EFFECT OF WATER STRESS AND PLANT DEFENSE STIMULATION ON MONOTERPENE EMISSION FROM A HISTORICAL AND A NEW PINE HOST OF THE MOUNTAIN PINE BEETLE

INKA LUSEBRINK [1, 2] *

[1] *Department of Biological Sciences*

University of Alberta

CW405 Biological Science Building

Edmonton, Alberta

Canada T6G 2E9

e-mail: lusebrin@ualberta.ca

MAYA L. EVENDEN [1]

[1] *Department of Biological Sciences*

University of Alberta

CW405 Biological Science Building

Edmonton, Alberta

Canada T6G 2E9

F. GUILLAUME BLANCHET [2]

[2] *Department of Renewable Resources*

University of Alberta

751General Service Building

Edmonton, Alberta

Canada T6G 2H1

JANICE E. K. COOKE [1]

[1] *Department of Biological Sciences*

University of Alberta

CW405 Biological Science Building

Edmonton, Alberta
Canada T6G 2E9

NADIR ERBILGIN [2]
[2] *Department of Renewable Resources*
University of Alberta
4-42 Earth Science Building
Edmonton, Alberta
Canada T6G 2E3

Abstract - The mountain pine beetle (*Dendroctonus ponderosae*, MPB) has killed millions of lodgepole pine (*Pinus contorta*) trees in Western Canada and recent range expansion has resulted in attack of jack pine (*Pinus banksiana*) in Alberta. Establishment of MPB in the Boreal forest will require use of jack pine under a suite of different environmental conditions than it typically encounters in its native range. Lodgepole and jack pine seedlings were grown under controlled environment conditions and subjected to either water deficit or well watered conditions and inoculated with *Grosmannia clavigera*, a MPB fungal associate. Soil water content, photosynthesis, stomatal conductance and emission of volatile organic compounds (VOCs) were monitored over the duration of the six-week study. Monoterpene content of bark and needle tissue was measured at the end of the experiment. β-Phellandrene, the major monoterpene in lodgepole pine, was almost completely lacking in the volatile emission profile of jack pine. The major compound in jack pine was α-pinene. The emission of both compounds was positively correlated with stomatal conductance. 3-Carene was emitted at a high concentration from jack pine seedlings which is in contrast to monoterpene profiles of jack pine from more southern and eastern parts of its range. Fungal inoculation caused a significant increase in total monoterpene emission in water deficit lodgepole pine seedlings right after its application. By four weeks into the experiment, water deficit seedlings of both species released significantly lower levels of total monoterpenes than well-watered seedlings. Needle tissue contained lower total monoterpene content than bark. Generally, monoterpene tissue content increased over time independent from any treatment. The results suggest that monoterpenes that play a role in pine-MPB interactions differ between lodgepole and jack pine, and also that they are affected by water availability.

Key Words - *Pinus contorta*, *Pinus banksiana,* VOCs, monoterpenes, tree defense, *Grosmannia clavigera*, mountain pine beetle.

INTRODUCTION

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) has destroyed 16.3 million ha of mainly lodgepole pine (*Pinus contorta* Dougl. ex Loud.) forests in British Columbia, Canada (www.for.gov.bc.ca) during this current outbreak, which began over a decade ago. In the last five years, MPB has moved eastward into Alberta, where in the northern part of the province the ranges of lodgepole pine and jack pine (*Pinus banksiana* Lamb.) overlap, resulting in a zone of hybridization (Cullingham et al. 2011). Lodgepole pine is one of the historical hosts of MPB in Western Canada, however, as its range expands from its historical habitat to new areas in northern Alberta (Carroll et al. 2006), MPB has spread across the lodgepole × jack pine hybrid zone into stands of pure jack pine (Cullingham et al. 2011). Studies show that beetles can reproduce and develop in jack pine (Cerezke 1995), as do its fungal associates (Rice et al. 2007). Jack pine is the most abundant pine species in the boreal forest which could potentially lead to the spread of MPB across the boreal forest to eastern Canada.

Global climate change might allow MPB to expand its range into formerly unoccupied lodgepole pine habitat and also enable further eastward invasion of jack pine (Logan and Powell 2001; Williams and Liebhold 2002). Climate change is expected to produce longer and more frequent droughts in many regions of the world (Breshears et al. 2009) which may influence the ability of trees to defend themselves against invading insects and diseases (reviewed in Franceschi et al. 2005). For plant species that are conservative water users, prolonged droughts will decrease carbon dioxide uptake and associated photosynthetic carbon assimilation due to stomata closure. Reduced carbon gain can result in the depletion of carbohydrate reserves for biosynthesis of defensive compounds, particularly carbon-based compounds such as terpenoids that could make trees more susceptible to biotic stress factors, such as bark beetles (McDowell et al. 2008; Breshears et al. 2009). Water deficit is one of the climatic variables used in climate-suitability models for MPB populations; in these models, it reduces the resistance of lodgepole pine to attack and subsequent development and survival of the beetle (Safranyik et al. 2010).

Increasing temperature predicted under a global climate change scenario could also alter volatile emission by potential host trees, since monoterpene emission is temperature dependent (Kesselmeier and Staudt 1999), and might influence host finding by MPB. Two mechanisms are

proposed to explain host finding by pioneering MPB. Beetles could visually orient towards and randomly land on potential host trees and then use gustatory cues to assess the suitability of the host (Raffa and Berryman 1982). Alternatively, recognition and direct flight towards a host could be based on orientation to volatile organic compounds (VOC) emitted from the host (Moeck and Simmons 1991) in combination with visual cues. MPB is able to detect and avoid non-hosts, which supports the hypothesis of direct flight as a host finding mechanism (Huber et al. 2000). Both mechanisms might act together; differences in VOC emission from different host trees grown under different conditions and with different levels of beetle infestation may be an indication of host suitability to orienting beetles. Successful attack and colonization of the host may be further influenced by the chemical composition of the bark of the host tree (Faccoli et al. 2005; Raffa et al. 2005).

Once a suitable host is found by a female pioneer beetle, α-pinene – one of the most abundant host monoterpenes in most pine species examined to date – is hydroxylated into the major aggregation pheromone *trans*-verbenol to attract both sexes of MPB to initiate a mass attack (Pitman et al. 1968; Pureswaran et al. 2000; Blomquist et al. 2010). As the colonization progresses, arriving males produce *exo*-brevicomin to attract additional females until the optimum attack density is achieved at which point both male and female beetles emit anti-aggregation pheromones to prevent further recruitment to the host tree (Rudinsky et al. 1974; Ryker and Libbey 1982).

At least two MPB associated blue stain fungi, *Grosmannia clavigera* and *Ophiostoma montium*, assist beetles in depleting tree defenses and killing their host (Reid et al. 1967; Solheim and Krokene 1998; Rice et al. 2007) during the host colonization process. *G. clavigera* is more virulent than *O. montium* (Yamaoka et al. 1990) and is often used experimentally to stimulate tree defenses (Reid et al. 1967; Lieutier et al. 2009).

Trees that are mass attacked by the bark beetle plus associated fungi defend themselves with resin, a mixture of monoterpenes, sesquiterpenes, diterpenoid resin acids, and phenolic compounds characteristic of conifers (Gershenzon and Croteau 1991; Keeling and Bohlmann 2006) that act as physical and chemical defense mechanisms. Resin monoterpene composition differs between species, and the composition of individual trees in various pine species correlates with resistance to bark beetle attack (Sturgeon 1979; Gollob 1980).

In order to test the hypothesis that tree responses differ between lodgepole and jack pine

trees, and are also affected by environmental conditions, we conducted a controlled environment experiment on seedlings subjected to water deficit (simulating drought conditions) and used the MPB fungal associate *Grosmannia clavigera* in conjunction with wounding to stimulate tree defenses.

The objectives were to (1) develop a chemical profile of volatiles released from the two different host tree species; (2) evaluate if volatile chemical profiles vary within and between tree species when subjected to different environmental (water deficit vs. well watered) and biological (fungal inoculation, mechanical wounding, and control) treatments; and (3) determine if the monoterpenoid content of tissues is affected by the treatments and differs between tree species.

METHODS AND MATERIALS

Seedling Source and Treatments Seedlings in their second growth cycle were used for all experiments. Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. ex Wats.) originated from west-central Alberta, while jack pine was from provenances in north-central Saskatchewan. Seedlings were grown through their first growth cycle under near-identical conditions at PRT Vernon, then packaged and placed in cold storage for approximately three months to satisfy dormancy requirements. Dormant seedlings were shipped overnight to Edmonton, where n=84 seedlings per species were planted (pot size: 15×18 cm) in Sunshine Mix #4 (Sungro, Vancouver BC Canada) and maintained throughout the experiment in a walk-in growth chamber, with 16 h light/8 h dark, 200 µmol m^{-2} full spectrum light intensity, 20 °C constant temperature and approximately 50 % humidity. Fertilizer was applied with irrigation weekly (0.5 g/l 20N -20P -20K), with additional watering provided as necessary until one week before the start of the experiment at which point both species had completed shoot elongation with an average height of 21.5 cm (± 2.9 S.D) for lodgepole pine and 32.5 cm (± 4.0 S.D) for jack pine seedlings. Once terminal bud formation had been initiated seedlings of each species were randomly divided into two groups and received one of the following two environmental treatments: water deficit or well watered. Seedlings in the water deficit group received only 50 ml of water and seedlings in the well watered group received 400 ml of water twice a week for the duration of the 6-week experiment. In order to ensure that the water deficit seedlings were experiencing mild water stress conditions at the onset of the 6-week experiment, water was withheld from these seedlings prior

to the application of biological treatments. Seedlings in both environmental treatment groups were randomly assigned to one of the following three biological treatments: (1) mechanical wounding plus inoculation with *G. clavigera*; (2) mechanical wounding alone; and (3) untreated control. The aim of the first treatment was to stimulate tree defenses with the MPB fungal associate and the last two treatments were positive and negative control treatments, respectively. Seedlings were inoculated with 5µl of a *G. clavigera* spore suspension injected into a pouch made with a 23G1Precicsion Glide needle. The spore suspension was prepared by using a sterile inoculation loop and transferring two loopfuls (20 µl) of spores of *G. clavigera* from a well sporulating strain collected from Fox Creek Alberta (54°24'N, 116°48'W) into sterile saline. Seedlings were inoculated at three points, equally distributed along the whole length of the stem. Mechanical wounding was applied in a similar manner but without the fungal inocula (Fig.1).

