 ml boiling double distilled water, and added 4 g of ground phloem and sapwood (ratio 9:1) to the mixture as described in Wallin & Raffa (2000) and Kopper et al. (2005).

Since all 11 families contained the same compounds, we developed a blend of compounds representing each of the 11 families based on the constitutive concentrations of individual

compounds (Fig. 3A; Table S2). Each blend contained 14 compounds, including α-pinene (98%), camphene (98%), 3-carene (95%), myrcene (96%), α-terpinene (95%), γ-terpinene (97%), *p*-cymene (98%), terpinolene (94%), bornyl acetate (97%), 4-allylanisole (98%), α-terpineol (90%), borneol (99%), (−)-β-pinene (94%), and limonene (95%). Liquid monoterpenes were measured using a micropipette and solid monoterpene weighed in an analytical balance (Mettler Toledo, XPE105 Delta Range®, Max.=41g/120g, d=0.01mg/0.1mg). After allowing the artificial diet to cool down to room temperature, we prepared a stock solution of monoterpenes representing each of the 11 families by mixing all 14 chemical compounds including 1 ml of ethanol (for dilution) in the artificial diet. We used diet without monoterpenes (blank-control) and diet with 1 ml of ethanol (ethanol-control) as the two control treatments in all experiments (n=18 per cluster, n=6 per family; total 108 plates: 72 treated +18 blank-control + 18 ethanol-control).

We allowed the assay unit to dry under a fume hood for 4 h at room temperature. We then flipped the dishes to allow the diet to fall onto the lid in order to leave 2 mm space around the diet and the edge of the dish. We weighed one adult female MPB in an analytical balance (Mettler Toledo, XPE105 Delta Range®, Max.=41g/120g, d=0.01mg/0.1mg) and placed it at the center of each assay unit. Beetles were randamoly selected and were not significantly different from each other. After 48 h at room temperature, we examined the female egg gallery length and determined the post-treatment beetle weight from each dish. We compared female egg gallery length and beetle weight change (difference between post and pre-feeding weights) among different clusters and pine families.

Testing the effects of different β-phellandrene concentrations on MPB host acceptance

Since β-phellandrene was not included in the blend above, we conducted a separate study on different concentrations of β-phellandrene to test its impacts on MPB host acceptance behavior. We used low (960.0 ng/mg), medium (2,690.0 ng/mg), and high (4,079.0 ng/mg) concentrations of β-phellandrene (n=5/treatment). The concentrations represented the lowest, median and the highest concentrations of all pine trees sampled in the current study. We prepared the same beetle diet as described above and mixed low, medium, or high concentrations of β-phellandrene separately in the diet. We used diet without monoterpenes and diet with ethanol as our two control treatments.

Testing the effects of different concentrations of monoterpenes on G. clavigera growth

The blend used in the beetle bioassays above was tested for effects on the fungal growth. We prepared the malt extract agar (MEA: malt extract 30 g and agar 20 gL^{-1}), and then autoclaved it at Liquid20 autoclave cycle. It was then cooled down at room temperature. After that, we added the monoterpene blend of each family into the stock MEA media separately according to the family-specific concentrations (Table S2). Then, we shook the stock media for three minutes before pouring into the Petri dishes (100 mm diam. × 15 mm ht.). We poured 20 ml MEA media from stock solution into Petri dishes by using sterilized syringes. The media was dried for 24 h at 23 °C. These dishes were ready for fungal inoculation after 24 h. The two different isolates of *G. clavigera*, tested are stored in the Erbilgin laboratory (Table S3). We inoculated the nine-day-old culture from the advancing edge of an actively growing colony of each isolate into each dish separately. We used media without monoterpenes and diet with ethanol as our two control

treatments in all experiments (n=18 per cluster, n=6 per family; total 108 plates: 72 treated +18 blank-control + 18 ethanol-control).

After four days we assessed the growth of *G. clavigera* in the media, digitally scanned the fungal growth in the dishes, and quantified the growth by using ImageJ software (National Institute of Health, Bethesda, MD, USA) (Abràmoff et al. 2004). Finally, we determined the are of growth of fungi on the media and compared this among pine families and clusters.

Effects of different enantiomers of α-pinene, limonene, and camphene on G. clavigera growth

The aim of this experiment was to determine whether the chirality of constitutive monoterpenes of lodgepole pine matters for the growth of *G. clavigera*. The concentrations 340.0 ng/mg for (+)-limonene and (−)-limonene, 880.0 ng/mg for (+)-α-pinene and (−)-α-pinene, and 73.0 ng/mg for (+)-camphene and (−)-camphene were used. The concentration of 680.0 ng/mg for racemic limonene, 1760.0 ng/mg for racemic α-pinene, and 146.0 ng/mg for racemic camphene were also tested. Liquid monoterpenes were measured using a micropipette and solid monoterpene such as camphene weighed in an analytical balance (Mettler Toledo, XPE105 Delta Range®, Max.=41g/120g, d=0.01mg/0.1mg). The growth of *G. clavigera* was observed in Petri dishes (100 mm diam. × 15 mm ht.) with MEA amended media one of the above monoterpenes. The concentrations were dissolved in ethanol. The media without monoterpenes and with ethanol served as the two control treatments. Each treatment was replicated five times. The data was recorded after four days, traced and measured through ImageJ software.

Testing the effects of different concentrations of β-phellandrene on G. clavigera growth

Since β-phellandrene was not included in the blend in the fungal bioassay described above, we conducted a separate study on different concentrations of β-phellandrene against *G. clavigera* growth. We used the same concentrations used in the beetle bioassays described above (n=5/treatment). We prepared the same fungal media and fungal isolates as described earlier for this study and mixed each concentration separately in the media. We used media without monoterpenes and media with ethanol as our two control treatments.

Statistical analysis of bioassay results

We calculated descriptive statistics for female beetle egg gallery lengths and weight change, and growth of *G. clavigera.* We checked data for the assumptions of homoscedasticity and normality by using Levene's and Shapiro–Wilk tests, respectively. Where necessary, we (log+1) transformed data prior to analysis.

We tested the impact of cluster and family on MPB egg gallery length, MPB weight change, and fungal growth for statistical significance by ANOVA, followed by *post-hoc* pair-wise differences using Tukey's HSD test. We used one-way ANOVA for different lodgepole pine family treatments tested against fungal growth, MPB egg gallery length and MPB weight change. In addition, we used ordinations to explore and visualize relationships among different chemotypes and families against MPB egg gallery length, MPB weight change and on fungal growth. We considered significant differences at $P< 0.05$. Statistical software R v3.4.3 (R Core Team 2017) was used for all statistical analyses.

Results

Lodgepole pine clusters

The cluster analysis identified four separate clusters of 40 lodgepole pine families based on monoterpene concentration estimated breeding values (Fig. S1). Although each cluster has its own unique profile, the general description of each cluster is based on how biologically important monoterpene compounds are to the cluster differentiation (Erbilgin et al. 2017; Raffa et al. 2017; Erbilgin 2019). Therefore, we described the clusters visually assessed from the heatmap (Fig. 3A) as follows: The cluster-1 is characterized by lower concentrations of limonene, myrcene, and α-terpineol. The cluster-2 is characterized by higher concentrations of terpineol and lower concentrations of terpinolene and α-pinene. The cluster-3 is characterized by higher concentrations of limonene, γ-terpinene 4-allylanisole, β-pinene, terpinolene, and cymene and lower concentrations of α-pinene, camphene, and bornyl acetate. The cluster-4 is characterized by higher concentrations of α-pinene, limonene, camphene, 3-carene, bornyl acetate and boreneol, and lower concentrations of cymene, 4-allylanisole and β-pinene (Fig. 3A).

Relationship between MPB egg gallery length and weight change

Overall, we observed a positive correlation between MPB egg gallery length and weight change before and after the feeding bioassays, suggesting that both egg gallery length and weight change of beetles similarly affected by the host monoterpenes amended in the media (Fig. 4). In Figure 4, the points on the negative scales indicate that beetle weight was significantly reduced in some monoterpene mixtures.

Effects of lodgepole pine clusters on MPB egg gallery length

Overall, MPB egg gallery length significantly varied among the four clusters and both control treatments (Fig. 5). Bark beetle galleries in all clusters were significantly lower than either blank- or ethanol-control treatments. Cluster-3 was the least suitable for MPB and had the shortest egg gallery length, followed by clusters 4, 2, and 1. There was no difference among the remaining three clusters. The cluster-3 is characterized by higher concentrations of limonene, γ-terpinene 4-allylanisole, β-pinene, terpinolene, and cymene and lower concentrations of α-pinene, camphene, and bornyl acetate (Fig. 3A; Table S2).

Effects of lodgepole pine family-specific chemical profiles on MPB egg gallery length

The lodgepole pine families having different concentrations of monoterpenes significantly influenced the MPB egg gallery construction (Fig. 6). Beetles did not construct any galleries in family-1799. Among the remaining families, beetles excavated the shortest egg galleries in family 2255 and 2266, followed by 1867. Beetles excavated the longest galleries in family-1046, which was not different from ethanol-control but was significantly shorter than the blank-control. The remaining families had significantly shorter egg gallery lengths than either control treatments. Overall, it appears that as the concentrations of limonene, 4-allylanisole, 3-carene, cymene increased, MPB egg gallery construction decreased (Fig. 3B; Table S2). In contrast, increased concentrations of α-pinene and decreased concentrations of limonene, 4-allylanisole, 3-carene, and cymene resulted in increased construction of egg galleries.

Effects of lodgepole pine clusters on MPB weight change

Weight change of beetles was significantly different among clusters and both control treatments (Fig. 7). We observed a minimum weight change in clusters 3 and 2; these two clusters were also significantly different from either control treatments and from the remaining two clusters. The clusters 1 and 4 were statistically similar; of these two clusters, only the former was different from the blank-control treatment. Clusters 3 and 2 are characterized by higher concentrations of limonene, 3-carene, β-pinene as compared to clusters 1 and 4 (Fig. 3A; Table S2).

Effects of lodgepole pine families on MPB weight change

Overall, MPB weight change significantly varied among ten families and both control treatments (Fig. 8). Beetle weight was significantly reduced in family-1799 which is characterized by the high concentrations of limonene, 4-allylanisole, and cymene, and the lower concentration of α-pinene. Among the remaining nine families, the minimum MPB weight change was observed in family-2266, followed by 1050 and 2255. These three families were not significantly different from each other but were different from either control treatments. Families 1046, 1105, and 2258 did not show any differences from either control.

Effects of β-phellandrene concentrations on MPB egg gallery length

Different concentrations of β-phellandrene significantly influenced the MPB egg gallery length (Fig. 9). Overall, increased concentrations of β-phellandrene resulted in decreased beetle gallery construction and at the highest concentration; beetles excavated the shortest egg gallery. Medium

concentration did not vary from either control treatments, while low concentration was significantly different from control but not from medium concentration.

Effects of β-phellandrene concentrations on MPB weight change

Similar to the beetle egg gallery length, at the highest and lowest β-phellandrene concentration, we observed the minimum MPB weight change (Fig. 10). Medium concentration of β-phellandrene did not result in different MPB weight change from either control treatments.

Effects of pine clusters on G. clavigera growth

The *G. clavigera* growth was significantly influenced by pine clusters (Fig. 11). We observed the lowest fungal growth in cluster-4, followed by cluster-3. These two clusters were significantly different from either control treatments and from the other two clusters. There were no significant differences among the two control treatments and clusters 1 and 2. Overall, higher concentrations of α-pinene, camphene, myrcene, γ-terpinene, bornyl acetate, borneol, and total monoterpenes decreased the *G. clavigera* growth (Fig. 3A; Table S2).

Effects of pine families on G. clavigera growth

Similar to the pine clusters above, pine families also significantly influenced the growth of *G. clavigera* (Fig. 12). With the exception of three families (1048, 1005, and 1046), all other families had significantly lower fungal growth than either control treatments. Among families, two families, namely 1867 and 1865 (both in cluster 4), had the lowest fungal growth and were least

suitable to *G. clavigera.* Overall, families with the lowest fungal growth had a higher concentration of α-pinene, camphene, myrcene, γ-terpinene, bornyl acetate, borneol, and total monoterpenes whereas families with higher fungal growth had lower concentrations of α-pinene and total monoterpenes (Fig. 3 B; Table S2).

