Reaching new heights: Chemical signatures of lodgepole pine trees change with elevation, but not with latitude

by
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ABSTRACT

The lodgepole pine (*Pinus contorta var. latifolia*) is Alberta's provincial tree and critical to the forest industry. This pine species is the historical host for mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae). In western Canada, the mountain pine beetle is expanding its range facilitated, in part, by climate change, and has invaded areas that were historically climatically unsuitable for its survival. As a result of this range expansion, novel lodgepole pine stands in Alberta are being invaded. Thus, it is timely to predict if the vulnerability of lodgepole pine trees varies across the province's elevational and latitudinal gradients. Elevational and latitudinal gradients can be used as space-for-time gradients in climate change studies. Host susceptibility to bark beetles is usually assessed via tree defenses. The primary defenses of lodgepole pine against bark beetles are the constitutive concentration of oleoresin terpenoids. Production of these defense chemicals relies in part on tree reserves, and non-structural carbohydrates (sum of total sugars and starch). I investigated whether the concentration of monoterpenes, diterpene resin acids, and non-structural carbohydrates of lodgepole pine trees change as a function of elevation or latitude. I characterized the chemical profile of trees along an elevational gradient of 1,251 m, and a latitudinal gradient of 736 km. I also determined age, growth rates, basal area index values, stand density, and basal area. I found that concentrations of terpenes increased with elevation while soluble sugars decreased. Latitude had no effect on the chemical signatures of trees. Overall, this project shows that pine trees occurring at higher elevations have a greater concentration of constitutive defense compounds, and a lower concentration of glucose and sucrose. These findings call for future research to determine inducibility of the same defense compounds at high elevations, and stress the importance of considering plant defenses against range expanding insect herbivores in forest pest management.

PREFACE

This thesis includes two studies (“elevation” and “latitude”), both of which are presented in Chapter 1. This chapter represents collaborative work with Dr. Nadir Erbilgin at the University of Alberta. For all studies, I designed the experiments, processed samples, collected and analyzed data, and wrote the thesis manuscript. Dr. Erbilgin contributed to the experimental design and revised the thesis in both content and composition. During my studies, I acquired all necessary permits based on the provincial and federal regulations.

All research conducted for this thesis is an original work, and none of it had been published yet. Chapter 1 is being prepared as journal article as: Mullin, M., Cale, J., Klutsch, J., Zhao, S., Whitehouse, C. and Erbilgin, N. “Reaching new heights: Chemical signatures of lodgepole pine trees change with elevation, but not with latitude”. I designed the experiments, collected data, processed samples, analyzed data, wrote the thesis, and am currently writing the manuscript. Dr Cale contributed to design of lab work associated to diterpene resin acids, soluble sugars, and starches, and revised the associated portions of the manuscript. Dr Klutsch contributed to design of lab work associated to monoterpenes. Drs. Cale, Klutsch, and Zhao provided help on data analysis. Caroline Whitehouse provided guidance in the ecological analysis. Dr. Erbilgin contributed to the experimental design and revised the manuscript in both content and composition.

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CHAPTER 1: Reaching new heights: Chemical signatures of lodgepole pine trees change with elevation, but not with latitude

1 INTRODUCTION

1.1 Evaluating impacts of climate change on insect outbreaks

While natural disturbance provides a variety of ecological benefits to forests, the invasion of novel forest habitats by bark beetles can also have negative ecological and economic consequences. Ecological consequences include, for instance, host plant mortality and associated changes to local biodiversity, food web structure, and carbon dynamics (Kurz et al. 2008, Hobbs et al. 2009, Bentz et al. 2010, Bartley et al. 2019). Such changes have the potential to affect watershed hydrology, which may lead to loss of fish and wildlife habitat (Safranyik et al. 2006, Dhar et al. 2018). Economic consequences include, for instance, loss of commercial revenue from decreased merchantable wood products, and decreased tourism and recreation due to the impacted area's reduced aesthetic appeal (Cole and Amman 1980, Safranyik et al. 2006, Dhar et al. 2018).

With warming climate, the geographical range of mountain pine beetle has shifted northward and to higher elevations, particularly in Alberta (e.g. Carroll et al. 2004, Raffa et al. 2013, Cudmore et al. 2010). This range expansion, combined with a large area of contiguous suitable host species (susceptible age and density), contribute to ideal conditions for insect outbreaks including mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae) (Bentz et al. 2010, Cudmore et al. 2010, Raffa et al. 2013, 2017, Erbilgin 2019). Thus, accurate predictions of climate change impacts on insect range expansion and associated host susceptibility are critical to guide research directions and inform effective forecast management actions.