For each tree species and environmental x biological treatment combination, four seedlings were randomly selected (total of 24 seedlings per species) and monitored for volatile emission, photosynthetic rate, stomatal conductance and soil water content the day before inoculation (t=0), and at five time points post inoculation (t= 1 day, t= 1 week, t= 2 weeks, t= 4 weeks, and t= 6 weeks). Twelve of the remaining seedlings of each treatment combination per species were destructively harvested at each time point (n=2) for chemical analysis of the bark (primarily constituting phloem tissue) of the current year and previous growth of the stem (referred to as current year and previous year bark) and needles. After 6 weeks, we harvested the seedlings that were used for acquiring volatile emission and physiology data for tissue analyses (below).

Soil Water Content and Gas Exchange Parameters. Soil water content was measured using time domain reflectrometry (TDR) (Hillel 1998). We measured the apparent dielectric constant of the soil with a Tektronix 1502B (Beaverton, Oregon, USA) and used the empirical equation for organic soils in Robinson et al. (2003) to relate it to water content. Rate of photosynthesis and stomatal conductance were measured using a Li-Cor 6400 (LI-COR Biosciences, Lincoln, Nebraska, USA). The instrument settings were: leaf area 1.5 cm^2, flow 300 µmol/sec, and Quantum flux lamp at 200 µmol/m^2sec (equivalent to light intensity in growth chamber).

Volatile Collection. Volatile organic compounds (VOCs) released from seedlings were collected one day after the physiological data were recorded from the same plant. An oven bag (LOOK®, 45×55cm) was imposed over the whole seedling and closed near the base of the stem with a cable tie. An absorbent tube (Porapak Q (OD 6mm, length 110mm; absorbent: front layer 150 mg, back up layer 75 mg; separated by glass wool) SKC Inc., Pennsylvania, USA) was inserted in the bag at the top of the seedling and affixed with Parafilm®. A small hole was placed on the other side of the oven bag to maintain constant air pressure inside the bag. Volatile emissions were collected for four hours at a constant flow rate of 200 ml/min. After collection, the sorbent tubes were capped and stored at -40°C until extraction. Air samples were collected at two locations of the growth chamber to control for possibility of contamination with chamber air.

Porapak Q tubes were extracted with 1 ml of dichloromethane (Sigma-Aldrich, St. Louis, Missouri, USA) spiked with 0.01% (v/v) tridecane (Sigma-Aldrich, St. Louis, Missouri, USA) as surrogate standard and subsequently stored at -40°C before GC analysis.

Tissue Extracts. Seedling were harvested outside of the chamber in which VOCs were collected. During seedling harvest, bark and needle samples were rapidly frozen in liquid nitrogen, and stored at -40°C prior to extraction. Tissue was ground in liquid nitrogen, and 100 mg of the tissue was transferred to a 1.5 ml microcentrifuge tube. The samples were extracted twice with 0.5 ml dichloromethane and 0.01% tridecane as surrogate standard. After adding the solvent the samples were vortexed for 30 sec, sonicated for 10 min, subsequently centrifuged at 13200 rpm and 0°C for 15 min, and placed in a freezer for at least two hours to let the pellet freeze. Extracts were transferred into an amber GC vial and stored at -40°C before GC analysis.

GC Analysis. Samples (1µl) were injected in an Agilent 7890A Gas Chromatograph (Agilent Technologies, Santa Clara, California, USA) with an HP Innowax (Agilent Technologies) column (I.D. 0.32 mm, length 30m), helium carrier gas flow at 1.8ml/min, temperature 50°C for 2 min, increased to 160°C by 5°C per min and then to 250°C by 20°C.

Peaks were identified using the following standards: Borneol, pulegone, α-terpinene, γ-terpinene, α-terpineol (Sigma-Aldrich, St. Louis, Missouri, USA), camphor, 3-carene, α-humulene, terpinolene, α-thujone and ß-thujone, (-)-α-pinene, (-)-β-pinene, (S)-(-)-limonene, sabinene hydrate, myrcene, (-)-camphene, p-cymene (Fluka, Sigma-Aldrich, Buchs, Switzerland),

bornyl acetate, *cis*-ocimene (SAFC Supply Solutions, St. Louis, Missouri, USA), β-phellandrene (Glidco Inc., Jacksonville, Florida, USA).

Calibration with standards allowed for analysis of quantitative differences among samples of differently treated seedlings at different time points. For all samples, the peaks were integrated and peak area was compared for qualitative and quantitative differences among samples of differently treated seedlings at different time points.

After all samples were analyzed with the GC, subsequent analysis with GC-mass spectrometry showed that our method was unable to separate myrcene and α-phellandrene. From a different experiment we know that α-phellandrene hardly occurs in jack pine seedlings and makes up to 35% of the peak in lodgepole pine seedlings.

Statistical Analyses. The VOC emission data and the seedling physiology data collected from the same trees were analyzed using the R statistical language (R development core team, 2010). In order to address how volatile emission of the seedlings changed due to the environmental and biological treatments and their associated change in physiology we carried out a canonical redundancy analysis (RDA; Legendre and Legendre 1998) using the rdaTest package (Legendre and Durand 2010). RDA axes were tested for significance by permutations with the vegan package (Oksanen et al. 2010) also through the R statistical language. Explanatory variables included environmental and biological treatments, as well as soil water content, photosynthesis and stomatal conductance. Total and individual monoterpenes of all time points were the response variables. Due to technical difficulties during the extraction process, two time points (week 1 and 6) were discarded from the jack pine dataset. In order to evaluate whether the resulting lack of correlation between soil water content and physiological parameters in jack pine was due to the missing two time points, we also carried out an RDA for lodgepole pine with the same time points omitted, the results remained the same.

To assess the effect of environmental treatments on total monoterpene emission from both tree species over time, Mann-Whitney *U* tests were used. The effect of the biological treatments was assessed using repeated measures ANOVAs with subsequent pairwise comparison with Bonferroni correction on water deficit and well watered seedlings separately using SPSS Statistics 17.0. Soil water content and plant physiology results are presented using bar graphs

with error bars showing 95% confidence intervals, non-overlapping bars represent a significant difference (Field 2009).

All tissue extract data was analyzed using SPSS Statistics 17.0. Total monoterpenes were analyzed using repeated measure ANOVA. Individual monoterpene data was transformed with log(x+1) to meet the assumptions of normality and analyzed for all tissues separately using ANOVA. In cases in which the data violated the assumptions of an ANOVA, non parametric tests were conducted on non transformed data. Interspecific differences were analyzed using *t*-test.

RESULTS

Soil Water Content and Gas Exchange Parameters. The water deficit seedlings were not watered the week before the experiment started; therefore there was a significant difference in soil water content between the well watered and water deficit seedlings on the first day of the experiment (Fig. 2). Through the duration of the experiment, the water content of the soil in the water deficit treatment decreased to almost 0%, whereas soil water content of well-watered seedlings stayed constant between 20-30%.

Independent of the biological treatments, the photosynthesis rate in lodgepole pine decreased over time in the water deficit group, but not in the well watered group (Fig. 3a). In jack pine, neither the biological nor the environmental treatments had an effect on photosynthesis rate. Stomatal conductance was generally higher in lodgepole than jack pine but it decreased sooner due to environmental treatment in lodgepole pine and reached 1/3 of the jack pine stomatal conductance by the end of the experiment (Fig. 3b).

Chemical Profiles. The main differences in the chemical profile emitted by seedlings of the two pine species are that lodgepole pine emits a higher percentage of β-phellandrene (27% ± 2.25 SE) than jack pine (1% ± 0.26 SE) and jack pine releases more α-pinene (27% ± 3.25 SE) than lodgepole pine (7% ± 0.80 SE) (Fig. 4). 3-Carene makes up more of the volatile chemical profile in jack pine than lodgepole pine (21% ± 3.99 SE in JP, 7% ± 1.76 SE in LP).

Volatile Emission. The first axis of the lodgepole pine RDA ($p<0.001$) and of the jack pine RDA ($p=0.006$) was significant. RDA triplots (Fig. 5) illustrate the relationship between the gas exchange parameters and volatile emission of the seedlings and environmental and biological treatments for each tree species.

In both species, 3-carene emission was correlated with fungal inoculation. Emission of the major compounds, β-phellandrene in lodgepole and α-pinene in jack pine, is correlated with the physiological state (photosynthesis and stomatal conductance) of the seedling. The physiological parameters of stomatal conductance and photosynthesis were positively correlated with soil water content in lodgepole but not jack pine seedlings.