Effects of different enantiomers of α-pinene, limonene, and camphene on the growth of G. clavigera

The growth of *G. clavigera* on different enantiomers of α-pinene varied significantly (Fig. 13). Overall, the fungal growth was reduced by the addition of α-pinene. The lowest growth was observed in (+)-α-pinene, followed by (–)-α-pinene and the racemic α-pinene. There was no difference between (–)-α-pinene and the racemic mixture. The addition of ethanol also reduced the growth, relative to the control, but it is much lower than any of the α-pinene treatments.

Likewise, limonene significantly reduced the fungal growth relative to both control treatments (Fig. 14). The lowest growth occurred on the media amended with (+)-limonene, followed by the racemic mixture, and (–)-limonene treatments. Similarly, the addition of ethanol reduced the fungal growth but much lower than any of the limonene treatments.

While all camphene treatments significantly reduced the fungal growth relative to either control treatments, fungal growth was similar among all three camphene treatments (Fig. 15).

Effects of β-phellandrene concentrations on G. clavigera growth

Again, the highest concentration of β-phellandrene significantly reduced the growth of *G. clavigera* relative to either control treatments and the other two concentrations (Fig. 16). The low and medium concentrations did not show any significant differences from either control treatments.

Discussion

Host chemical defenses are critical components of tree resistance to bark beetles and their fungal symbionts and can determine the host colonization success of the bark beetle-fungus complex (Raffa & Smalley 1995; Raffa et al. 2005, 2008; DiGuistini et al. 2011; Erbilgin et al. 2017; Cale et al. 2019). However chemical, and supporting anatomical, defenses of conifers vary substantially, resulting in an array of tree responses to bark beetle attacks (Erbilgin et al. 2014, 2017; Six 2019; Zhao & Erbilgin 2019; Zhao et al. 2019). In order to address whether such variation differentially affects MPB and its primary fungal symbiont *G. clavigera*, we characterized different monoterpene chemotypes in lodgepole pine at the constitutive level and described them by four clusters. These clusters are in agreement with Forrest (1981), who investigated the lodgepole pine monoterpenes across its natural range and found different chemotypes at constitutive level. Following bioassays that incorporated the composition of each cluster demonstrated that MPB and *G. clavigera* differentially responded to each cluster. Although several studies examined the effect of monoterpenes against MPB and its fungal symbionts (Hughes 1973; Gries et al. 1990; Huber et al. 2000; Miller & Borden 2000, 2003; Pureswaran et al. 2004; Cale et al. 2017; Chiu et al. 2017; Reid et al. 2017), these studies only focused on fewer monoterpenes of the host trees. Thus, the outcome of this study has significant impacts on improving host plant-insect interactions

particularly those pertaining to bark beetle-conifer interactions and provides strong empirical evidence for the results of field studies reported earlier (Miller & Borden 2000; Boone et al. 2011; Erbilgin et al. 2014, 2017; Six et al. 2018; Cale et al. 2019).

First, host selections by female MPB begins with random selection of a potential host tree (Hynum & Berryman 1980; Borden et al. 2008). Gustatory and olfactory receptors help the female beetles to assess host suitability (Raffa & Berryman 1982; Bruce et al. 2005). After this stage of host selection, a female beetle either accepts or rejects the host on the basis of mainly monoterpene concentrations and composition (Raffa & Berryman 1983; Wallin & Raffa 2000; Franceschi et al. 2005; Raffa et al. 2005; Boone et al. 2011; Erbilgin et al. 2017). Even after the initial host acceptance, bark beetle success in the host still depends on the quality of host substrate (Borden et al. 2008), resulting in successful and non-successful MPB attacks on host trees even in the same forest stand (Waring & Pitman 1985; Yanchuk et al. 2008; Erbilgin et al. 2017; Six et al. 2018; Zhao & Erbilgin 2019). Currently there is little or no empirical evidence to support the role of host chemical defenses during the early stages of host colonization and establishment (Erbilgin 2019). Thus, our research provides the first empirical evidence explaining why the outcome of bark beetle attacks on apparently suitable host trees shows so much variation in nature. In particular, we showed that biologically important chemicals such as limonene, 4-allylanisole, and 3-carene can influence the initial host colonization success of MPB by reducing its host acceptance and feeding (Erbilgin 2019) and that apparently this is a concentration-dependent process (Erbilgin 2019). Off monoterpenes, limonene is the most toxic to MPB even at low concentration (Chiu et al. 2017; Reid et al. 2017). 4-Allylanisole and 3-carene can also have an inhibitory effect on beetles and negatively affect their feeding and host choice (Pitman 1971; Hayes & Strom 1994; Miller & Borden 2000; Joseph et al. 2001; Erbilgin et al. 2017).

Second, the combination of several monoterpenes together can be toxic to MPB and can interfere with their host colonization process as suggested by earlier studies in different plant-pest systems (Berenbaum et al. 1991; Whitehead et al. 2013). Similar results were also reported by Reid et al. (2017) but in this earlier study, only four individual monoterpenes were mixed together. In general, conifers show high diversity of monoterpenes that make them defensive to herbivores and their symbionts (Phillips & Croteau 1999; Zulak & Bohlmann 2010; Erbilgin 2019). But these monoterpenes can have different effects on MPB based on their concentration individually or collectively (Seybold et al. 2006; Chiu et al. 2017; Reid et al. 2017). Moreover, Khan et al. (2013) reported that chemical compounds used in combination can increase their toxicity compared to their individual use. These results suggest that using a combination of host defense compounds in bioassays gives a better proxy to simulate host quality effects on insect herbivores, including bark beetles.

Third, we found that adult beetles did not construct any gallery and reduced its weight in media mixed with the monoterpene profiles of some host trees. In fact, having positive correlations between MPB egg gallery length and beetle weight change suggests that monoterpenes can have reduced fitness consequences on attacking beetles as well their population dynamics. For instance, longer galleries usually result in a higher number of broods on pines (Ayres et al. 2000; Bleiker & Six 2007). Likewise, greater MPB weight likely leads to greater reproduction as there is a positive relationship between adult beetle weight and brood produced (Elkin & Reid 2005; Lusebrink et al. 2016). Furthermore, beetle size is an important part of beetle flight capacity, since larger beetles can fly longer than smaller beetles (Evenden et al. 2014). Also, larger beetles are more likely to produce larger offspring which make them more successful to detoxify and tolerate host chemical defenses (Graf et al. 2012; Manning & Reid 2013; Erbilgin et al. 2014; Evenden et al. 2014). All

these results suggest that population models should incorporate the host substrate quality in predicting population densities and even dispersal abilities of beetles.

Fourth, similar to adult MPB, we found that *G. clavigera* growth was also affected by the lodgepole pine clusters, in particular 4, but not by the same clusters that inhibited feeding and reduced fitness of beetles (e.g., cluster 3). Pine trees in cluster 4 contained higher concentrations of α-pinene, camphene, bornyl acetate, and borneol and had the most inhibitory effects on the fungal growth. Similarly, Adams et al. (2011) reported that MPB symbionts showed opposite responses to monoterpenes that were toxic to MPB; their growth was greatly inhibited by α-pinene and 3-carene, while they tolerated the limonene which was toxic to the beetles. Interestingly, while (−)-limonene was the most toxic to adult MPB, α-pinene showed the least toxicity among individual monoterpenes tested (Chiu et al. 2017). We also found that (+)-limonene was more toxic to *G. clavigera* than (−)-limonene. Although there is some evidence that *G. clavigera* uses monoterpenes including limonene as a carbon source to support its growth and pathogenicity (Wang et al. 2013; Cale et al. 2017), growth of the fungus is also concentration-dependent (Davis et al. 2018).

In lodgepole pine, β-phellandrene is the most abundant monoterpene (Smith 2000; Shrimpton & Reid 1973; Pureswaran & Borden 2005). In the current study, this particular monoterpene was inhibitory against both MPB and *G. clavigera* at its highest concentration tested, suggesting its dual role in lodgepole pine defenses. This is the first study demonstrating the concentration-dependent effects of β-phellandrene on the fungal symbionts of MPB. Monoterpene concentrations can affect MPB and its symbiont (Chiu et al. 2017; Reid et al. 2017). Mass attack by MPB and its symbiont reduce the secondary compounds more specifically monoterpenes in trees, while their higher concentrations may be attractive to beetles for host identity and host

quality (Boone et al. 2011; Miller & Borden 2000). Our results show that low β-phellandrene concentration was not attractive to beetle which caused lower gallery construction and weight gain. It is possible that low concentration was not more attractive for the beetle as compare to medium dose which shows more positive response. Furthermore, it is also possible that *G. clavigera* uses concentrations of β-phellandrene less than 2.69 μg/mg as a carbon source and that higher concentrations are above the tolerance threshold of the fungus (DiGuistini et al. 2011; Wang et al. 2013; Cale et al. 2017).

Finally, *G. clavigera* can discriminate among different enantiomers of host tree monoterpenes. Our experiments testing the effects of individual chiral compounds including α-pinene, limonene, and camphene on the growth of *G. clavigera* showed that with the exception of camphene, enantiomers can differentially affect the growth of *G. clavigera*, which is in agreement with the results reported by Cale et al. (2017). Although we did not use different enantiomers of monoterpenes against MPB host acceptance, Chiu et al. (2017) revealed that both (–) and (+) enantiomers of limonene were toxic to the beetle (but the former was more toxic) and that both (–)- and (+)-α-pinene were the least toxic. Similar results were also reported by Reid et al. (2017).

Overall, lodgepole pine families having different concentrations of monoterpenes differentially affect MPB and *G. clavigera*, suggesting the co-evolutionary development of plant defenses against their enemy complex, enabling them to target multiple agents in the same mixture. In this co-evolutionary response, both the symbiotic fungi and bark beetles have evolved in detoxification of terpenes, more specifically monoterpenes (Ayres et al. 2000; Franceschi et al. 2005; Raffa et al. 2005, 2008; DiGuistini et al. 2011; Wang et al. 2013; Cale et al. 2017). Apparently, the family-1799 (cluster 3) had the most potent profile against MPB as the beetle did not construct any galleries in the diet and reduced its weight. In contrast, two lodgepole pine

families 1867 and 1865 (both in cluster 4) were least suitable to *G. clavigera* which showed much lower growth as compared to other families or clusters. These results likely reflect the differences in the monoterpene profiles of these families. Interestingly, in this study, some lodgepole pine families showed similar suitability to both MPB and *G. clavigera*.

Conclusion

Defensive traits of lodgepole pine consist of constitutive terpenoids. Here, we have shown not only that lodgepole pine has various chemotypic variations in constitutive terpenoids, but also exhibits differential resistance or susceptibility to MPB and *G. clavigera*. The recent MPB outbreak in western Canada killed a large number of mature lodgepole pine trees across the landscape. Yet, even in the midst of massive die-off, some trees survived from this outbreak (Erbilgin et al. 2017; Six 2019; Zhao & Erbilgin 2019; Zhao et al. 2019). This is likely a result of the large diversity of secondary compounds in lodgepole pine trees across the landscape, along with anatomical and genetic differences among trees. Our study revealed that these secondary compounds can have positive and negative impacts on MPB and *G. clavigera*. Furthermore, our studies showed that chemicals toxic to the MPB may not be toxic to its fungal symbionts or vice versa, suggesting that trees produce different toxic chemicals in various concentrations targeting multi-species enemy complex.

Management implications

The Canadian boreal forest is particularly vulnerable to climate change, facing unprecedented moisture deficits and is subject to frequent climatic extremes (Trumbore et al. 2015; Seidl et al.