In order to evaluate the impacts of climate change, two broad strategies are commonly employed; predicting and testing. In order to predict the impacts of climate change on insect outbreaks, researchers can make use of tools such as insect outbreak forecasting software, historical databases, and models (e.g., Régnière et al. 1995, Logan et al. 2003, Elmendorf et al. 2015). To test the impacts of climate change, researchers commonly employ tactics such as manipulative experiments or space-for-time sampling (Elmendorf et al. 2015). Space-for-time

sampling "encompasses analyses in which contemporary spatial phenomena are used to understand and model temporal processes that are otherwise unobservable, most notably past and future events" (Blois et al. 2013). This sampling method can be used for example to predict and detect climate-driven changes in species distribution, richness, and biodiversity (Blois et al. 2013, Elmendorf et al. 2015, Bartley et al. 2019). Such changes in high elevation areas are considered "sensitive indicator[s]" of ecological responses to global climate change (Roots 1989, Pauli 2016). Thus, in the current study, space-for-time sampling along elevational and latitudinal gradients will be used as a proxy for climate change.

Even with predictive tools and experimental methods, contradictory hypotheses exist to predict how host plants (of insect herbivores) respond to climate change. Some hypotheses predict that species (or conspecifics) inhabiting lower elevation and latitude ecosystems will fare better in response to climate change (e.g., Logan and Powell 2001, Logan et al. 2010, Raffa et al. 2013, 2017). For instance, the "elevational gradient for plant defense" hypothesis and the "latitude gradient for plant defense" hypothesis would predict that plants occurring in lower ecosystems have enhanced defenses due to increased rates of herbivory (Moles el al. 2011, Mitton and Ferrenberg 2012, Ferrenberg et al. 2017, Hahn et al. 2019). In contrast, other hypotheses predict the opposite. For instance, the "resource availability hypothesis" would predict that species occurring in higher ecosystems have greater constitutive defenses, due to the high cost of tissue replacement following herbivore attack (Coley et al. 1985). Further complicating the ability to predict how plants respond to the changing climate, is the uncertainty in how trees living on high elevation or latitude gradients prioritize their resources for growth vs. defense (Stamp 2003, Moreira et al. 2014, Hahn et al. 2019). There is also uncertainty as to whether insect herbivory is truly greater in lower ecosystems (Moles et al. 2011).

1.3 The lodgepole pine (*Pinus contorta var. latifolia*)

1.3.1 Biology and importance

The lodgepole pine is Alberta's provincial tree and has both economical and ecological importance. Economically speaking, lodgepole pines' physical, visual and working properties make it a key tree species in the western Canadian forest industry (Forintek Canada 2006). Specifically, these trees have a tall, slender, and straight silhouettes (Government of British

Columbia 2017). They reproduce quickly in exposed mineral soil after a fire, due to their serotinous cones (Rowe 1972).

Ecologically speaking, lodgepole pines are common in western Canadian forests, as they are a colonizing, early reproducing species which can occur in a variety of habitats (Rowe 1972, Cole and Amman 1980, Archibald et al. 1996, Hansen et al. 2016). Their presence represents an important part of the successional pathway of the boreal forests in western Canada (Rowe 1972). Within Alberta, lodgepole pine trees can occur from 550 m to 2,100 m elevation, and from 49ºN to 60ºN (Figure S.1) (Little 1971, Archibald et al. 1996). Research has been conducted regarding the ecology of lodgepole pine trees, providing valuable information to the scientific community and resource managers alike. However, there is still much work to be done, especially where possible vulnerability in high elevation and latitude systems are concerned.

At high elevations, lodgepole pine trees can occur in mixed stands with the endangered whitebark pine (*Pinus albicaulis*) species (COSEWIC 2010). Of special concern in this zone of distributional overlap is the potential for increased transfer of mountain pine beetle from a historical host to a novel host species (Raffa et al. 2013, 2017). Lodgepole pine trees having co-evolved with mountain pine beetles, and thus have more thoroughly integrated defense compounds than whitebark pine trees. In other words, whitebark pine are expected to be comparatively less capable of defending themselves when under attack (Raffa et al. 2017).

1.3.2 Defense strategies against bark beetles

Plant defenses are commonly studied in the context of host susceptibility to invading pests or pathogens. Pine trees are defended from mountain pine beetle attack by tough outer bark, a network of resin ducts, and both constitutive and induced chemical defenses (Klepzig et al. 1996, Raffa et al. 2005, 2017, Keeling and Bohlmann 2006, Erbilgin 2019). Constitutive defense compounds are those which are present at the time of insect attack, whereas induced defense compounds are produced in response to insect colonization attempts (Karban and Myers 1989). In other words, the concentration of constitutive defense compounds represents the first line of defense against attacks by any species of insect herbivores including bark beetles.