In both lodgepole pine and jack pine, water deficit seedlings released significantly less total monoterpenes starting at week 4 of the experiment (Mann-Whitney *U*, lodgepole pine: $U=21$, $z=-2.94$, $p=0.003$; jack pine: $U=26$, $z=-2.66$, $p=0.008$) and thus, all further analyses were conducted on water deficit and well watered seedlings separately. Biological treatments had a significant effect on total monoterpene emission in water deficit lodgepole pine seedlings (repeated measures ANOVA, $F(2,8)=6.329$, $p=0.022$; Fig. 6). Subsequent pairwise comparison with Bonferroni correction of the timepoints revealed that one day after biological treatment application more VOCs were emitted from lodgepole pine than at any other timepoint ($p=0.006$; Fig. 6). Further, pairwise comparison of the biological treatments showed that lodgepole pine seedlings that were inoculated with *G. clavigera* emitted significantly more monoterpenes than controls ($p=0.029$). Mechanically wounded seedlings also emitted more volatiles than control seedlings but not at a significant level ($p=0.066$). Well-watered lodgepole pine demonstrated the same trends but showed no significant differences. In jack pine, the biological treatments had no significant influence on VOC emission. Lodgepole pine seedlings emitted less VOC than jack pine but the difference was not significant.

Tissue Extracts. The number of detectable monoterpenes in the tissue extracts of both species was higher than in the volatile profiles (Table 1). Sabinene hydrate, *cis*-ocimene, α-thujone, and pulgeone occurred only in lodgepole pine and were never detected in jack pine.

The overall needle monoterpene content in lodgepole pine seedlings is lower compared to previous year and current year growth bark (repeated measures ANOVA, $F(1.80,127.70)=118.37$, $p<0.001$; Fig. 7). *cis*-Ocimene is the only terpenoid that did not follow this pattern and occurred

in higher concentration in the needles. All further analyses were conducted on the different tissues separately. The environmental treatment affected terpinolene, which occurred in higher concentrations in the current year and previous year bark of watered seedlings (ANOVA, $F(1,70)=3.999$, $p=0.028$ and $F(1,70)=5.059$, $p=0.049$, respectively) than the water deficit seedlings. Less bornyl acetate occurred in the previous year's bark of watered seedlings (ANOVA, $F(1,70)=5.265$, $p=0.025$) compared to seedlings subjected to water deficit. The biological treatments had no effect on any of the measured terpenoids in the tissue extracts.

Like lodgepole pine, jack pine needles had a significantly less overall monoterpene content than current year or previous year bark (repeated measures ANOVA, $F(2,142)=115.66$, $p<0.001$; Fig. 7). The environmental treatment had no effect on the monoterepene content of jack pine tissues. The bornyl acetate content in the previous year bark of fungal-inoculated seedlings was significantly higher than in wounded seedlings (Kruskal-Wallis, $H(2)=16.467$, $p<0.001$; Fig. 8).

The overall monoterpene content of all tissues increased in both species over time independent from any manipulation (Fig. 9). Both species exhibit similar terpenoid content in collected bark tissues, but jack pine needles contain significantly more terpenoids compared to those from lodgepole pine (*t*-test, $t(117.664)=3.413$, $p=0.001$; Fig. 7).

DISCUSSION

Water regime manipulation and stimulation of plant defenses affected the emission of VOCs differently in the two pine species. In general, jack pine emitted higher amounts of 3-carene (21%), compared to lodgepole pine (7%) while lodgepole pine released higher amounts of β-phellandrene (27%) than jack pine (1%) (Fig. 4). β-Phellandrene is the only monoterpene that attracts MPB in the absence of aggregation pheromones when released at high doses (Miller and Borden 1990), which could mean that pure jack pine stands might fail to attract pioneer beetles flying actively into the region compared to lodgepole pine stands.

The chemical profile of jack pine seedlings tested in the current study contained 21% of 3-carene on average, similar to previous reports of jack pine from central Saskatchewan, Canada (Pauly and von Rudolf 1971) and consistent with the profile of mature jack pine in the Smoky Lake region of Alberta (unpublished data). Within the wide range of jack pine in North America,

there are surprisingly few records available on chemical profiles of jack pine and almost all records are from the most southern extension of the jack pine forest in Wisconsin, USA. In those studies, the chemical profiles consist primarily of α- and β-pinene; 3-carene was either not found or occurred only in trace amounts (Zavarin 1969; Wallin and Raffa 1999; Erbilgin et al. 2001; Aukema et al. 2010). An early study that directly compared resin monoterpene composition among lodgepole pine, jack pine and their hybrids found 3-carene to be a signature compound in lodgepole pine that could distinguish it from jack pine (Zavarin 1969). This large variability of 3-carene in jack pine monoterpene profiles suggests that there are two or more phytochemical phenotypes in jack pine, similar to lodgepole pine (Forrest 1980), or that there is a greater degree of introgression of lodgepole pine genes into jack pine in the western extent of the jack pine range than originally assumed. A similar variability of 3-carene content occurs in Scots pine seedlings from different provenances in Turkey and Finland (Semiz et al. 2007), suggesting that terpenoid synthesis is under genetic control and differs by geographical origin of the seeds. Clarification of the role, if any, of 3-carene in jack pine profiles is essential in order to predict the potential interaction between MPB and jack pine in the boreal forest.

The chemical profile measured from lodgepole pine seedlings in the current study is in agreement with existing published profiles (Zavarin 1969; Pauly and von Rudolf 1971; Pureswaran et al. 2004a). The consistency in monoterpene profiles of lodgepole pine resin enabled the use of chemosystematics across the geographic range of the species (Forrest 1980). Chemical profiles are used to distinguish subspecies/varieties of lodgepole pine, which are: subsp. *contorta*, *bolanderi*, *murrayana*, and *latifolia* (Critchfield 1957). Chemotyping only failed to distinguish the lodgepole pine subspecies *murrayana* and *latifolia* (Forrest 1980; Bohm 2009). Our results underscore the need for a similar thorough study to chemotype jack pine trees across its broad range.

Seedlings of both species emitted an overall lower quantity of VOCs under water deficit conditions which was evident at day one of the experiment but significant at week four (Fig. 6). Although emission of VOCs is not thought to be controlled by stomatal conductance (Sharkey 1991; Kesselmeier and Staudt 1999; Blanch et al. 2007), in our study stomatal conductance may have indicated the general condition of seedlings and indirectly affected the efficiency of monoterpene synthesis in the plant to increase monoterpene emission rates. Similarly, VOC emission was strongly reduced by severe drought conditions and linked to stomatal closure in

common Mediterranean woody species including *Pinus halepensis* (Llusià and Peñuelas 1998). Although in a similar study, *Pinus halepensis* seedlings exposed to moderate drought conditions exhibited no change in VOC emission as a result of water deficit (Blanch et al. 2007). In the current study, stomatal conductance explained the release of some of the most abundant monoterpenes in lodgepole pine and jack pine. Likewise, Niinemets et al. (2002) concluded that stomatal conductance in *Pinus pinea* affected the emission of alcohols, aldehydes, carboxylic acids, and oxygenated monoterpenoids. This finding should be considered in monoterpenoid emission models that currently use a simple equation that relies mainly on temperature to describe the monoterpene efflux from foliage (Guenther et al. 1991) and does not consider any role of stomatal conductance in VOCs emission (Sharkey 1991; Kesselmeier and Staudt 1999).

Stomatal conductance and the resulting gas exchange that is essential for the carbon reactions of photosynthesis depend on the water transport from soil to leaf (Hubbard et al. 2001). Plant species categorized as isohydric exhibit tight control over stomatal aperture in response to soil water deficit as a means to regulate water loss; whereas species classified as anisohydric do not (Tardieu and Simonneau 1998; McDowell et al. 2008). Stomatal conductance in lodgepole pine seedlings in the current study was higher than that in jack pine and responded more quickly to a decrease in soil water content (Fig. 3). Jack pine appears to be better adapted to low water availability than lodgepole pine which is consistent with the success of jack pine in dry and nutrient poor soils across its natural range (Vidaković 1991). In the current study, soil moisture content influenced the physiological state of pine species differently. The RDA results (Fig. 5) support the findings that jack pine is better adapted to water deficit conditions as the physiological state of lodgepole pine but not that of jack pine was correlated with soil water content.