2017; Moreno-Fernández et al. 2019). In nature, trees show resistance against their enemies due to the abundance and diversity of secondary compounds, specifically monoterpenes in the case of lodgepole pine. We found that lodgepole pine families differentially affect MPB and *G. clavigera*. This research contributes to the pest management implications by screening out the most and least suitable lodgepole pine families against MPB and *G. clavigera*. These chemotypes can further be used for development of insects and fungal resistant lodgepole pine phenotypes in the future. Additional studies are needed to determine the effect of lodgepole pine families on MPB and *G. clavigera* under field conditions before putting them into practice. Our new findings can be helpful in the process of integrating plant defense compounds with genomics to develop host plant

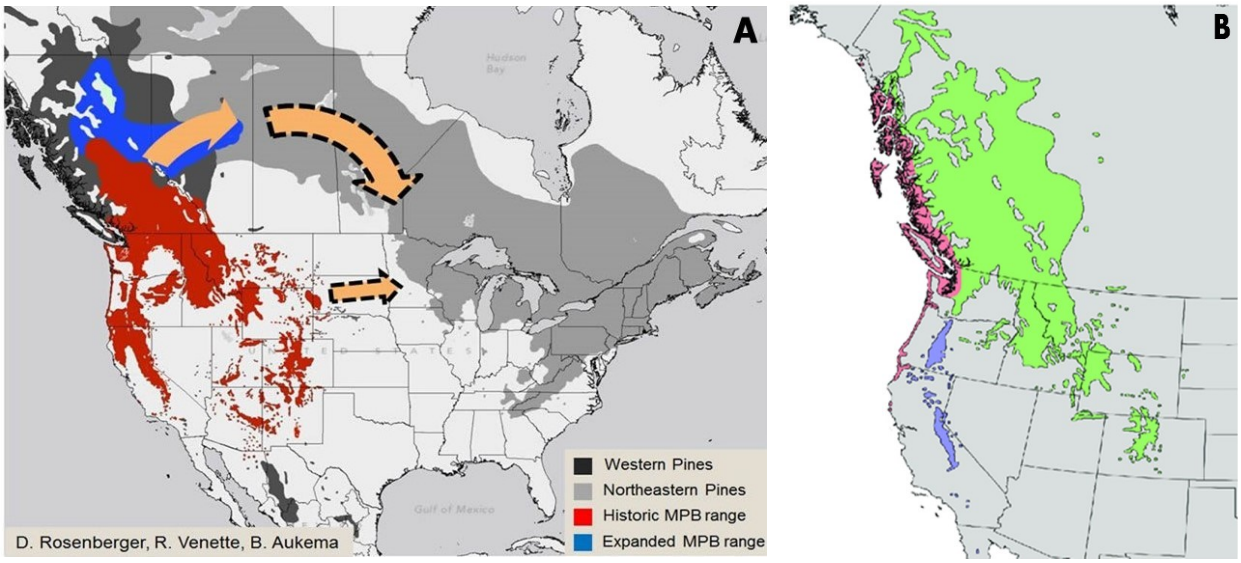


Figure 1. A) Distributions of different pine (*Pinus*) species, the historical and recent range expansion by mountain pine beetle (*Dendroctonus ponderosae*) (Credit: Rosenberger et al. 2017**)**, **B)** The distribution of lodgepole pine (*Pinus contorta*). Green portion denotes the spp. *latifolia*, pink portion denotes the spp. *contorta* and blue portion denotes the spp. *murrayana* (Credit: USGS, USA).

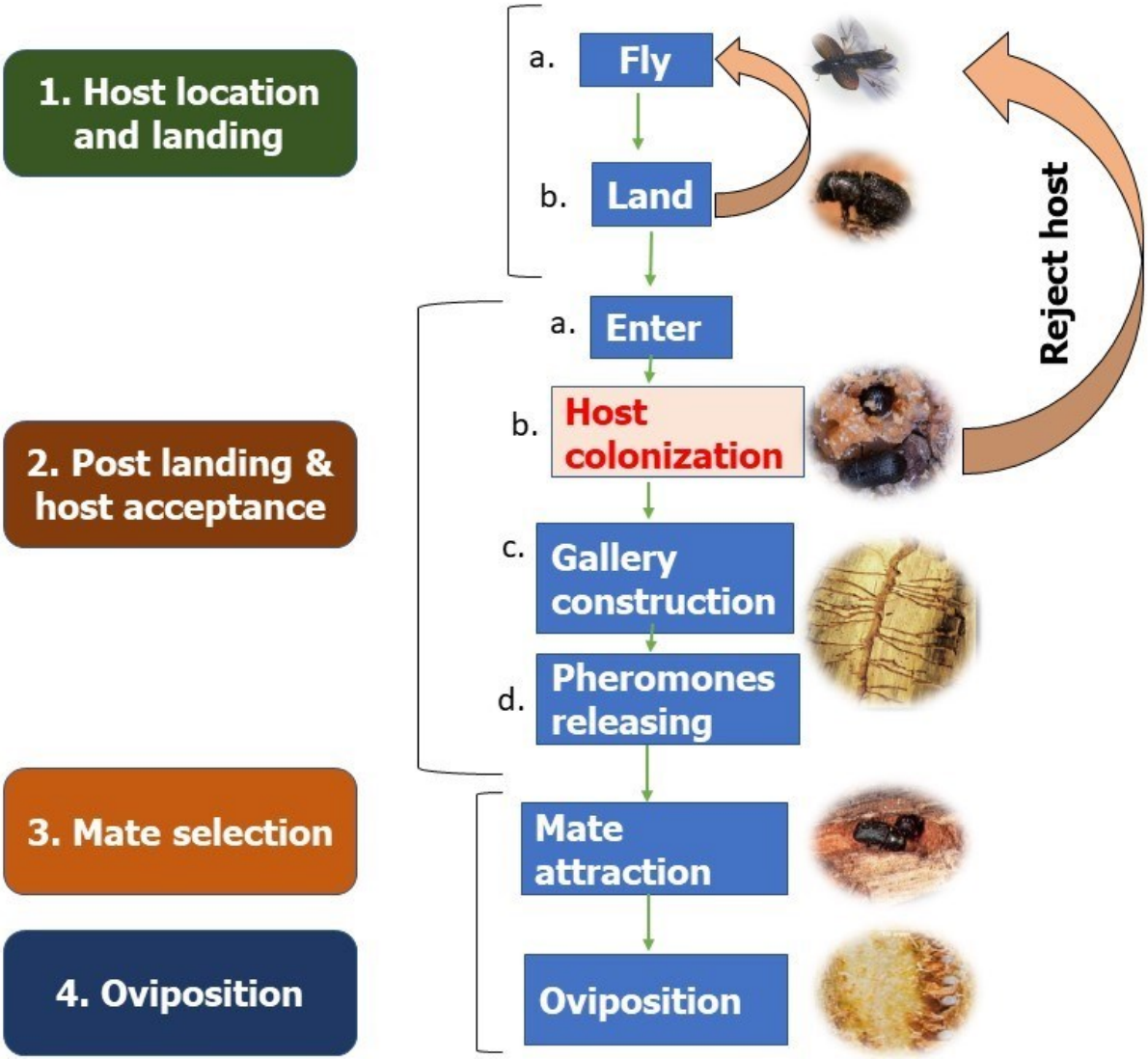


Figure 2. Several steps involved in host acceptance by mountain pine beetle (*Dendroctonus ponderosae*) that lead to survival or death of the host tree (modified from Wallin & Raffa 2000).

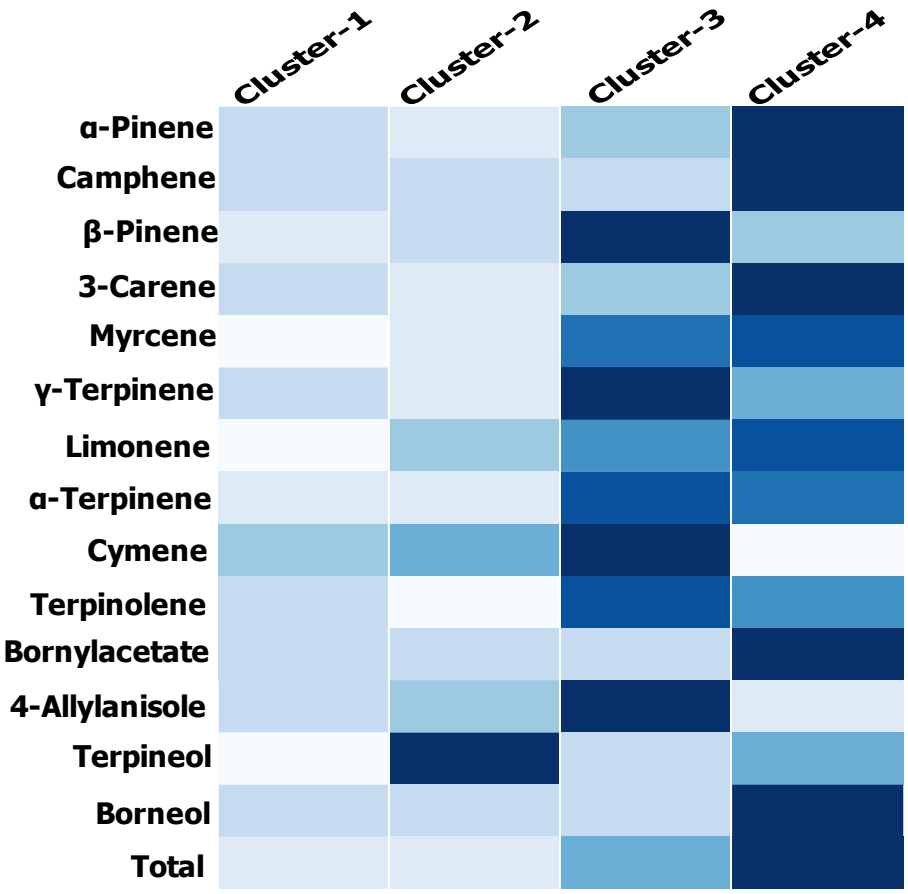


Figure 3A. A heatmap showing the chemical profiles of four clusters of lodgepole pine (*Pinus contorta*). Lowest and highest concentrations (ng/mg) were demonstrated by light to dark colours. To compare relative concentrations among clusters for each compound, colours are based on concentration of each compound across clusters. This heatmap was generated using the R ggplot2 package.

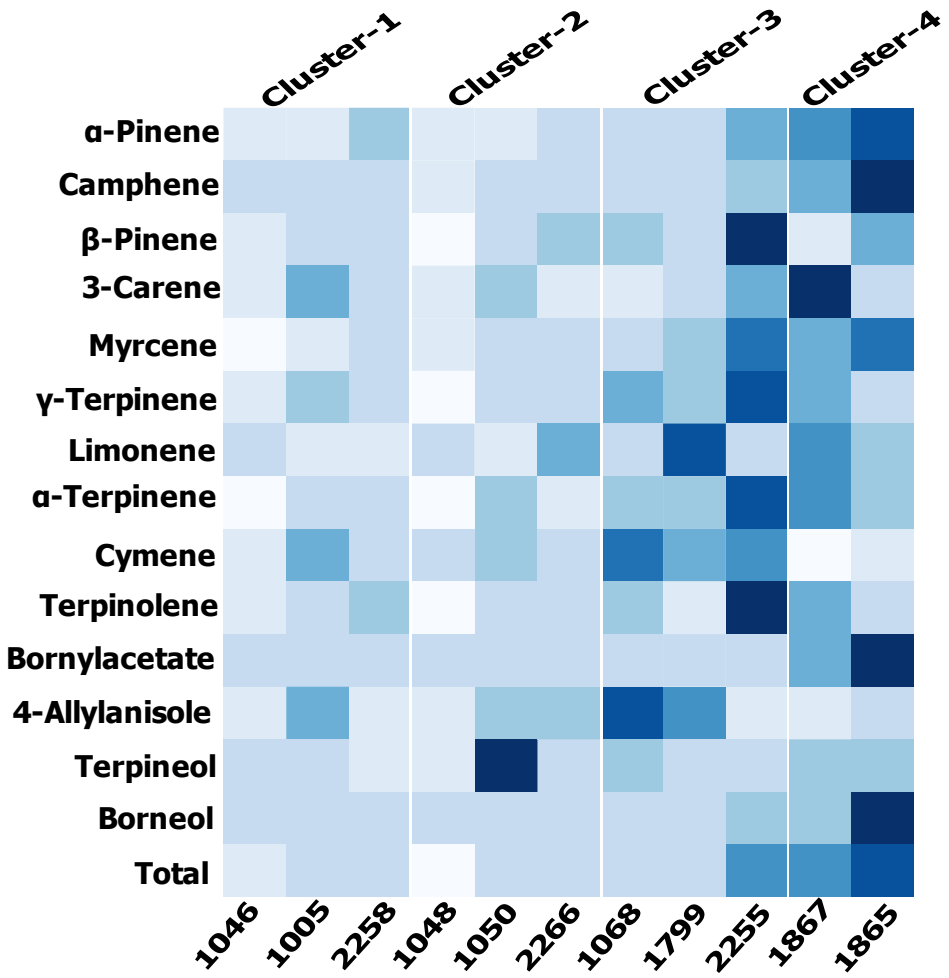


Figure 3B. A heatmap showing the chemical profiles of 11 lodgepole pine (*Pinus contorta*) families in four clusters. Lowest and highest concentrations were demonstrated by light to dark colours. To compare relative concentrations among families for each compound, colours are based on concentration of each compound across families. This heatmap was generated using the R ggplot2 package.