Previous studies have analyzed the chemical defense compounds of pine trees using phloem, as this is the tissue that bark beetles feed on (e.g., Erbilgin and Raffa 2001, and Boone et al. 2011, Erbilgin et al. 2017a). Within the phloem tissues, the constitutive oleoresin

phytochemicals (defense compounds) are composed of mainly monoterpenes, sesquiterpenes, and diterpenes acids (Moreira et al. 2014). Monoterpenes appear to be the most toxic to the beetles, bearing the strongest insecticidal properties and moderate antifungal properties (Keeling and Bohlmann 2006, Chiu et al. 2017, Raffa et al. 2017, Erbilgin 2019). The role of sesquiterpenes is unclear in the beetle-fungal complex (Raffa et al. 2017). Diterpene resin acids have the strongest antifungal properties, limiting both fungal germination, and linear growth of mycelium (e.g., Klepzig et al. 1996, Kooper et al. 2005, Boone et al. 2013, Raffa et al. 2017). In addition to reducing fungal germination and growth, diterpene resin acids can also polymerize to seal bark beetle attack site wounds (Keeling and Bohlmann 2006).

Previous studies have compared the chemical signatures of lodgepole pine against those of other pine species (e.g., Raffa et al. 2013, 2017, Ishangulyyeva et al. 2016, Erbilgin 2019). However, very few studies have compared the chemical signatures of lodgepole pine *conspecifics*, along the elevational or latitudinal gradients occupied by the species. It is worth acknowledging that a recent study compared the chemical signatures of high- versus low-elevation lodgepole pines, finding that individuals at higher elevation had lower monoterpene diversity than conspecifics at lower elevation (Ferrenberg et al. 2017). This study, however, was based on resins collected from stems, not phloem tissues. Thus, in the current study, I will sample phloem tissues to analyze the constitutive concentration of monoterpenes and diterpene resin acids of lodgepole pine.

The lodgepole pine's internal reserves also impact success in defense against bark beetle attack. It was shown that the amount of "photosynthate available for defense", will impact the tree's resistance to herbivory (Millar and Berryman 1986, Christiansen et al. 1987, Roth et al. 2017). An individual tree's reserves are comprised of non-structural carbohydrates, which are constituted by the soluble sugars and starches arising from photosynthesis (Pallardy 2008). These non-structural carbohydrates can be mobilized and transformed into either other primary metabolites, or defense chemicals. Transformations of non-structural carbohydrates to defense compounds are energetically costly (Moreira et al. 2014). In theory, the plant converting its reserves should do so in a manner which maximizes allocation of photosynthates to respiration, structural growth, reproduction, storage, and defense.

Studies looking to establish relationships between concentration of defense chemicals and non-structural carbohydrates of pine trees, are in their infancy (e.g., Roth et al. 2017). In

addition, studies investigating non-structural carbohydrates have yet to distinguish among the compounds which make up these reserves: starch, glucose, fructose, and sucrose. Thus, in the current study, I will sample phloem tissues to analyze the concentration of individual non-structural carbohydrates (starch, glucose, fructose, and sucrose) of lodgepole pine.

1.3 The mountain pine beetle

1.3.1 Population phases and range expansion

Mountain pine beetles are a tree-killing beetle species, whose historical host includes the lodgepole pine (*Pinus contorta var. latifolia)*. Mountain pine beetles are native to conifer forests of western North America and their endemic range extends from northern Mexico (latitude 31ºN) to central British Columbia in Canada (latitude 56ºN) (Safranyik et al. 2010). Within Canada, the endemic range includes a small portion of lodgepole pine stands in southern Alberta (Safranyik and Carroll 2006, Hansen et al. 2016). Historically, high elevation and latitude ecosystems were unsuitable to the survivability of the bark beetles, due to temperature extremes which killed larvae and halted any range expansion (Amman 1972, Macfarlane et al. 2013). In these higher ecosystems, the previous outbreaks which did occur were infrequent and short-lived (Logan et al. 2010, Hansen et al. 2016, Raffa et al. 2017).

Currently, mountain pine beetles are invading areas which were historically climatically unsuitable to their survival, including higher elevations and more northern latitudes. This most recent range expansion of mountain pine beetles has been attributed to shortened generation times linked to above-average temperatures, as well as favorable forest conditions (Logan and Powell 2001, Carroll et al. 2004, Hansen et al. 2016). This range expansion allows for increased bark beetle population growth, which in turn, allows for increased efficiency in host colonization, further perpetuating the outbreak conditions (Hicke and Logan 2009, Cudmore et al. 2010, Raffa et al. 2017). The current epidemic range of the mountain pine beetle extends into novel lodgepole pine stands. Increased pine mortality is likely to occur if these novel stands are unable to deter beetle colonization (Clark et al. 2010, Cudmore et al. 2010, Raffa et al. 2013, Rosenberger et al. 2017).