Fungal inoculation of seedlings with an MPB associated fungus also altered the VOC emissions in both pine species. 3-Carene emission increased and was correlated with fungal inoculation in both lodgepole and jack pine (Fig. 5). Mature jack pine trees inoculated with the pine engraver, *Ips pini*, associated fungus *Ophiostoma ips* had increased 3-carene phloem content (Raffa and Smalley 1995). Jost et al. (2008) inoculated mature lodgepole and jack pine trees as well as their hybrids with MPB associated fungi and collected bark volatiles after a 6- week inoculation period. Unfortunately, they did not measure 3-carene and the only significant difference they found was a strong reduction in α-pinene emission in fungal inoculated lodgepole

406 pine. Direct comparison of the two studies is hard to make since they did not quantify their
407 results but only compared ratios. During our experiment, the fungal inoculated lodgepole pine
408 seedlings always released more α-pinene than the control seedlings. Resin monoterpenes from
409 ponderosa pine including α- and β-pinene, 3-carene, limonene, and terpinolene have anti-fungal
410 properties (Himejima et al. 1992). Monoterpenes and other chemical compounds in the resin of
411 host pine inhibit the growth of bark beetle-associated fungi in the southern US coniferous system
412 (Bridges 1987; Klepzig et al. 1996). There is growing evidence that plants respond to microbial
413 attack by releasing volatiles which initiates defense mechanisms by signaling within as well as
414 possibly between plants (Kesselmeier and Staudt 1999). Volatile organic compounds released
415 from inoculated plants induce the resistance to fungi in *Arabidopsis* (Kishimoto et al. 2006), but
416 hardly anything is known about plant communication in conifers (Heil and Karban 2009). The
417 increased emission of 3-carene after fungal inoculation in our study might be due to allocation of
418 that compound towards the inoculation site by the plant to fight the fungus and the wound
419 facilitating its release. In particular, damaged bark can be a major source for VOC emission in
420 conifer seedlings (Heijari et al. 2011).
421 In the current study, we sampled both volatile and tissue chemistry of both pine species in
422 response to environmental and biological treatments. There are fewer detectable monoterpenes in
423 the seedling volatile profiles compared to monoterpene content in bark and needles. Monoterpene
424 concentration in bark (phloem) and needles can also respond to drought stress (Hodges and Lorio
425 1975; Kainulainen et al. 1992; Llusià and Peñuelas 1998). Monoterpenes and resin acids in the
426 woody tissue of Scots pine and Norway spruce seedlings significantly increased as a result of
427 drought exposure (Turtola et al. 2003). In our study, water deficit had no effect on overall
428 monoterpene content in either tissue type. In fact, terpinolene concentration decreased in the bark
429 as a result of water deficit in the lodgepole pine seedlings. The only compound that significantly
430 increased due to water deficit treatment was bornyl acetate in the previous year's bark of
431 lodgepole pine seedlings. The levels of bornyl acetate was also associated with fungal inoculation
432 in jack pine previous year's bark (Fig. 8). Increased bornyl acetate concentrations in the resin of
433 lodgepole pine are associated with Armillaria root disease (*Armillaria mellea*) (Nebeker et al.
434 1995). Bornyl acetate levels also increase in root and stem tissue of Douglas-fir seedlings
435 (*Pseudotsuga menziesii*) treated with methyl jasmonate, a phytohormone implicated in mediating
436 defense responses (Huber et al. 2005). In some areas MPB attack is linked to pines infested with

A. mellea (Tkacz and Schmitz 1986). Mountain pine beetle antennae do not respond to bornyl acetate (Pureswaran et al. 2004b), but it is possible that it can taste this compound since olfactory and gustatory information are processed in different areas of the insect brain (DeBruyne and Warr 2006). Gustatory assessment might be an important way for MPB to evaluate the health status of a host tree and to determine how well defended it is.

The primary goal of this study was to evaluate the chemical profiles of jack pine and lodgepole pine trees and to make reasonable assumptions about jack pine suitability as a potential host to MPB based on its chemistry. All volatile monoterpenes emitted by lodgepole and jack pine seedlings in the current study can be detected by the MPB (Huber et al. 2000; Pureswaran et al. 2004b). Some of these host monoterpenes synergize MPB response to the aggregation pheromones *trans*-verbenol and *exo*-brevicomin in trapping studies (Borden et al. 1986; Borden et al. 2008). There is strong evidence that myrcene, 3-carene, terpinolene and α-pinene synergize MPB aggregation pheromone response (Seybold et al. 2006; Borden et al. 2008). In olfactometer tests, 3-carene was most attractive to MPB out of 20 tested compounds (Conn 1981). In our study, 3-carene emission increased with plant defense stimulation by *G. clavigera.* This compound might function as an indicator of a weakened tree and could support the aggregation of beetles on susceptible hosts. MPB is able to distinguish between host and non-host volatiles, and may also use host volatiles to identify weak hosts and avoid the risks associated with trying to overcome tree defenses of a healthy host (Keeling and Bohlmann 2006). Jack pine emits about three times more 3-carene than lodgepole pine, suggesting that it may be more prone to MPB attacks (Conn 1981; Borden et al. 2008). Further, jack pine bark contains higher concentrations of α-pinene than lodgepole pine, and α-pinene can be a synergist for the MPB aggregation pheromone and it is also a precursor for production of *trans*-verbenol, the primary component of MPB aggregation pheromone (Borden et al. 2008).

This study enabled us to compare VOC emission and monoterpene content between lodgepole and jack pine exposed to various environmental and biological treatments under controlled and easy to manipulate conditions. The results from these studies suggest that monoterpenes that play a role in pine – MPB interactions differ between lodgepole and jack pine, and are also affected by water availability. Shrimpton and Watson (1971) recommended the use of lodgepole pine seedling for studying wound response, since seedlings and mature trees are identical in their resistant response. Nevertheless, inferences made from our study to MPB host

use are limited as beetles do not attack trees at the seedling stage. However, the results from this study are critical for developing the models that guide rational design of field studies, with their inherently greater degree of difficulty and experimental variance. Additional field experiments to understand chemically-mediated interactions among MPB, its associated fungus and its pine hosts under water deficit conditions are currently being conducted in mature lodgepole, jack and hybrid pine stands. Future studies will assess the behavioral importance of the host monoterpenes from the two species in host detection and acceptance by MPB.

Acknowledgements – We thank two anonymous reviewers for their helpful suggestions on an earlier version of this manuscript. We acknowledge the lab members of Janice E. K. Cooke as well as Boyd Mori for helping potting the seedlings; Miles Dyck for providing us with the TDR equipment; Celia Boone for sharing β-phellandrene with us; Patrick James for an introduction to R; and Joanne Mann (West Fraser Mills Ltd., Hinton Wood Products), Michael Bendzsak (Saskatchewan Forest Centre), and Glenn Goodwill, Candace Kent and Stewart Haywood-Farmer (PRT) for providing seedlings. We particularly acknowledge Adrianne Rice for providing fungal culture and knowledge, Jeremiah Bolstad and Andrew Ho for the help during the sample extraction process.
Funding for this research has been provided through grants from the Government of Alberta through Genome Alberta, the Government of British Columbia through Genome BC and Genome Canada in support of the Tria 1 and Tria 2 projects (http://www.thetriaproject.ca) of which MLE, JEKC, and NE are co-investigators.

REFERENCES

AUKEMA, B. H., POWELL, J. S., CLAYTON, M. K., and RAFFA, K. F. 2010. Variation in complex semiochemical signals arising from insects and host plants. Environ. Entomol. 39(3):874-882.

BLANCH, J.-S., PEÑUELAS J., and LLUSIÀ, J. 2007. Sensitivity of terpene emissions to drought and fertilization in terpene-storing *Pinus halepensis* and non-storing *Quercus ilex*. Physiol. Plantarum 131:221-225.

BLOMQUIST, G. J., FIGUEROA-TERAN, R., AW, M., SONG, M., GORZALSKI, A., ABBOTT, N. L., CHANG, E., and TITTIGER, C. 2010. Pheromone production in bark beetles. Insect Biochem. Molec. 40:699-712.

BOHM, B. A. 2009. The geography of phytochemical races. Springer, New York.

BORDEN, J. H., CONN, J. E., OEHLSCHLAGER, A. C., PIERCE JR., H. D., and CHONG, L. J. 1986. Composition for attracting, and method of combating mountain pine beetles. Canadian patent no. 1212044. 1986 September 30.

BORDEN, J. H., PURESWARAN D. S., and LAFONTAINE, J. P. 2008. Synergistic blends of monoterpenes for aggregation pheromones of the mountain pine beetle (Coleoptera: Curculionidae). J. Econ. Entomol. 101(4):1266-1275.

BRESHEARS, D. D., MYERS, O. B., MEYER, C. W., BARNES, F. J., ZOU, C. B., ALLEN, C. D., McDOWELL, N. G., and POCKMAN, W. T. 2009. Tree die-off in response to global change-type drought: Mortality insights from a decade of plant water potential measurements. Front. Ecol. Environ. 7(4):185-189.

BRIDGES, J. R. 1987. Effects of terpenoid compounds on growth of symbiotic fungi associated with the southern pine beetle. Phytopathology 77: 83-85.

CARROLL, A. L., RÉGNIÈRE, J., LOGAN, J. A., TAYLOR, S. W., BENTZ, B. J., and POWELL, J. A. 2006. Impacts of climate change on range expansion by the mountain pine beetle. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC. Mountain Pine Beetle Initiative Working Paper 2006-14. 20 p.

CEREZKE, H. F. 1995. Egg gallery, brood production, and adult characteristics of mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in three pine hosts. Can Entomol. 127:955-965.

CONN, J. E. 1981. Pheromone production and control mechanisms in *Dendroctonus ponderosae* Hopkins. M.Sc. thesis, Simon Fraser University, Burnaby, B.C.

CRITCHFIELD, W. B. 1957. Geographic variation in *Pinus contorta*. Harvard University, Cambridge, Massachusetts. Maria Moors Cabot Found. Publ. 3.

CULLINGHAM, C. I., COOKE, J. E. K., DANG, S., DAVIS, C. S., COOKE, B. J., and COLTMAN, D. W. 2011. Mountain pine beetle host-range expansion threatens the boreal forest. Mol. Ecol. 20:2157-2171.

DeBRUYNE, M. and WARR, C. G. 2006. Molecular and cellular organization of insect chemosensory neurons. BioEssays 28:23-34.