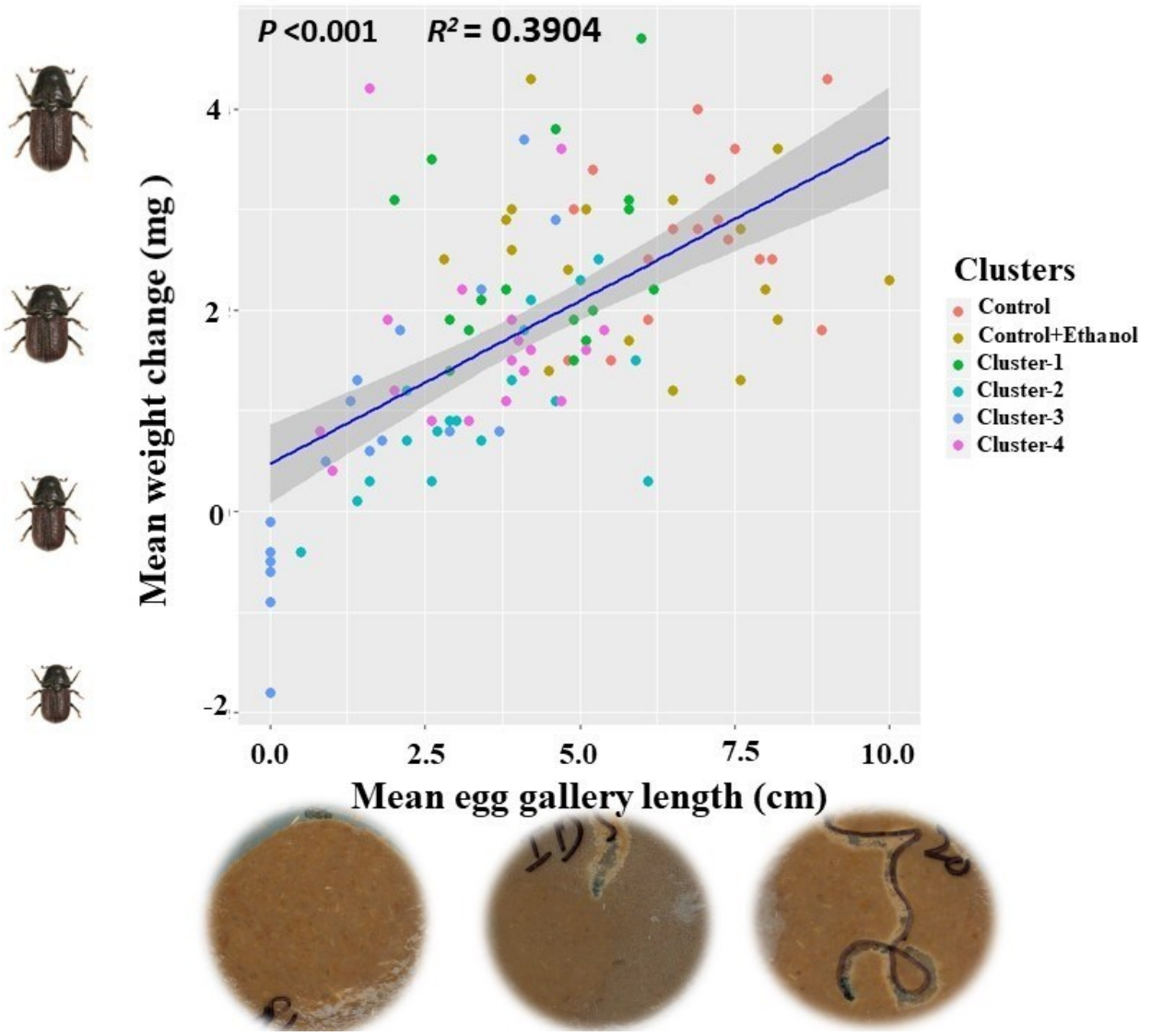


Figure 4. The correlation between *Dendroctonus ponderosae* egg gallery length (cm) and weight change (mg) on different *Pinus contorta* clusters and control (n=18).

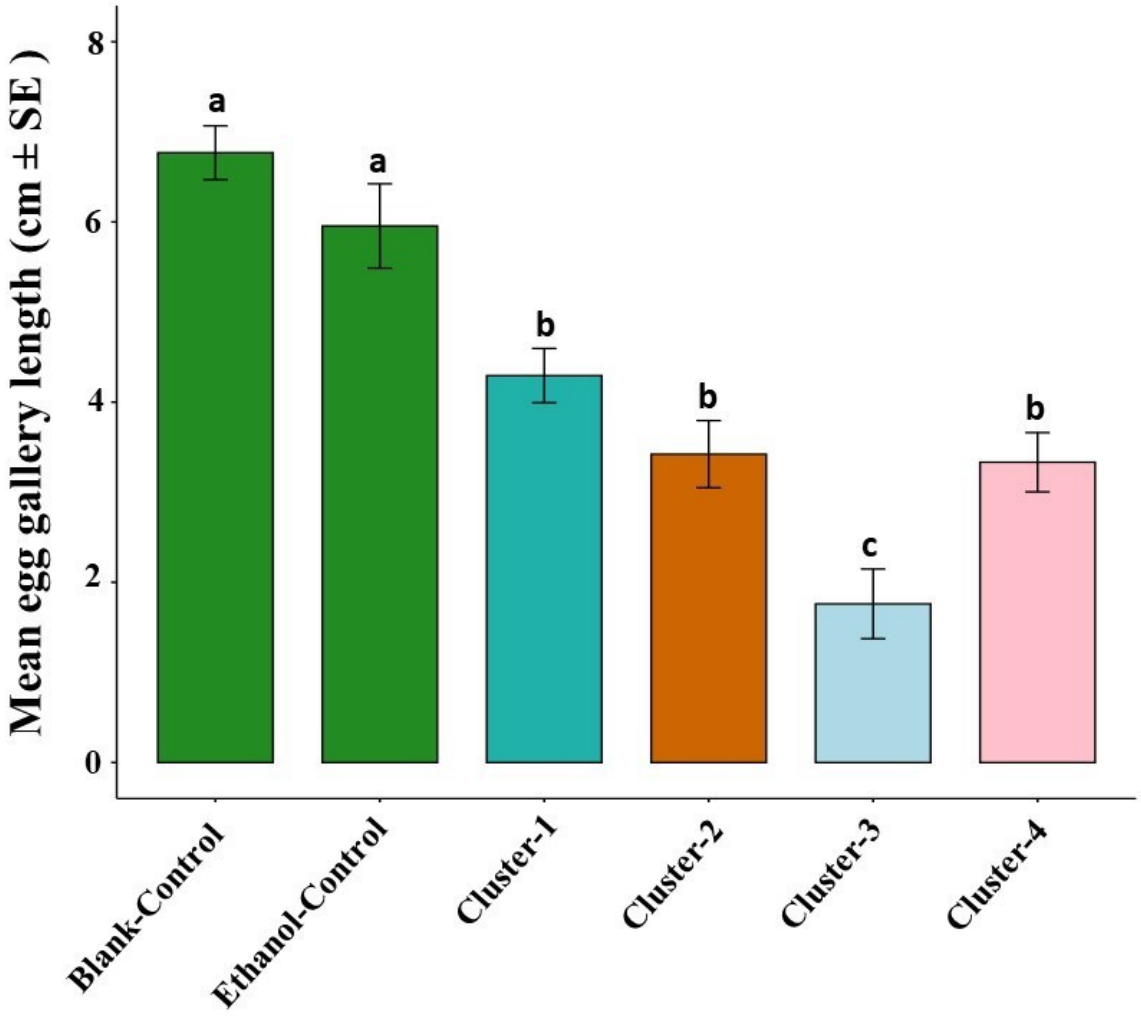


Figure 5. Mean (±SE) effect of four *Pinus contorta* clusters on egg gallery length (cm) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{5,102}$=25.61, P <0.001, n=18).

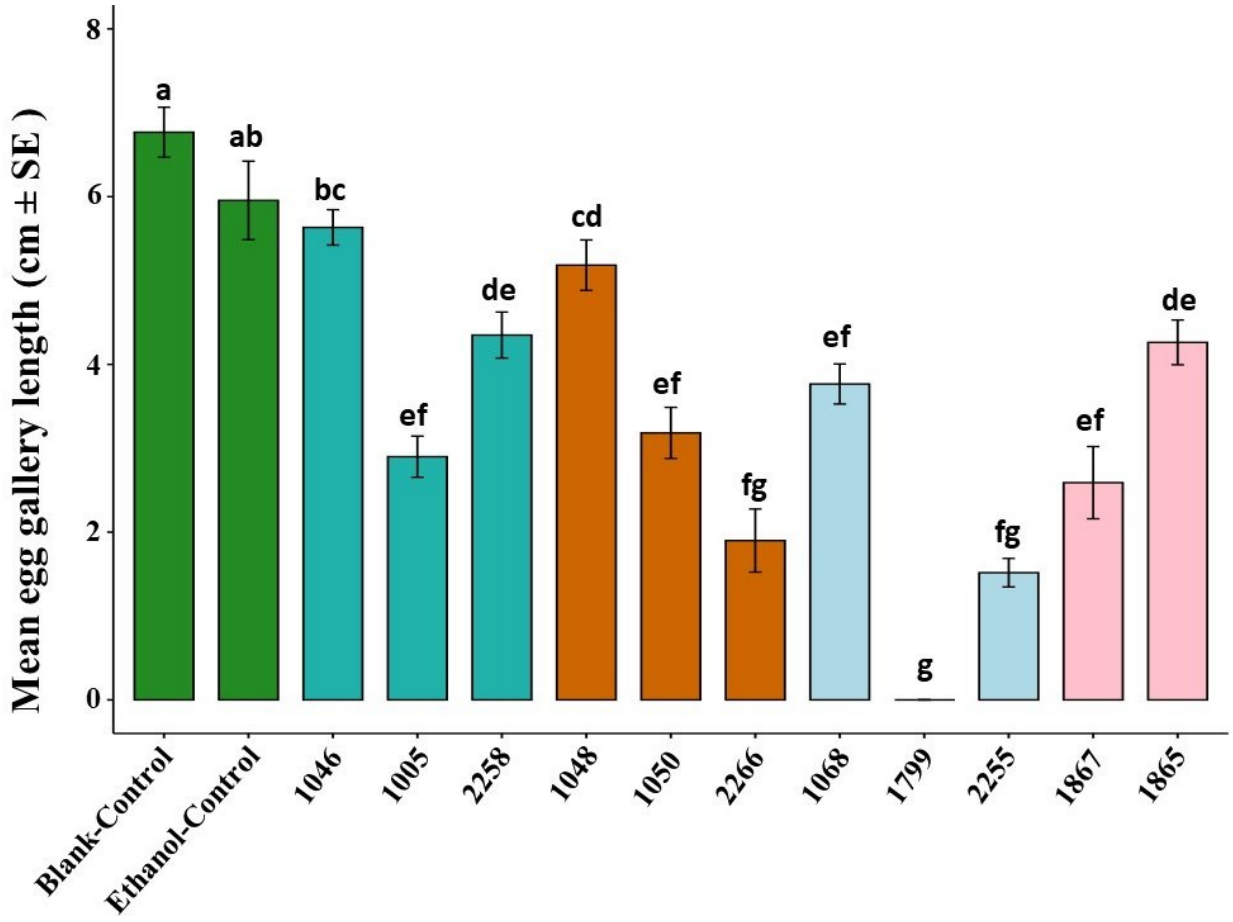


Figure 6. Mean (±SE) effect of *Pinus contorta* families on egg gallery length (cm) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{12,95}$=24.87, *P* <0.001, n ranged 6-9 for families).