1.3.2 Beetle-host tree interactions

When selecting a host tree to colonize, a female mountain pine beetle initiates the attack and thus is considered a "pioneering beetle". Once a potential host tree has been identified, the pioneering female will oxidize the host monoterpene α-pinene, to produce the aggregation pheromone *trans*-verbenol (Wood 1982). This aggregation pheromone is then synergized by host monoterpenes such as myrcene, 3-carene, or terpinolene (Borden et al. 1983, Jost et al. 2008, Erbilgin et al. 2014). The combination of aggregation pheromones and pheromone synergists attracts both sexes of beetles to the host tree, thereby initiating mass-attack on host trees.

The strategy of mass-attack is used to overwhelm pine defenses, which facilitates successful host colonization and reproduction (Safranyik et al. 2010, Boone et al. 2011). During the beginning of the of mass-attack, arriving male beetles produce their own aggregation pheromone, *exo*-brevicomin (Cole and Amman 1980). Both *exo*-brevicomin and *trans*-verbenol will continue to be emitted until beetle density is high enough to overcome tree defenses (Borden et al. 1987, Pureswaran et al. 2000, Raffa et al. 2005, Bentz et al. 2010). Once the optimal attack threshold is reached, anti-aggregation pheromones are emitted by the beetles. Specifically, males emit *frontalin*, and both sexes emit *verbenone* during the "host switching" phase (Borden et al. 1987, Pureswaran et al. 2000).

In addition to the mass attack strategy, mountain pine beetle also vectors bluestain fungi in the sapwood of the tree to overcome tree defenses (Safranyik and Carroll 2006, Safranyik et al. 2010). These fungi are mutualistic to the beetle and pathogenic to the host tree (Safranyik and Carroll 2006). Several species may be vectored: *Ophiostoma montium*, (Rumbold von Arx), or *Grosmannia clavigera* (Robinson-Jeffrey and Davidson Zipfel, de Beer and Wingfield), or *Leptographium longiclavatum* (Lee, Kim, and Breuil). Regardless of the species vectored, fungal spores germinate on phloem and xylem sapwood (Klepzig et al. 1996, Safranyik et al. 2010). The fungal growth contributes to host mortality by girdling the sapwood (stopping the transport of water), terminating resin production (stopping the flow of induced defense compounds), and providing increased nutrients (nitrogen and ergosterol) to developing larvae and teneral adults (Raffa et al. 2005, Safranyik and Carroll 2006, Jost et al. 2008, Erbilgin et al. 2017a).

If host tree defenses are overcome and colonization is successful, mating occurs. Following mating, eggs are laid in a maternal gallery excavated by both sexes. These eggs develop into larvae which overwinter in the phloem of the host tree. As the larvae overwinter,

they will feed on the phloem tissues, gain cold tolerance as the winter progresses, and emerge as beetles in the subsequent summer (Raffa et al. 2005, Régnière and Bentz 2007). Phloem thickness is important in "determining brood production" (Amman 1972, Cole and Amman 1980, Safranyik and Carroll 2006). If the tree is stressed, the phloem production will be very low, resulting in a thinner tissue and smaller beetle population (Bentz et al. 2010, Boone et al. 2011).

1.4 The knowledge gap and research question

Few studies have reported how host plant susceptibility to insect herbivores changes as a function of climate change. More specifically, there is conflicting information in the scientific literature regarding the impacts of elevation and latitude on changes in concentration of defense compounds or plant reserves of lodgepole pine trees.

The research question for the current study is: *are the chemical signatures of lodgepole pine trees occurring in higher elevation and latitudes different from those of conspecifics in lower systems?* In the current study, I explicitly consider how two space-for-time climate change gradients influence the defensive chemistry and internal reserves of a commercially and ecologically valuable tree species.

1.6 Project objectives and hypothesis

The current study is among the first to investigate the relationship between lodgepole pine defense compounds and plant reserves along two space-for-time climate change gradients: elevation and latitude. The overarching project goal is to contribute to research which would lead to the identification of geographic areas in Alberta where lodgepole pine trees may be more vulnerable to attack by range-expanding mountain pine beetle.

The project objectives are to determine if concentrations of constitutive monoterpenes, diterpenes, soluble sugars, and starches, of lodgepole pine phloem tissues vary along elevational and latitudinal gradients.