ERBILGIN, N., SZELE, A., KLEPZIG, K. D., and RAFFA, K. F. 2001. Trap type, chirality of α-pinene, and geographic region affect sampling efficiency of root and lower stem insects in pine. J. Econ. Entomol. 94: 1113-1121.

FACCOLI, M., BLAŽENEC, M., and SCHLYTER, F. 2005. Feeding response to host and nonhost compounds by males and females of the spruce bark beetle *Ips typographus* in a tunneling microassay. J. Chem. Ecol. 31(4):745-759.

FIELD, A. 2009. Discovering statistics using SPSS. SAGE Publications Ltd, London.

FORREST, G. I. 1980. Geographical variation in the monoterpenes of *Pinus contorta* oleoresin. Biochem. Syst. Ecol. 8:343-359.

FRANCESCHI, V. R., KROKENE, P., CHRISTIANSEN, E., and KREKLING, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytol. 167:353-376.

GERSHENZON, J. and CROTEAU, R. 1991. Terpenoids, pp.165-219, in: G. Rosenthal and M. Berenbaum (eds.). Herbivores: Their interaction with secondary plat metabolites, Vol 1: The chemical participants. Academic Press, San Diego, California.

GOLLOB, L. 1980. Monoterpene composition in bark beetle-resistant loblolly pine. Naturwissenschaften 67:409-410.

GUENTHER, A. B., MONSON, R. K., and FALL, R. 1991. Isoprene and monoterpene emission rate variability: Observation with Eucalyptus and emission rate algorithm development. J. Geophys. Res. 96:10799-10808.

HEIJARI, J., BLANDEA, J. D., and HOLOPAINEN, J. K. 2011. Feeding of large pine weevil on Scots pine stem triggers localised bark and systemic shoot emission of volatile organic compounds. Environ. Exp. Bot. 71: 390–398.

HEIL, M. and KARBAN, R. 2009. Explaining evolution of plant communication by airborne signals. Trends Ecol Evol. 25(3):137-144.

HILLEL, D. 1998. Environmental soil physics. Academic Press, San Diego, California.

HIMEJIMA, M., HOBSON, K. R., OTSUKA, T., WOOD, D. L., and KUBO, I. 1992. Antimicrobial terpenes from oleoresin of ponderosa pine tree (*Pinus ponderosa*): A defense mechanism against microbial invasion. J. Chem. Ecol. 18(10):1809-1818.

HODGES, J. D. and LORIO JR., P. L. 1975. Moisture stress and composition of xylem oleoresin in loblolly pine. Forest Sci. 21:283-290.

HUBBARD, R. M., RYAN, M. G., STILLER, V., and SPERRY, J. S. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ. 24:113-121.

HUBER, D. P. W., GRIES, R., BORDEN, J. H., and PIERCE JR., H. D. 2000. A survey of antennal responses by five species if coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. Chemoecology 10:103-113.

HUBER, D. P. W., PHILIPPE, R. N., MADILAO, L. L., STURROCK, R. N., and BOHLMANN, J. 2005. Changes in anatomy and terpene chemistry in roots of Douglas-fir seedlings following treatment with methyl jasmonate. Tree Physiol. 25:1075-1083.

JOST, R.W., RICE A.V., LANGOR D.W., and BOLUK, Y. 2008. Monoterpene emission from lodgepole and jack pine bark inoculatied with mountain pine beetle-associated fungi. J. Wood Chem. Technol. 28(1):37-46.

KAINULAINEN, P., OKSANEN, J., PALOMÄKI, V., HOLOPAINEN, J.K., and HOLOPAINEN, T. 1992. Effect of drought and waterlogging stress on needle monoterpenes of *Picea abies*. Can. J. Botany 70:1613-1616.

KEELING, C. I. and BOHLMANN, J. 2006. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence on conifers against insects and pathogens. New Phytol. 170:657-675.

KESSELMEIER, J. and STAUDT, M. 1999. Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. J. Atmos. Chem. 33:23-88.

KLEPZIG, K. D., SMALLEY, E. B., and RAFFA, K. F. 1996. Combined chemical defenses against an insect-fungal complex. J. Chem. Ecol. 22(8):1367-1388.

KISHIMOTO, K., MATSUI, K., OZAWA, R., and TAKABAYASHI, J. 2006. Analysis of defensive responses activated by volatile *allo*-ocimene treatment in *Arabidopsis thaliana*. Phytochemistry 67:1520-1529.

LEGENDRE, P. and DURAND, S. 2010. rdaTest: Canonical redundancy analysis (R package version 1.7). URL http://www.bio.umontreal.ca/legendre/.

LEGENDRE, P. and LEGENDRE, L. 1998. Numerical Ecology. Elsevier Science, Amsterdam.

LIEUTIER, F., YART, A., and SALLE, A. 2009. Stimulation of tree defenses by ophiostomatoid fungi can explain attack success of bark beetles on conifers. Ann. For. Sci. 66:801-823.

LLUSIÀ, J. and PEÑUELAS, J.1998. Changes in terpene content and emission in potted Mediterranean woody plants under severe drought. Can. J. Botany 76:1366-1373.

LOGAN, J. A. and POWELL, J. A. 2001. Ghost forests, global warming, and the mountain pine beetle (Coleoptera: Scolytidae). Am. Entomol. 47(3):160-172.

McDOWELL, N., POCKMAN, W. T., ALLEN, C. D., BRESHEARS, D. D., COBB, N., KOLB, T., PLAUT, J., SPERRY, J., WEST, A., WILLIAMS, D. G., and YEPEZ, E. A. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol. 178:719-739.

MILLER, D. R. and BORDEN, J. H. 1990. β-Phellandrene: Kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). J. Chem. Ecol. 16(8):2519-2531.

MOECK, H. A. and SIMMONS, C. S. 1991. Primary attraction of mountain pine beetle, *Dendroctonus ponderosae* Hopk. (Coleoptera: Scolitidae), to bolts of lodgepole pine. Can. Entomol. 123:299-304.

NEBEKER, T. E., SCHMITZ, R. F., TISDALE, R. A., and HOBSON, K. R. 1995. Chemical and nutritional status of dwarf mistletoe, armillaria root rot, and comandra blister rust infected trees which may influence tree susceptibility to bark beetle attack. Can. J. Botany 73:360-369.

NIINEMETS, Ü., REICHSTEIN, M., STAUDT, M., SEUFERT, G., and TENHUNEN, J. D.

2002. Stomatal constraints may affect emission of oxygenated monoterpenoids from the foliage of *Pinus pinea*. Plant Physiol. 130:1371-1385.

OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., O'HARA, R. B., SIMPSON, G. L., SÓLYMOS, P., STEVENS, M. H. H., and WAGNER, H. 2010. vegan: Community ecology package. R package version 1.17-4. http://CRAN.R-project.org/package=vegan.

PAULY, G. and VON RUDOLFF, E. 1971. Chemosystematic studies in the genus *Pinus*: the leaf oil of *Pinus contorta* var. *latifolia*. Can. J. Botany 49:1201-1210.

PITMAN, G. B., VITÉ, J. P., KINZER, G. W., and FENTIMAN JR., A. F. 1968. Bark beetle attractants: *trans*-Verbenol isolated from *Dendroctonus*. Nature 218:168-169.

PURESWARAN, D. S., GRIES, R., BORDEN, J. H., and PIERCE JR., H. D. 2000. Dynamics of pheromone production and communication in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). Chemoecology 10:153-168.

PURESWARAN, D. S., GRIES, R., and BORDEN, J. H. 2004a. Quantitative variation in monoterpenes in four species of conifers. Biochem. Syst. Ecol. 32: 1109-1136.

PURESWARAN, D. S., GRIES, R., and BORDEN, J. H. 2004b. Antennal responses of four species of tree-killing bark beetles (Coleoptera: Scolitidae) to volatiles collected from beetles, and their host and nonhost conifers. Chemoecology 14:59-66.

R Development Core Team. 2010. R: a language and environment for statistical computing (version 2.12.0). R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

RAFFA, K. F. and BERRYMAN, A. A. 1982. Gustatory cues in the orientation of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) to host trees. Can Entomol. 114:97-104.

RAFFA, K. F. and SMALLEY, E. B. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes. Oecologia 102:285-295.

RAFFA, K. F., AUKEMA, B. H., ERBILGIN, N., KLEPZIG, K. D., and WALLIN, K. F. 2005. Interactions among conifer terpenoids and bark beetles across multiple levels of scale: An attempt to understand links between population patterns and physiological processes. Recent Adv. Phytochem. 39:79-118.

REID, R. W., WHITNEY, H. S., and WATSON, J.A. 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Botany 45:1115-1126.

RICE, A. V., THORMANN, M. N., and LANGOR, D. W. 2007. Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole x jack pine hybrids in Alberta. Can. J. Botany 85:307-315.

ROBINSON, D. A., JONES, S. B., WRAITH, J. M., OR, D., and FRIEDMAN, S. P. 2003. A review of advances in dielectric and electrical conductivity measurement in soils using time domain reflectometry. Vadose Zone J. 2:444-475.

RUDINSKY, J. A., MORGAN, M. E., LIBBEY, L. M., and PUTNAM, T. B. 1974. Antiaggregative-rivalry pheromone of the mountain pine beetle, and a new arrestant of the southern pine beetle. Environ. Entomol. 3(1):90-98.