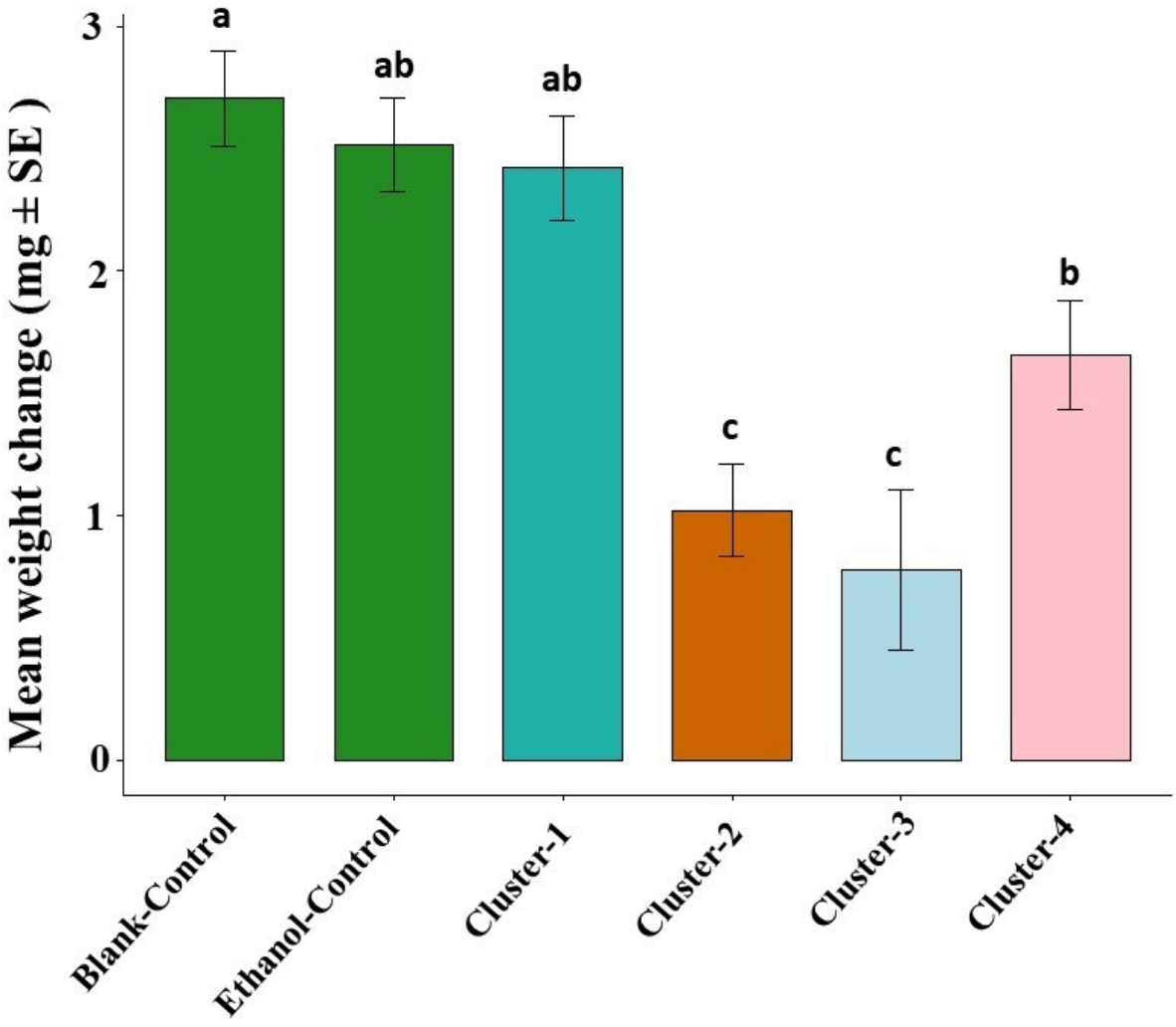


Figure 7. Mean (±SE) effect of *Pinus contorta* clusters on weight change (mg) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{5,102}$=12.98, P <0.001, n=18).

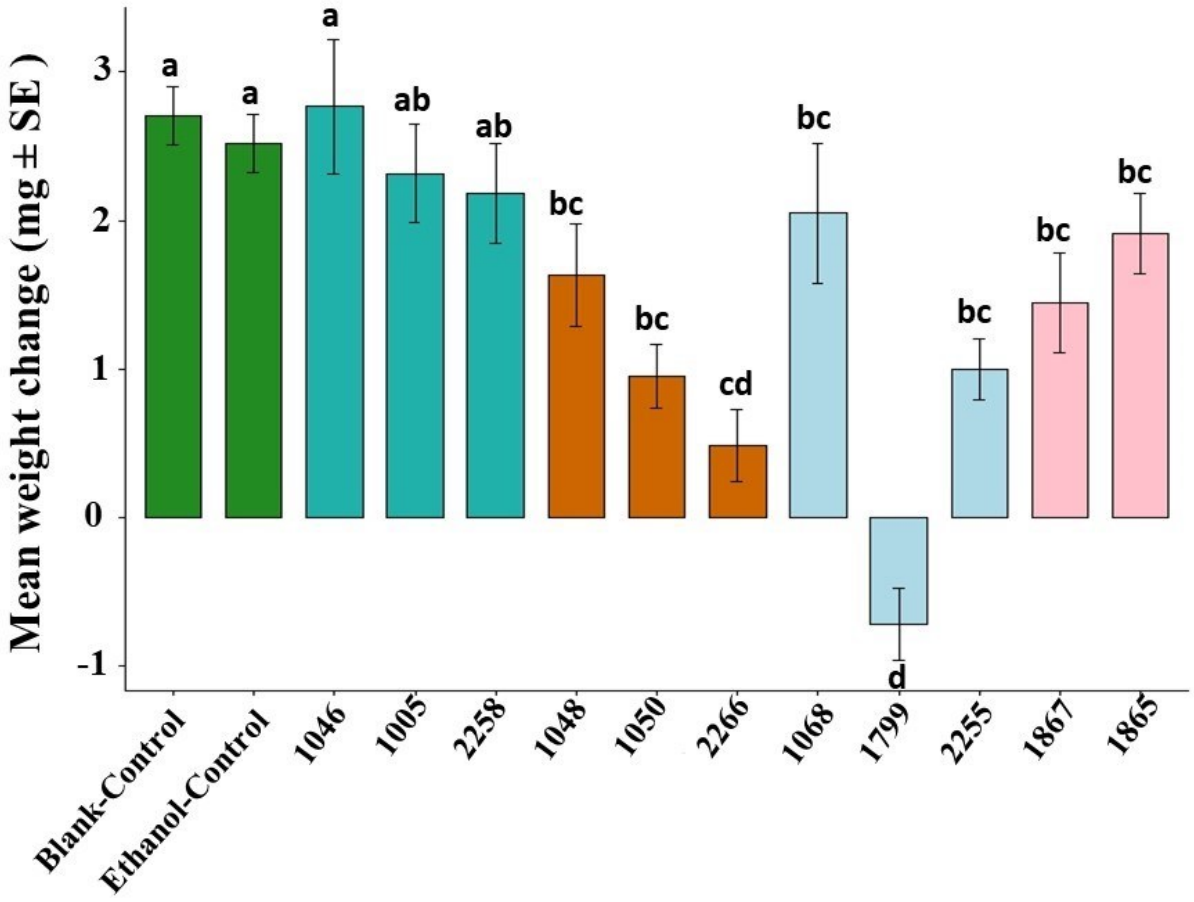


Figure 8. Mean (±SE) effect of *Pinus contorta* families on weight change (mg) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{12,95}$=10.82, *P* <0.001, n ranged 6 to 9 for families).

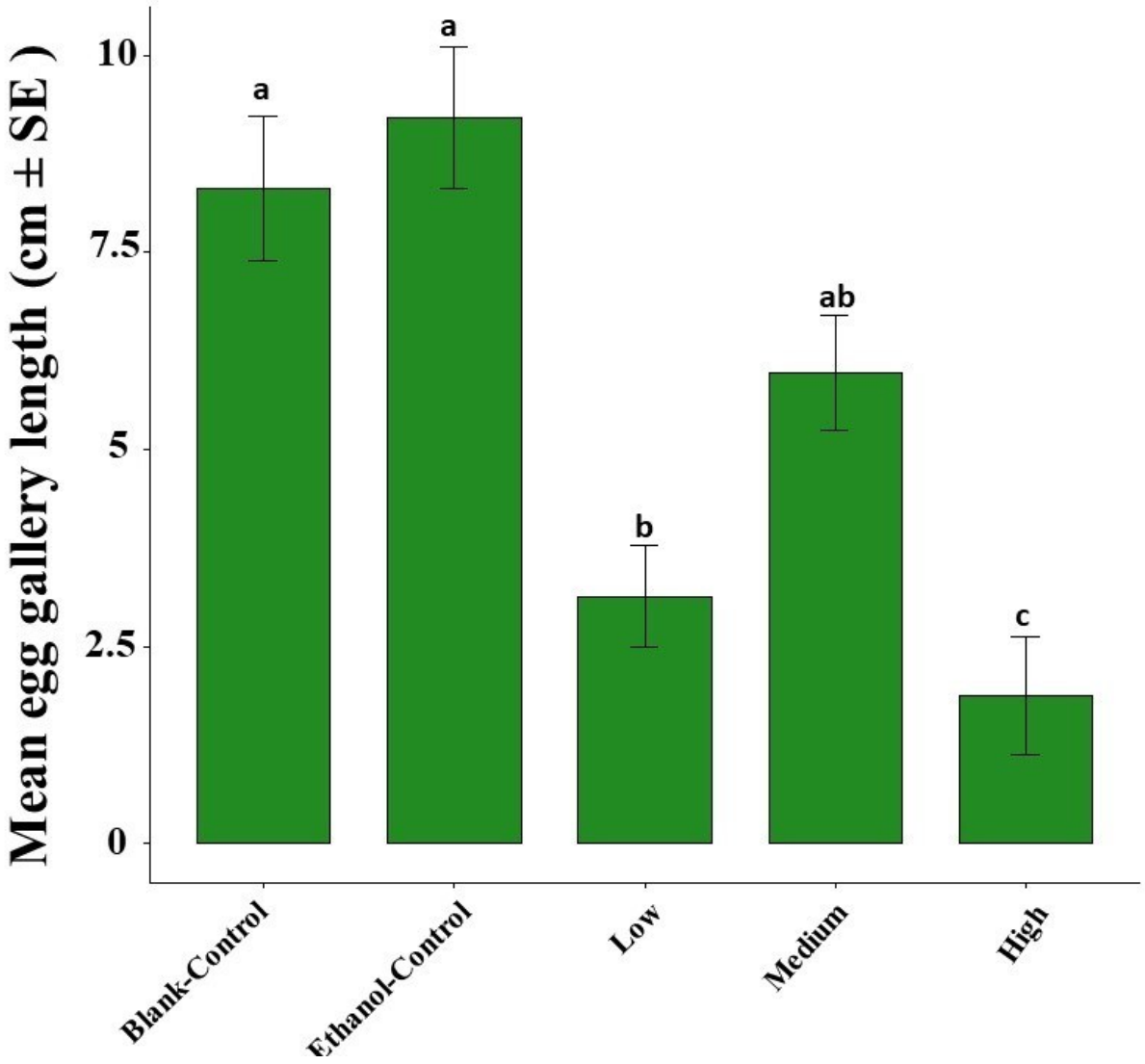


Figure 9. Mean (±SE) effect of β-phellandrene on egg gallery length (cm) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=4.92, P <0.001, n=5).