In relation to the project objectives, the hypotheses are as follows: lodgepole pine trees occurring in higher elevations or latitudes will have (1) a lower concentration of constitutive monoterpenes and diterpene resin acids, and (2) identical concentration of starch and soluble sugars as compared to conspecifics in lower ecosystems

2. MATERIALS AND METHODS

2.1 Overview

I sampled phloem tissues from lodgepole pine trees along two gradients: an elevational gradient of 1,251 m, as well as a latitudinal gradient of seven degrees. I selected healthy, dominant or co-dominant lodgepole pine trees, with a mean diameter at breast height (DBH) of 20.0 cm (± 0.211 SE). I ensured consistency across sites by sampling phloem from all four cardinal directions on the bole of each tree. I quantified phloem tissue concentration of defense compounds (monoterpenes and diterpene resin acids) as well as non-structural carbohydrates (soluble sugars, and starches). Finally, I analyzed the statistical differences between compounds along the elevation and latitudinal gradients sampled. This was done using both multivariate and univariate techniques.

2.2 Sampling design

Field sampling and measurements were carried out in the summer of 2018. Field activities took place when mountain pine beetles are active in Alberta: between June and July (Klutsch et al. 2017). By this time, new growth tissues would have already expanded and elongated (Hoch and Korner 2012). Tissue sampling during these months would reduce the chance of bias in the distribution of mobile carbon between trees.

I used ArcGIS software to determine coordinates of sites which should be suitable for sampling. I determined site selection criteria and used these criteria to define the potential sampling region. Selection criteria for both studies included: lodgepole pine stands, where percent cover pine >70%, and height of pines >10m, located in western Alberta, between 50 m and 500 m from a road. Additional constraints for the latitude study: not within the white zone (settled or agriculture portion), not within the lodgepole x jack pine hybrid zone (Cullingham et al. 2012), and elevation between 600 m – 1,050 m. Additional constraints for the elevation study: between south-facing and west-facing slope aspects, between 50 m and 500 m from a road or trail, and latitude between 51ºN and 52 ºN. Within this pre-selected area, ArcGIS randomly generated over 1,000 coordinates of potential sample sites per study. This was done to reduce sampling bias in the field. As each potential site was visited, notes were gathered on whether the site should be sampled or not. The final study sites are given in Figure 1.1.

For the sake of statistical power, the largest possible sample size was desired. The sample size was predicted to be constrained by post-field analyses. The goal was to collect samples from 105 trees in the latitude study, and 105 trees in the elevation study. One tree was sampled per site, in order to capture between-site variation more robustly. This plan was based largely on the methodology from earlier studies (Goodsman et al. 2013, Raffa et al. 2013, 2017, Ferrenberg et al. 2017, Roth et al. 2017).

In-field challenges restricted the target sample size to 61 sampled sites for the elevation study, and 69 sites for the latitude study. Of these, some trees served a "dual purpose", and were suitable to be used in statistical analyses for both studies. All told, the latitude study was comprised of 68 sites spanning seven degrees latitude, and the elevation study was comprised of 71 sites spanning 1,251 meters of elevational gain (Figure 1.1).

2.3 Fieldwork

I collected phloem samples from trees using a leather hollow punch (1.9 cm dia.). Specifically, samples were collected from all four cardinal directions on the stem of the tree and at a height of 1.3 m from the ground. These specifications helped to account for potential differences in solar radiation, as well as ensure consistency across trees sampled. Thickness of all four phloem samples was measured in field with digital calipers (to the nearest 0.1 mm), and the average thickness was recorded. Once the thickness was measured, these four phloem samples were immediately wrapped together in labelled aluminum foil and stored on dry ice. Once returned to the laboratory, these were stored at −40 ºC until chemical analysis.

I collected two increment cores from each tree, such that age may be measured directly, and growth rates for the last 10 years and basal area indices may be calculated. Specifically, I collected cores from both the north- and south-facing sides of the tree. Although DBH was held within a constant range in this study, it was expected that tree growth rates would decrease with increased elevation. Having accurate measures of age for all trees would allow for statistical control of a potential source variation. All tools (leather hollow punch, and increment core borer)