RYKER, L. C. and LIBBEY, L. M. 1982. Frontalin in the male mountain pine beetle. J. Chem. Ecol. 8(11):1399-1409.

SAFRANYIK, L., CARROLL, A. L., RÉGNIÈRE, J., LANGOR, D. W., RIEL, W. G., SHORE, T. L., PETER, B., COOKE, B. J., NEALIS, V. G., and TAYLOR, S.W. 2010. Potential for range

714 expansion of mountain pine beetle into the boreal forest of North America. Can. Entomol.
715 142:415-442.
716
717 SEMIZ, G., HEIJARI, J., ISIK, K., and HOLOPAINEN, J. K.. 2007. Variation in needle
718 terpenoids among *Pinus sylvestris* L. (Pinaceae) provenances from Turkey. Biochem. Syst. Ecol.
719 35: 652-661.
720
721 SEYBOLD, S. J., HUBER, D. P. W., LEE, J. C., GRAVES, A. D., and BOHLMANN, J. 2006.
722 Pine monoterpenes and pine bark beetle: a marriage of convenience for defense and chemical
723 communication. Phytochem. Rev. 5: 143-178.
724
725 SHARKEY T. D. 1991. Stomatal control of trace gas emissions, pp. 335-339, in: T. D. Sharkey,
726 E. A. Holland, and H. A. Mooney (eds.). Trace gas emissions by plants. Academic Press, San
727 Diego, California.
728
729 SHRIMPTON, D. M. and WATSON, J. A. 1971. Response of lodgepole pine seedlings to
730 inoculation with *Europhium clavigerum*, a blue stain fungus. Can. J. Botany 49: 373-375.
731
732 SOLHEIM, H. and KROKENE, P. 1998. Growth and virulence of mountain pine beetle
733 associated blue-stain fungi, *Ophiostoma clavigerum* and *Ophiostoma montium*. Can. J. Botany
734 76:561-566.
735
736 STURGEON, K. B. 1979. Monoterpene variation in ponderosa pine xylem resin related to
737 western pine beetle predation. Evolution 33(3):803-814.
738
739 TARDIEU, F. and SIMONNEAU, T. 1998. Variability among species of stomatal control under
740 fluctuating soil water status and evaporative demand: modeling isohydric and anisohydric
741 behaviours. J. Exp. Bot. 49:419-432.
742
743 TKACZ, B. M. and SCHMITZ, R. F. 1986. Association of an endemic mountain pine beetle
744 population with lodgepole pine infected by armillaria root disease in Utah. USDA For. Serv. Res.

Note INT-353.

TURTOLA, S., MANNINEN, A.-M., RIKALA, R., and KAINULAINEN, P. 2003. Drought stress alters the concentration of wood terpenoids in scots pine and norway spruce seedlings. J. Chem. Ecol. 29(9):1981-1995.

VIDACOVIĆ, M. 1991. Conifers: Morphology and variation. Graficki Zavod Hrvatske, Zagreb, Croatia.

WALLIN, K. F. and RAFFA, K. F. 1999. Altered constitutive and inducible phloem monoterpenes following natural defoliation of jack pine: Implications to host mediated interguild interactions and plant defense theories. J Chem. Ecol. 25(4):861-880.

WILLIAMS, D. W. and LIEBHOLD, A. M. 2002. Climate change and the outbreak ranges of two North American bark beetles. Agr. For. Entomol. 4(2):87-99.

[www.for.gov.bc.ca] British Columbia. Ministry of Forests, Lands and Natural Resource Operations. http://www.for.gov.bc.ca/hfp/mountain_pine_beetle/Updated-Beetle-Facts_Mar2010.pdf

YAMAOKA, Y., SWANSON, R. H., and HIRATSUKA, Y. 1990. Inoculation of lodgepole pine with four blue-stain fungi associated with mountain pine beetle, monitored by a heat pulse velocity (HPV) instrument. Can. J. Forest Res. 20:31-36.

ZAVARIN, E., CRITCHFIELD, W. B., and SNAJBERK, K. 1969. Turpentine composition of *Pinus contorta* × *Pinus banksiana* hybrids and hybrid derivatives. Can. J. Botany 47:1443-1453.

Figure 1: Methodology scheme showing the two different environmental treatments: water deficit and well watered, the three biological treatments: control, mechanical wounding and wounding plus inoculation with *G. clavigera*, and collection of VOCs.

Figure 2: Soil water content over time measured for pots containing lodgepole and jack pine seedlings. For each time point, data for the two species were combined. Error bars represent the 95% confidence interval. Bars with non overlapping error bars are significantly different from each other.

Figure 3: Changes in gas exchange parameters over time shown for both environmental treatments in lodgepole and jack pine seedlings **a**) rate of photosynthesis **b**) stomatal conductance. Error bars represent the 95% confidence interval. Bars with non overlapping error bars are significantly different from each other.

Figure 4: Chemical profiles presented as percent of volatile organic compounds emitted from lodgepole pine and jack pine seedlings based on peak area in GC analysis. Error bars indicate standard errors.

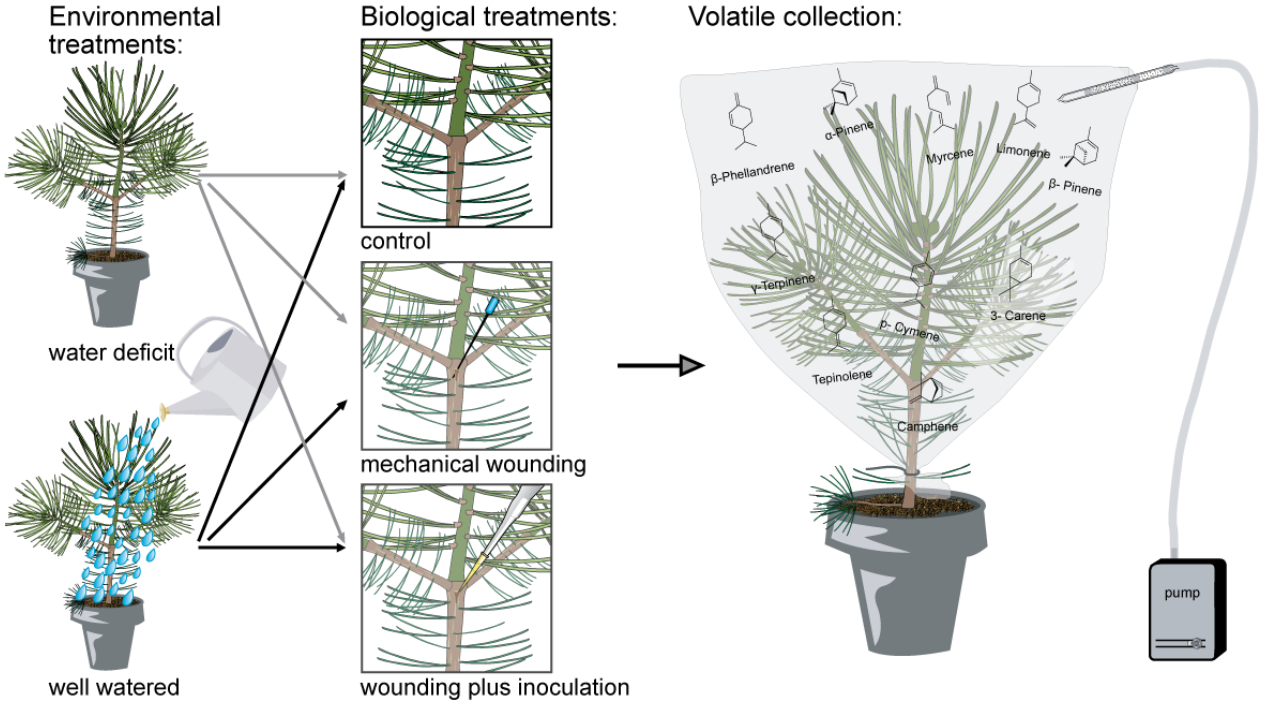
Figure 5: Canonical redundancy analysis (RDA) triplots (scaling 2) illustrating the effect of environmental and biological treatments as well as seedling physiology on volatile emission of individual and total monoterpenes in lodgepole and jack pine.

Figure 6: Effect of environmental and biological treatment on total monoterpene emission by lodgepole and jack pine seedlings over time. Error bars indicate standard errors. The asterisk indicates the significant difference of total monoterpene emission in fungal inoculated water deficit lodgepole pines compared to the control treatment (p=0.029).

Figure 7: Total monoterpene content in the three different tissues of lodgepole and jack pine seedlings. Error bars indicate standard error. The asterisk indicates the significant difference in monoterpene content between lodgepole and jack pine needles.