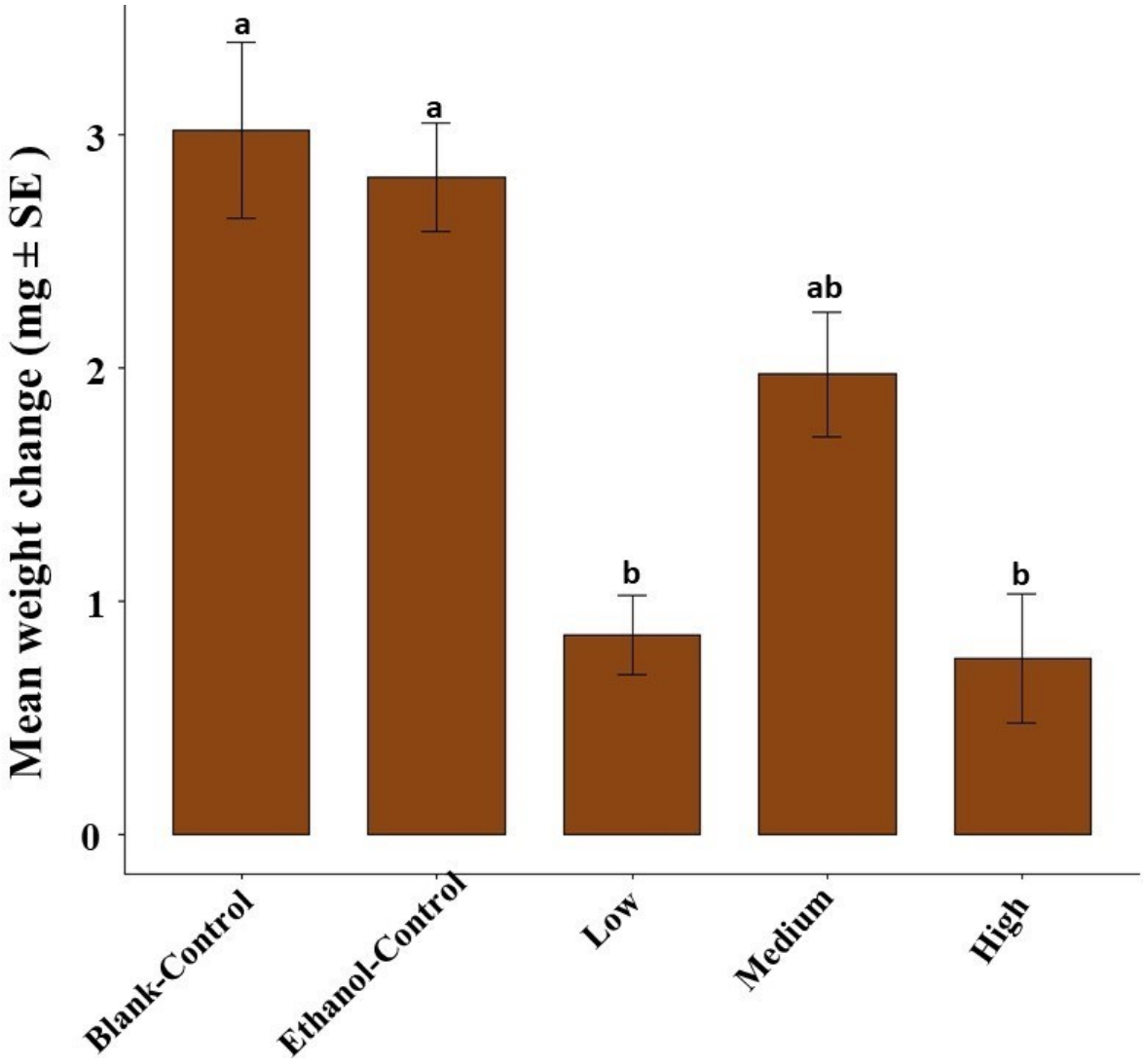


Figure 10. Mean (±SE) effect of β-phellandrene on weight change (mg) of *Dendroctonus ponderosae*. Assays were conducted for 48 hours. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=11.66, P <0.001, n=5).

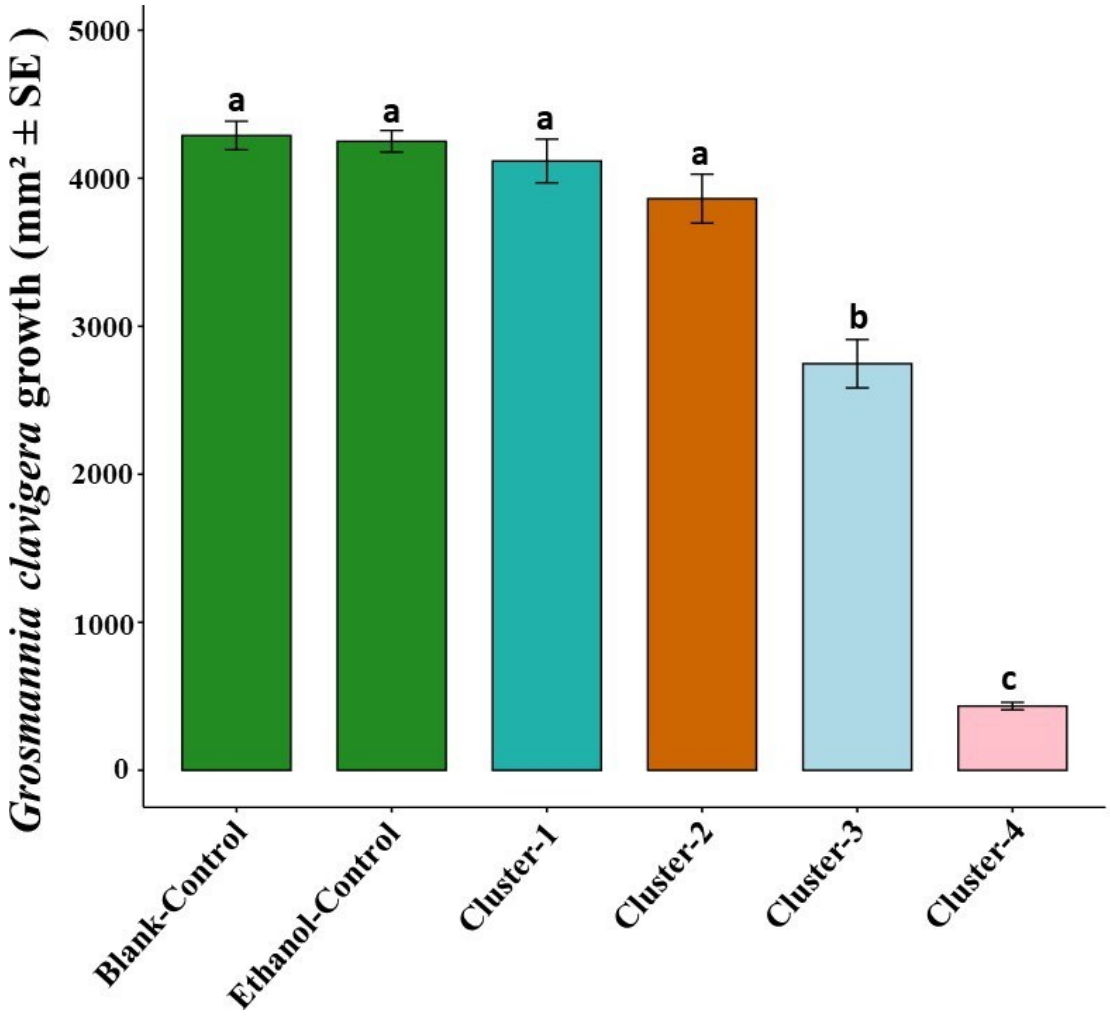


Figure 11. Mean (±SE) effect of *Pinus contorta* clusters on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{5,102}$= 50.32, P <0.001, n=18).

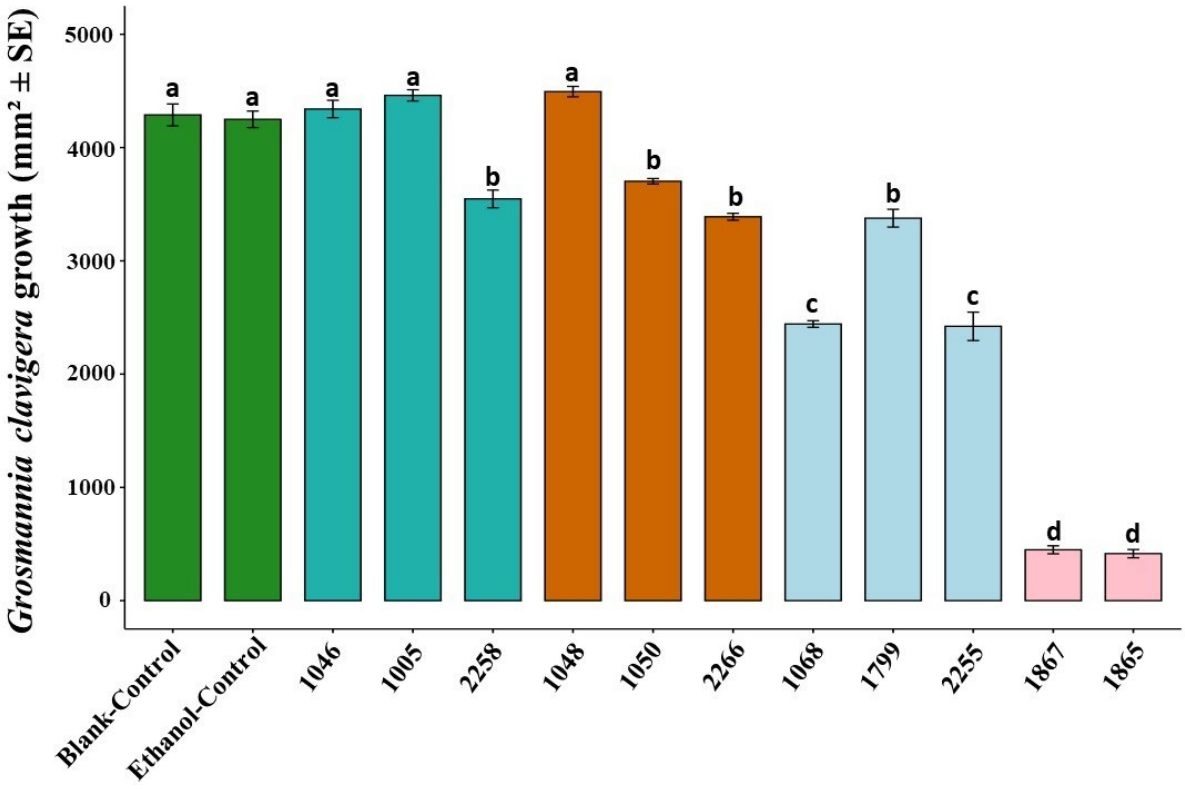


Figure 12. Mean (±SE) effect of *Pinus contorta* families on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{12,95}$=76.77, P <0.001, n ranged 6-9 for families).

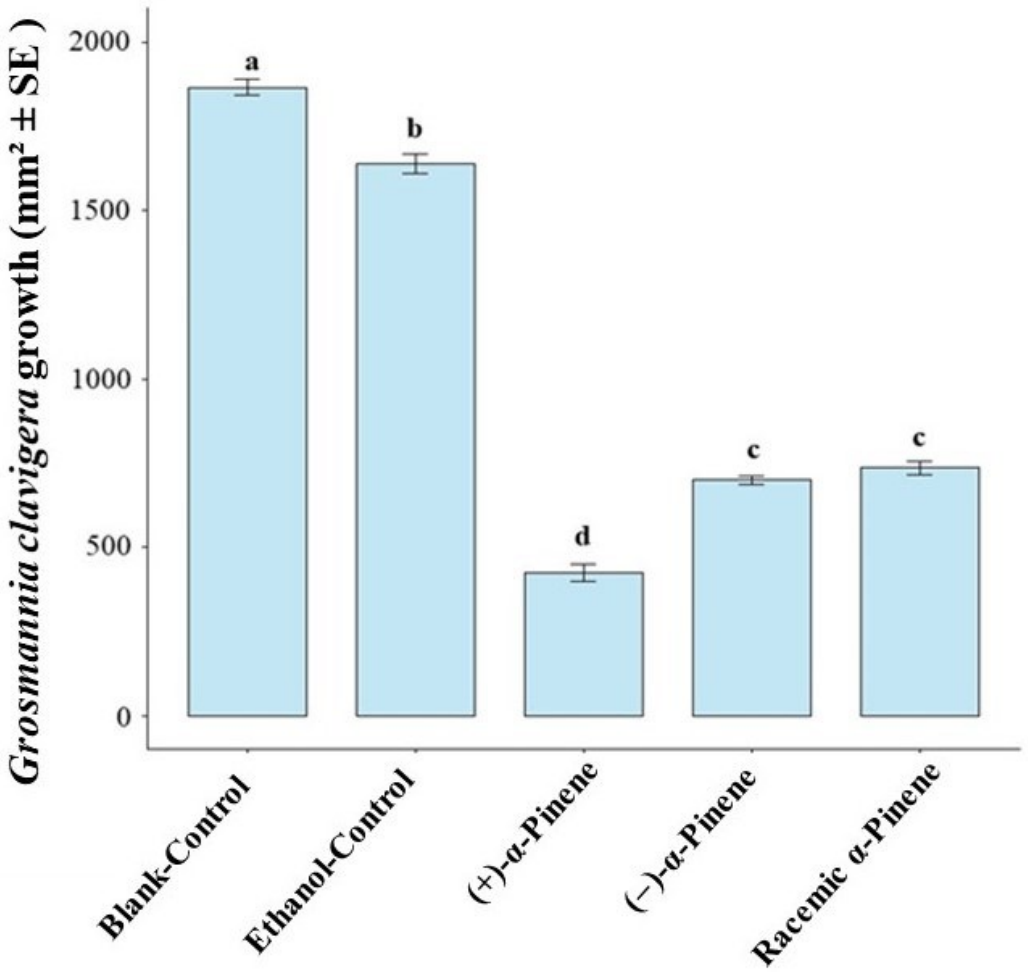


Figure 13. Mean (±SE) effect of enantiomers of α-pinene on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=150.37, P <0.001, n=5).