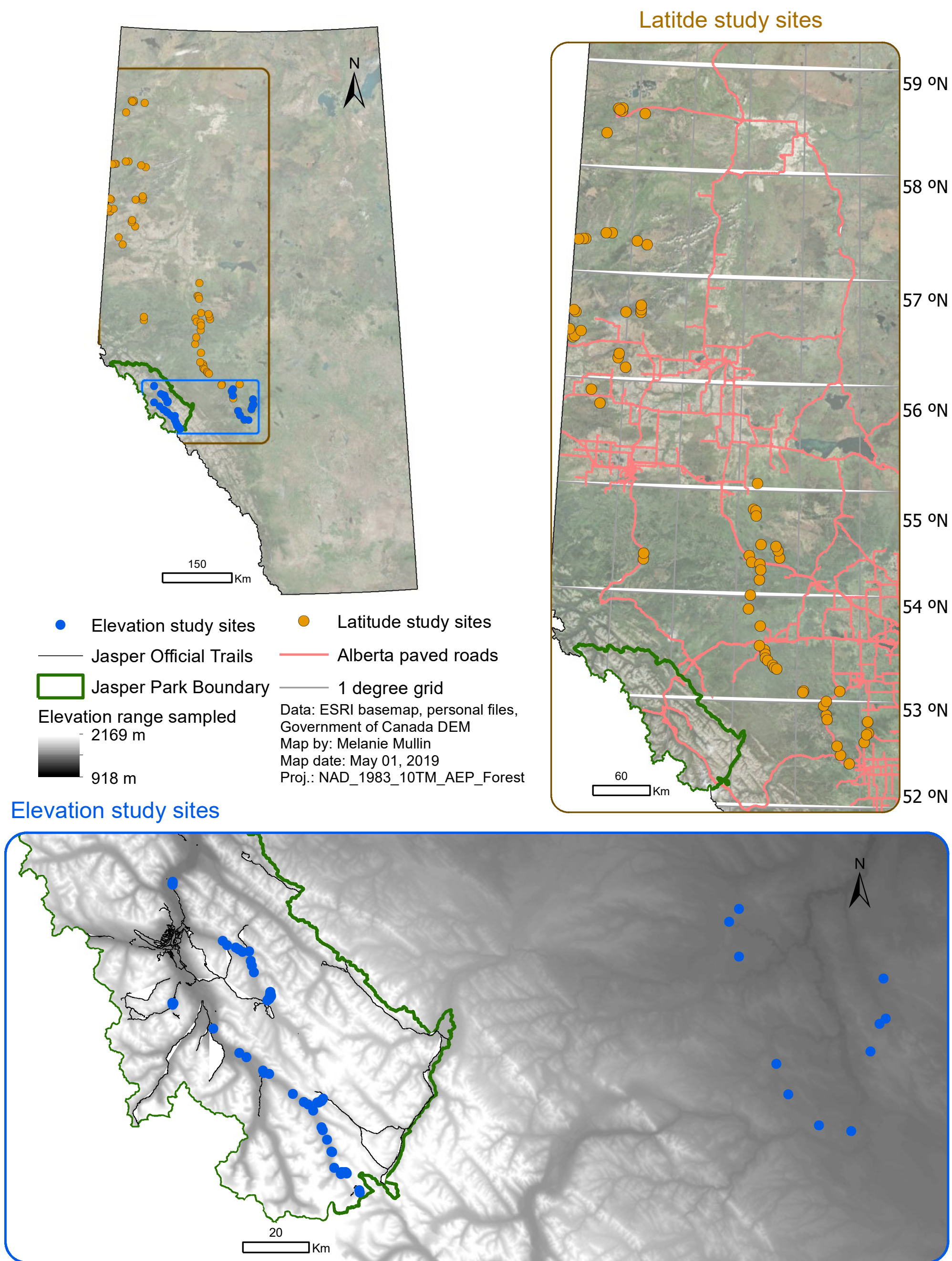

Figure 1.1 Map of sampled sites from summer 2018 fieldwork

Orange inset panel represents latitude study sites as orange circles, Alberta paved roads as coral lines, and world latitude and longitude grid in grey. Latitude range sampled: from 52ºN to 59ºN. Blue inset panel represents elevation study sites as blue circles, Jasper National Park boundary in green, Jasper National park trails in black. Underlain digital elevational model shows range sampled from grey to white: 918 m to 2,169 m asl.

were kept sterile by wiping surfaces with ethanol between trees.

At each site, the sampling date was noted. The coordinates of the site were both marked in a hand-held GPS unit, and recorded on the datasheet. The site aspect (in degrees north) was determined using a compass. The site slope degree, and the height of the tree were both determined using a clinometer and surveyor's tape.

Stand density was determined by a variable radius plot using an angle gauge with a basal area factor of 5. This basal area factor was selected on the first site, where 15 trees were counted as being "in". From this point on in both studies, the basal area factor 5 was used for consistency.

2.4 Laboratory work

For the purpose of clarity, laboratory work has been separated into two main categories: site considerations, and plant considerations. Plant considerations are further divided into defense chemicals and reserves, as per Figure 1.2.