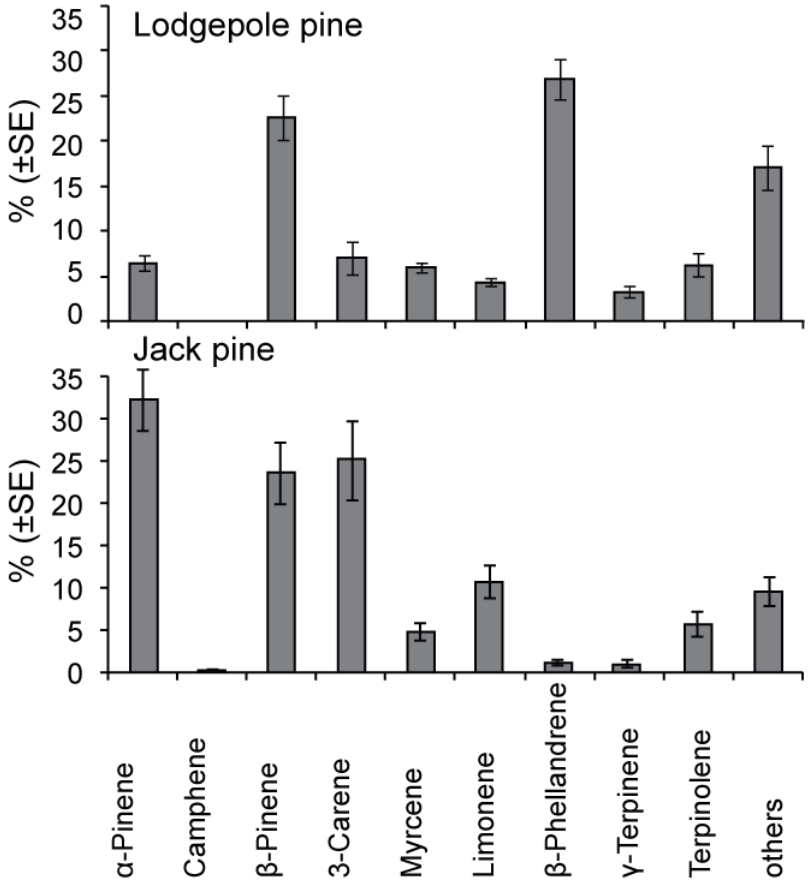
Figure 8: Different bornyl acetate concentrations in previous year's bark of jack pine caused by the biological treatments. Kruskal-Wallis test was used followed up by Mann-Whitney U tests. A Bonferroni correction was applied and so all effects are reported at a 0.025 level of significance. Different lowercase letters indicate a statistically significant difference.

Figure 9: Monoterpene increase over time in all tissues of lodgepole and jack pine seedlings across all treatments. Error bars represent the 95% confidence interval. Bars with non overlapping error bars are significantly different from each other.