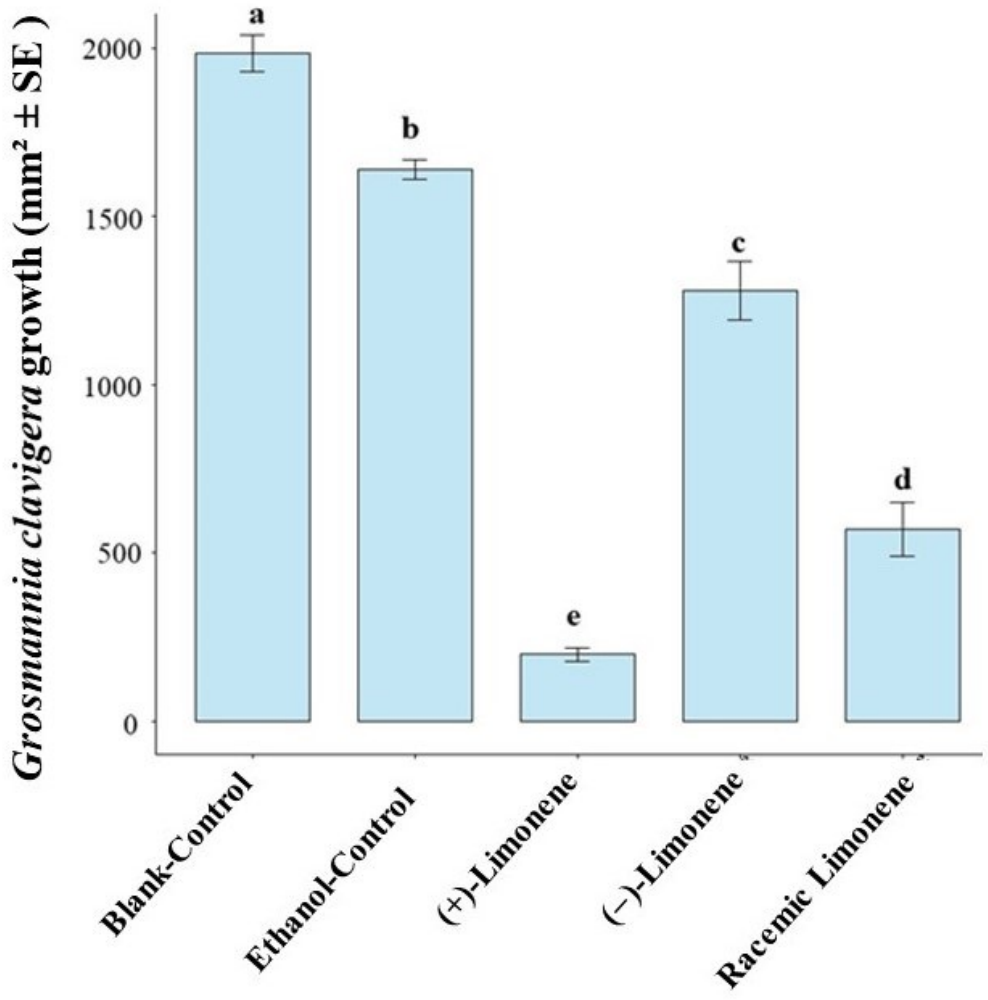


Figure 14. Mean (±SE) effect of enantiomers of limonene on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=150.79, P <0.001, n=5).

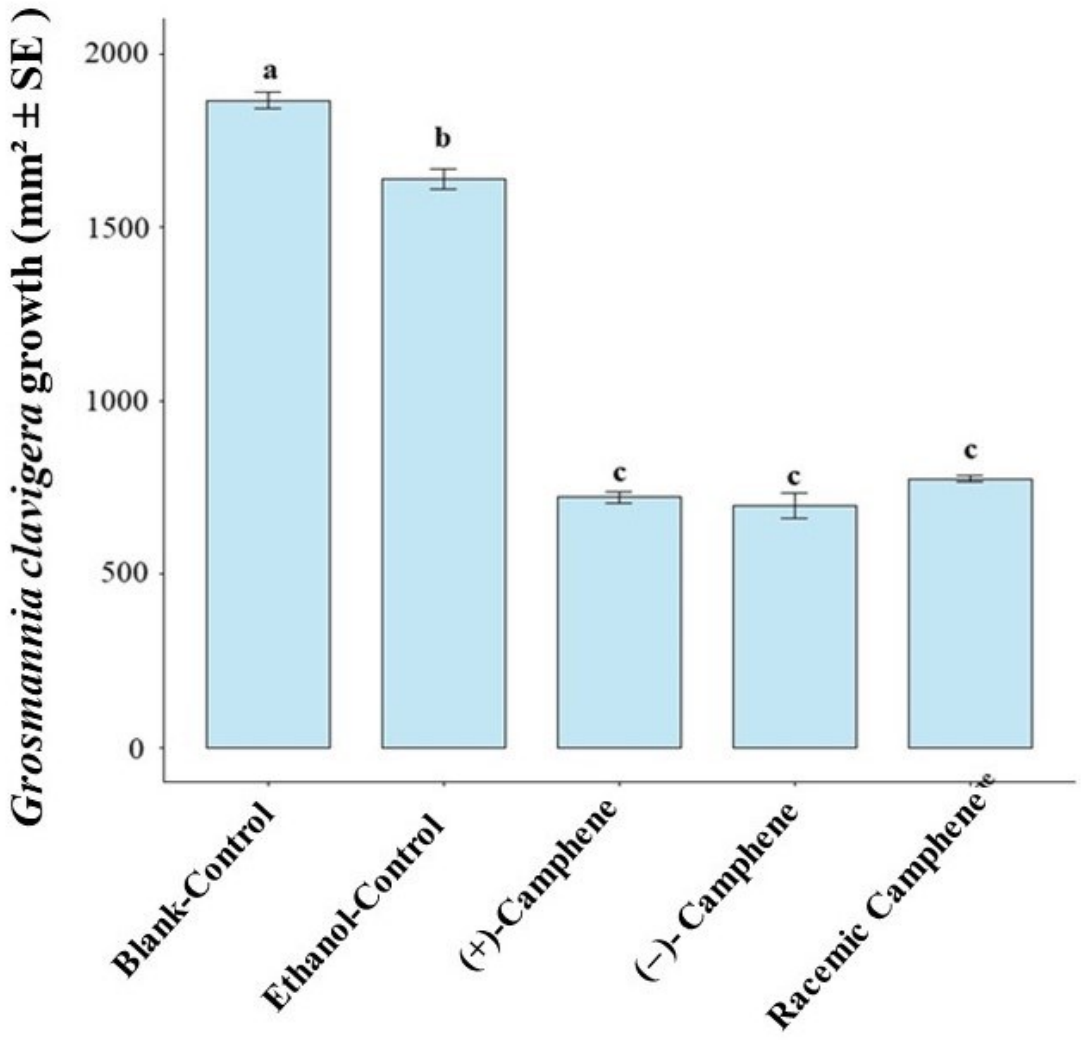


Figure 15. Mean (±SE) effect of enantiomers of camphene on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=150.79, P <0.001, n=5).

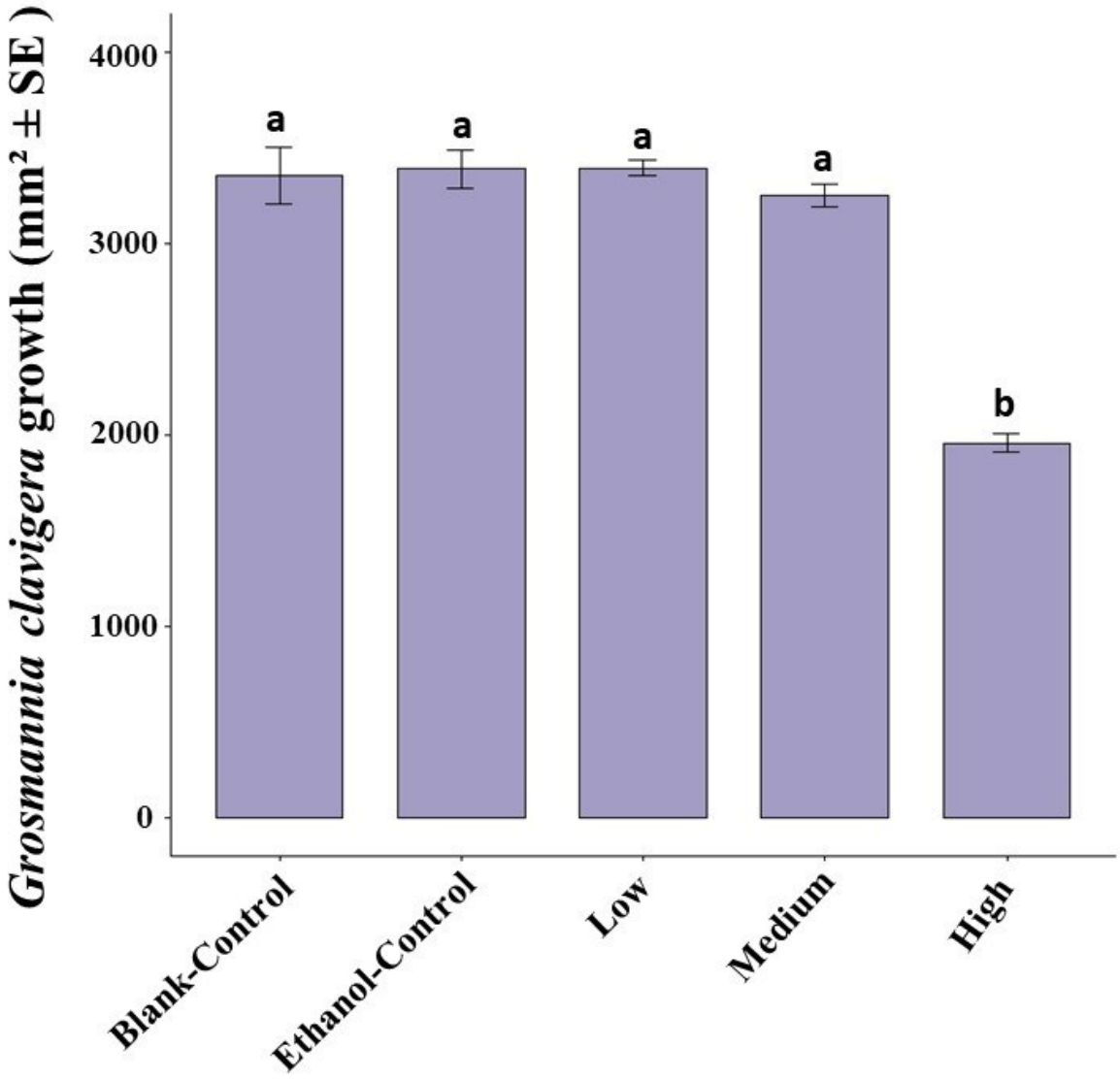
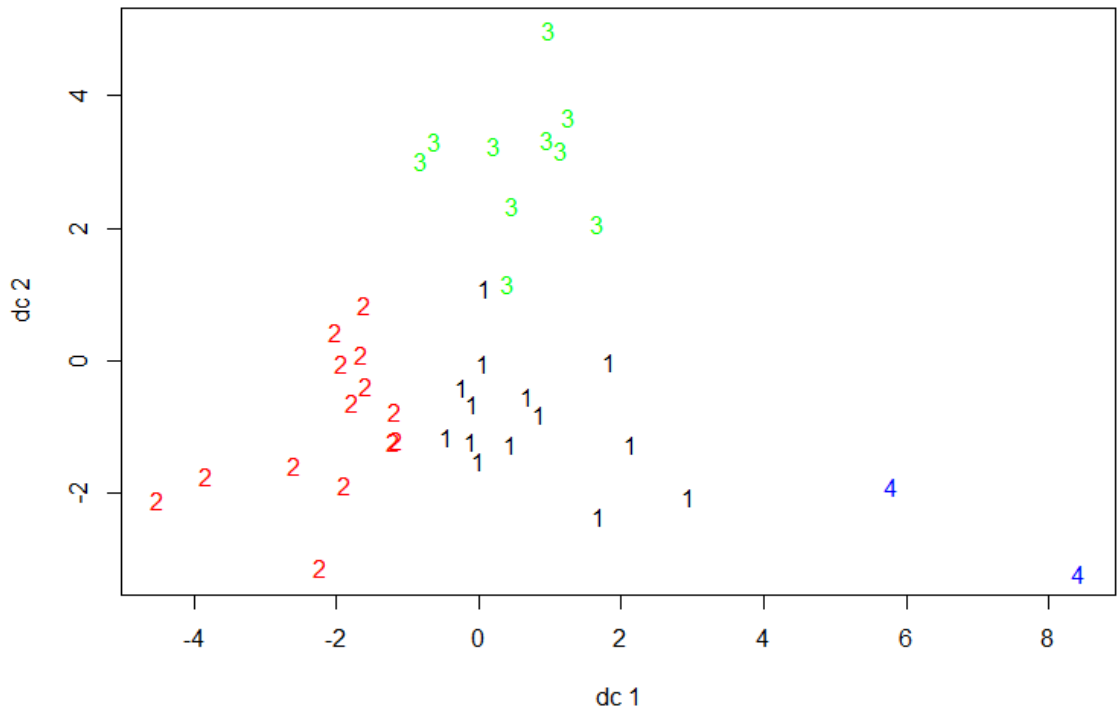