2.4.1 Site Considerations

I glued increment cores into grooved boards and sanded these with a palm sander and 400-grit sandpaper. Next, these boards were cleaned of all sawdust using a paintbrush and scotch tape. This allowed the annual growth rings to be seen clearly. I determined tree age by counting annual growth rings of a trees' north and south cores under a dissecting microscope. Age of trees was corroborated using the computer software, WinDendro (Regent Instruments Inc., ON, CAN 2008). There was no master chronology available, given the range of the geographic area being sampled. As such, I performed visual cross-dating to assess for false and pinch rings. I determined growth rate by measured the ring widths for the most recent 10 years of growth (2008-2017) from the north-facing core of each tree. In addition, I calculated basal area index for each tree from the ten-year growth rate and DBH.

2.4.2 Plant considerations

Phloem samples were cleaned of residual bark, to prepare for extraction of monoterpenes, diterpene resin acids, soluble sugars, and starches. Phloem was ground to a fine powder using a cryo-grinder (BioSpec, Bartlesville, OK, USA) fitted with stainless steel grinding jars. Samples

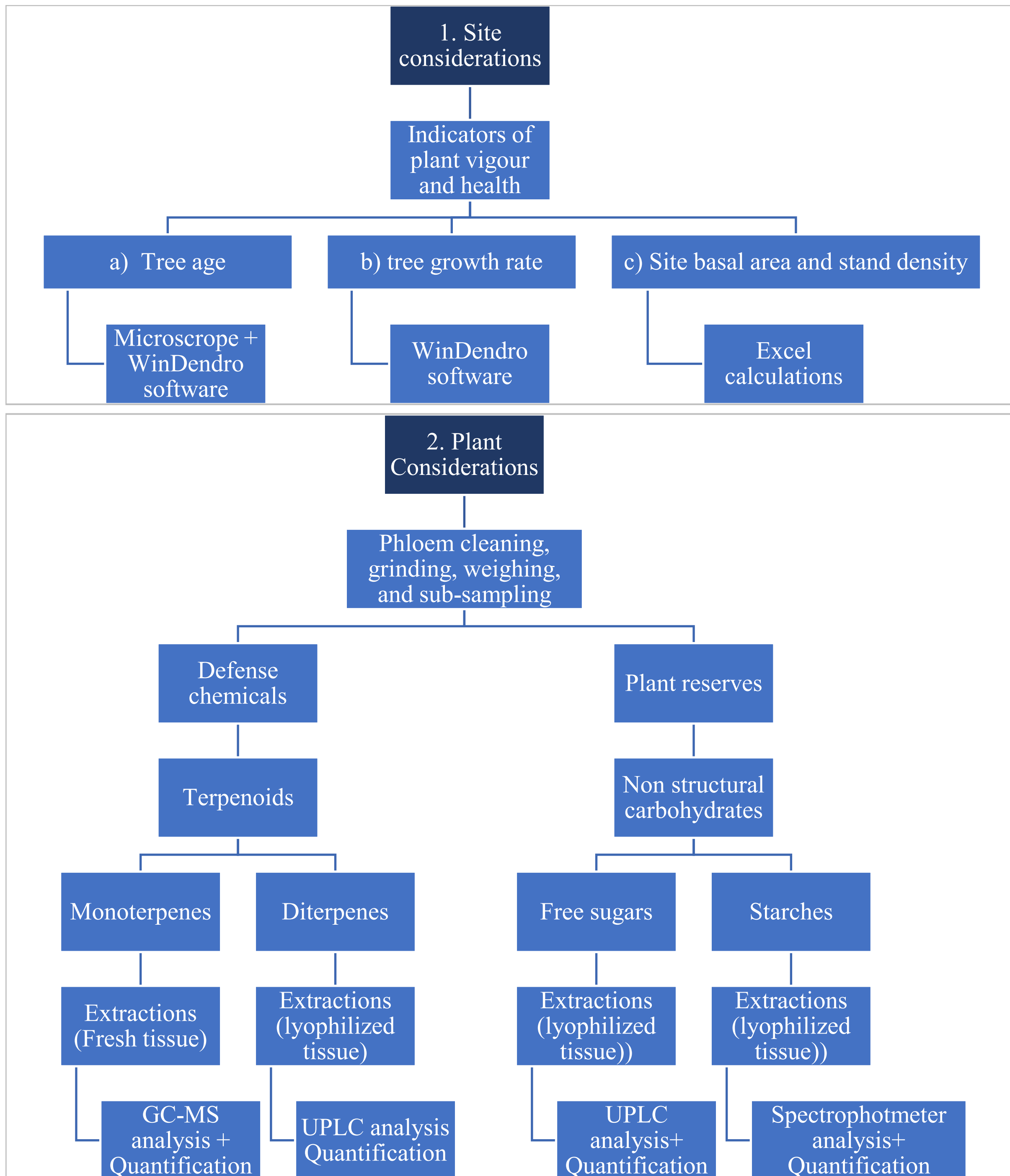

Figure 1.2 Schematic of 2018 – 2019 laboratory work

Schematic of laboratory workflow for processing of all samples collected in-field. Schematic highlights relationships between all measured variables, as well as the methods of analyses for each.