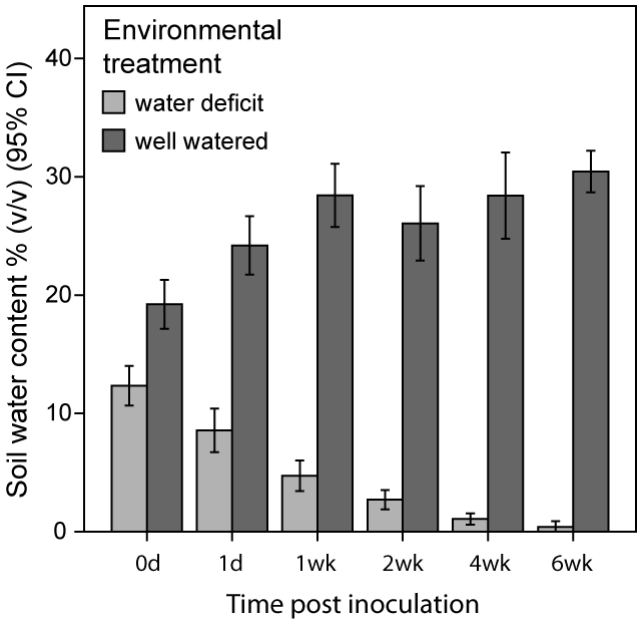
814

815 Figure 1



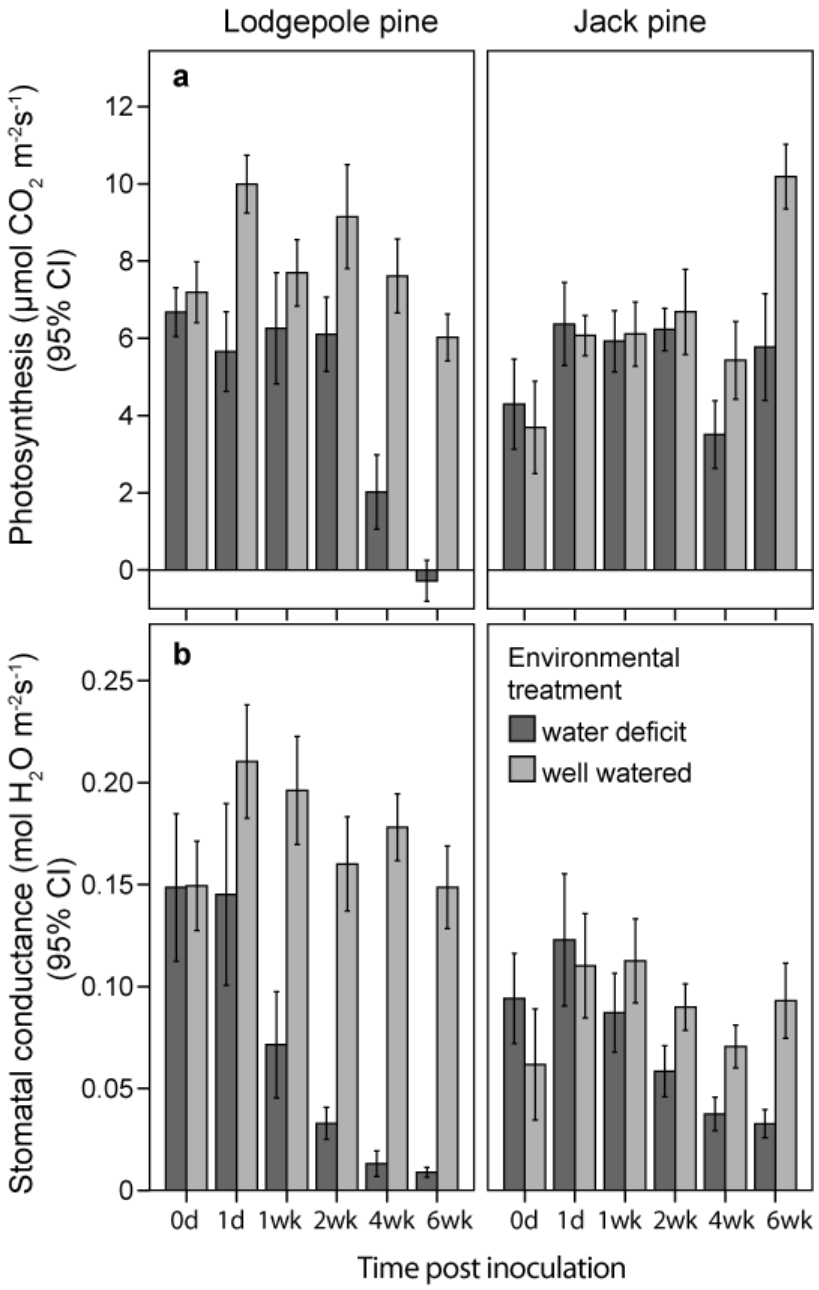
816

817 Figure 2:



818

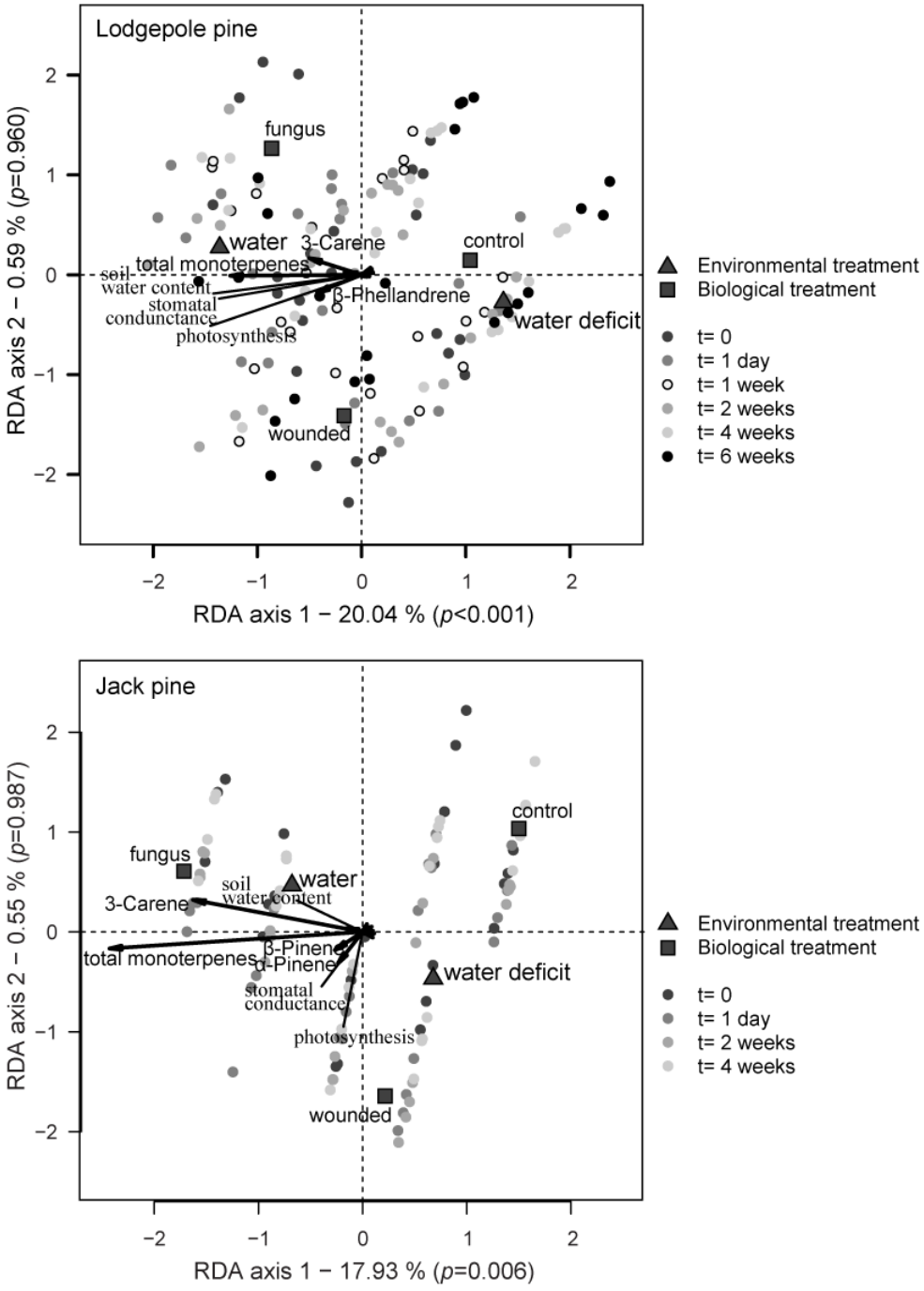
819 Figure 3



820

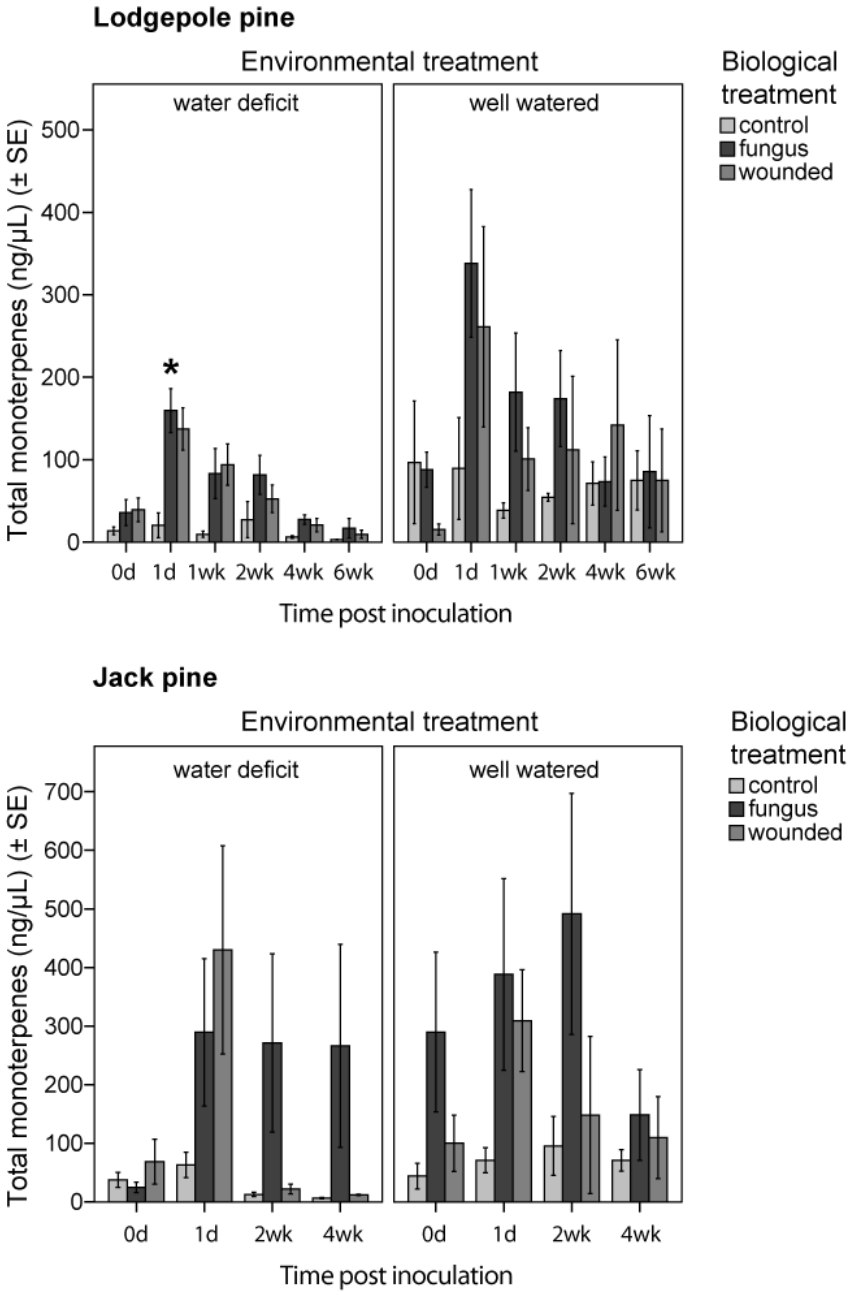
821

822 Figure 4



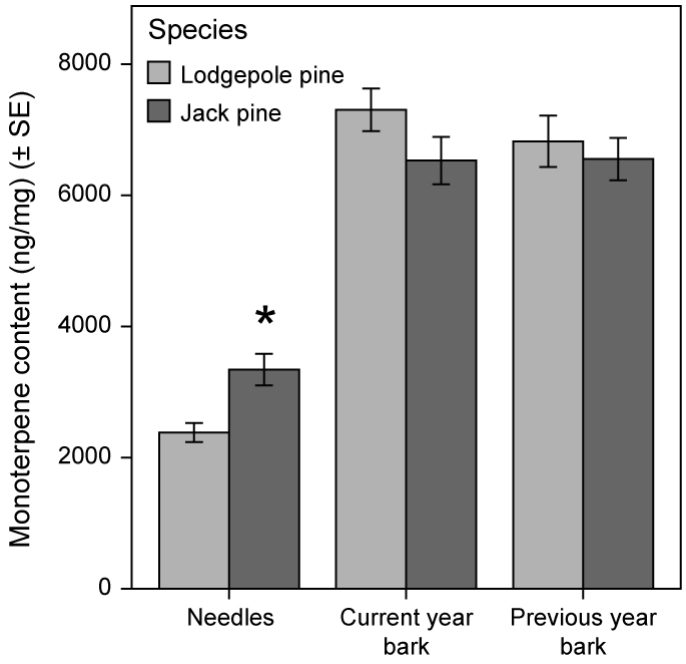
823

824 Figure 5:



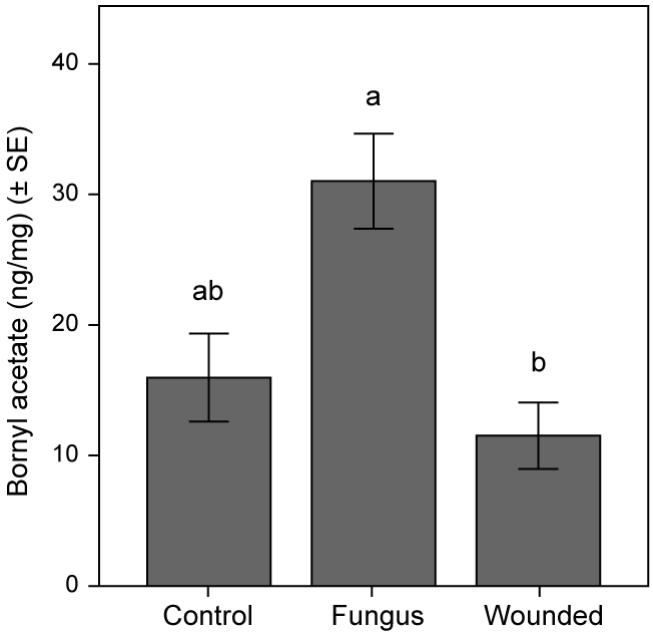
825

826 Figure 6:



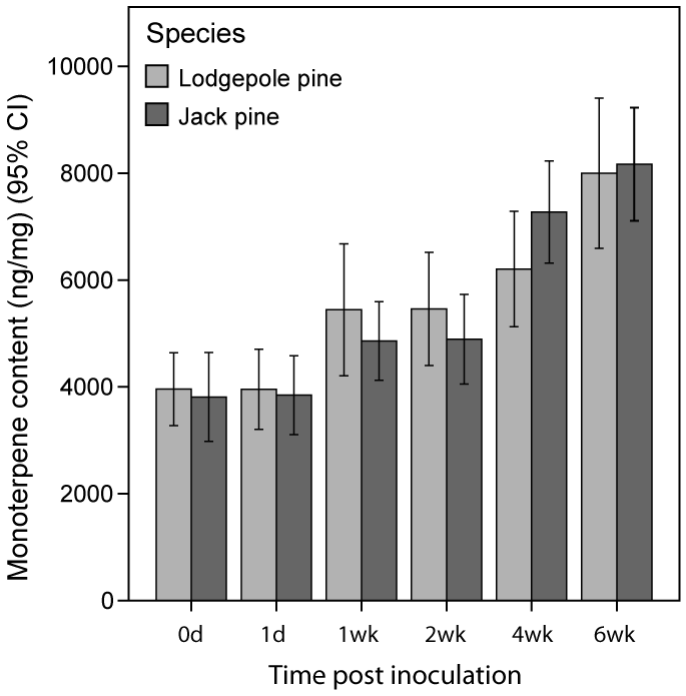
827

828 Figure 7



829

830 Figure 8



831

832 Figure 9