Figure 16. Mean (±SE) effect of β-phellandrene on growth (mm^2) of *Grosmannia clavigera*, a fungal associate of *Dendroctonus ponderosae*. Assays were conducted for four days. Bars with different letters are statistically different as indicated by Tukey Honest Significant Difference tests. *P* values indicate results of one-way ANOVA ($F_{4,20}$=9.86, P <0.001, n=5).

A.



B.

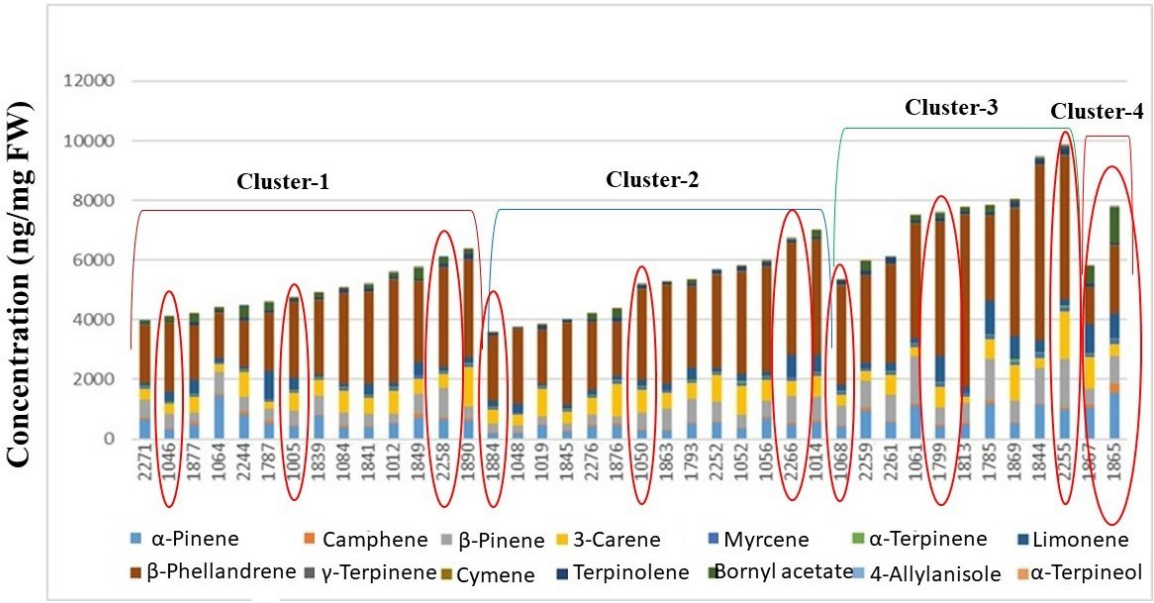


Figure S1. A) Display of results from partitioning around medeiod clustering analysis on *Pinus contorta* family estimated breeding values of monoterpene compounds, **B)** The concentration (ng/mg) of *Pinus contorta* families grouped in different clusters. The colours showing the concentration of monoterpenes and non-monoterpenes in each family. Oval red colours showing the families selected for the bioassays.

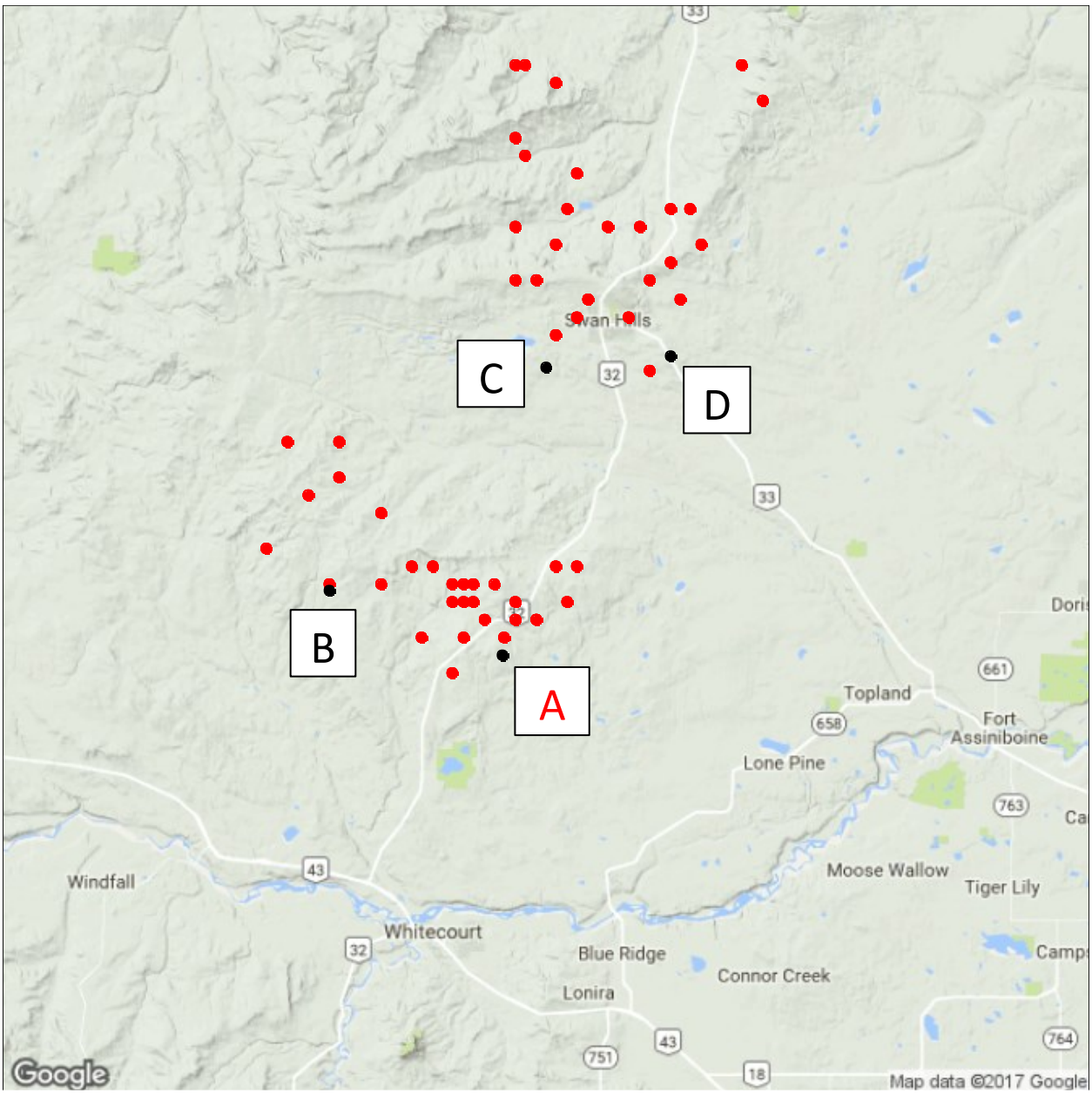


Figure S2. Location of Region C selections (red) and test sites (black). Letters identify test site identity as indicated in Table S1. Red A indicates site used for bioassays.

Table S1. Test site locations of the Region C G128 progeny trials.

SITE	Latitude(N)	Longitude(W)	Elevations(m)
Judy Creek (A)	54º24'	115º34'	1097
Virginia Hills (B)	54º28'	115º51'	1127
Swan Hills (C)	54º40'	115º30'	1033
Swan Hills (D)	54º41'	115º18'	1064

Table S2. The monoterpenes (ng/mg) in different *Pinus contorta* families and clusters used for MPB and *G. clavigera* bioassays.

Cluster	**1**			**2**			**3**			**4**	
Family	**1046**	**1005**	**2258**	**1048**	**1050**	**2266**	**1068**	**1799**	**2255**	**1867**	**1865**
α-Pinene	269.1	311.1	505.7	187.8	282.1	383.0	376.2	328.5	749.3	996.9	1418.6
Camphene	30.4	27.9	39.1	24.3	30.7	41.4	38.3	39.9	66.6	107.0	247.6
β-Pinene	358.1	434.2	489.2	199.4	491.5	644.8	583.1	392.6	1217.6	316.5	677.5
3-Carene	175.1	407.8	262.4	224.9	356.5	184.4	175.1	285.3	454.4	761.1	280.6
Myrcene	47.3	65.2	76.0	58.6	83.3	77.9	88.1	104.3	142.2	107.1	147.8
α-Terpinene	11.6	20.7	17.9	7.1	18.4	15.7	30.6	22.4	46.3	26.4	17.5
Limonene	171.6	131.3	120.1	182.2	164.0	309.4	169.9	509.3	182.5	360.8	281.1
γ-Terpinene	7.6	12.5	14.6	9.1	15.2	11.1	14.8	15.7	27.5	21.2	16.3
Cymene	11.0	16.7	12.9	13.1	16.2	14.5	21.8	17.0	18.9	8.9	11.8
Terpinolene	42.0	63.4	78.7	31.1	64.2	52.1	69.4	44.1	158.4	89.4	66.3

Bornyl acetate	36.7	34.4	30.2	2.7	14.1	7.9	15.7	17.4	15.4	359.1	1130.8
4-Allylanisole	4.9	15.2	3.9	2.3	13.5	10.2	25.5	21.2	4.7	4.1	7.3
α-Terpineol	1.4	1.4	0.9	0.9	7.4	1.5	2.2	1.4	1.5	2.1	2.7
Borneol	3.7	3.6	10.9	3.6	5.0	4.6	8.1	5.4	19.1	22.5	111.1
Total	**1170.5**	**1545.5**	**1662.8**	**947.1**	**1562.2**	**1758.4**	**1618.8**	**1804.8**	**3104.5**	**3183.3**	**4416.9**

All the monoterpenes of each family were diluted into 1 ml ethanol, or ethanol as a control were mixed in MBP diet for MPB bioassay and malt extract agar (MEA) media for fungal bioassay.

Table S3. Detailed source information about isolates of *Grosmannia clavigera* fungi used for fungal bioassay.

Accession Number	**Location Collection**	**Date Collected**	**Notes**
El004	Fox Creek, AB	1/25/2015	Isolated from *Dendroctonus ponderosae*
El035	Graham Fire Base, AB	5/17/2017	Isolated from a *Dendroctonus ponderosae* gallery in *Pinus banksiana* x *Pinus contorta* hybrid

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