were ground at 3,764 rcf for 1 min. and dipped in liquid nitrogen. This process was repeated three times per sample. Steel grinding jars were cleaned with 70% methanol between each sample. From this ground tissue, 110 mg of fresh tissue was set aside for monoterpene extraction, 190 mg was set aside as a backup for monoterpene extraction, and the rest of the tissue was freeze-dried (6 d). The lyophilized tissue was used for the extraction of diterpene resin acids, soluble sugars, and starches.

2.4.2.1 Monoterpene analysis

Monoterpenes were extracted twice from 110 mg of ground fresh tissue in 1.0 mL of hexane with 0.004% v/v pentadecane as an internal standard at room temp as described in Cale et al. (2019). Samples were vortexed at 30 sec, sonicated for 10 min, then centrifuged for 15 min at 0°C at 18,213g. Extracts were transferred to 2 mL amber glass GC vials and stored at −40 °C until analysis.

The enantiomeric composition of hexane-extractable compounds, mainly monoterpenes, one phenylpropanoid (4-allylanisole), and one acetate ester of borneol (bornyl acetate) (hereafter "monoterpenes") was analyzed by injecting 1 µL of extracts with a split ratio of 25:1 into a Gas Chromatograph/Mass Spectrometry (GC-MS, GC 7890A, MS 5975C; Agilent Tech., Santa Clara, CA, USA) fitted with an Agilent HP-Chiral-20B column (0.25 mm id x 30.0m width 0.25µm film). Injector temperature was 250°C. Inlet temperature was 205°C. Flow rate was 1.1 mL He min^{-1}. Temperature program was: an initial temperature of 50°C, increased at a rate of 75°C/min up to 72°C (held for 0.5 min), then increased at a rate of 30°C min^{-1} up to 90°C (held for 2.0 min), then increased at a rate of 3°C min^{-1} up to 95°C (held for 1.0 min), then increased at a rate of 5°C min^{-1} up to 100°C (held for 0.5 min), then increased at a rate of 8°C min^{-1}up to 150°C, then increased at a rate of 15°C min^{-1} up to 170°C (held for 0.5 min), then increased at a rate of 20°C min^{-1} up to a final temperature of 250°C, at which it was held for 4 min. Total run time was 24 min.

Monoterpenes were quantified using standard curves from dilutions prepared from analytical standards of (+)-α-pinene (chemical purity 98.5%), (1S)-(–)-α-pinene (98%), β-myrcene (94%), (+)-camphene (90%), (+)-3-carene (98.5%), (–)-β-pinene (99%), α-terpinene (95%), ocimine (90%), p-cymene (99%), (–)-limonene (99%), (+)-limonene (99%), β-phellandrene (77%), γ-terpinene (97%), terpinolene (90%), thujone (99%), (±)-camphor (95%),

4-allylanisole (98.5%), (R)-(+)-pulegone (97%), bornyl acetate (97%), α-terpineol (90%), thujone (99%), and (–)-borneol (99%). Compounds were identified by comparing retention times and mass spectra with those of the standard chemicals. Concentrations were calculated as µg/mg of fresh tissue, and later converted to µg/mg of dry tissue. For conversion of monoterpenes concentrations in terms of dry weight, see Appendix 1.

2.4.2.2 Diterpene resin acid analysis

Diterpene resin acids were extracted from 50 mg of lyophilized ground tissue in 1 mL of methanol, as adapted from Cale et al. (2019). Samples were vortexed at 30 sec, then centrifuged for 10 min at 4°C at 18,213rcf. Extracts were transferred to 2 mL amber glass GC vials and stored at −40 °C until analysis.

Diterpene resin acids were analyzed using an ultra-high-performance liquid chromatograph (UHPLC, 1290 Infinity, Agilent Tech.), fitted with an InfinityLab Poroshell 120 EC-C18 column (2.1 x 150 mm 1.9µm; Agilent Tech.) and a diode array detector (UV/Vis, 1290 DAD, Agilent Tech.). A gradient analysis was performed with a binary solvent system; ultra-pure water with 1.7% v/v acetic acid (A) and 100% methanol (HPLC grade) (B) flowing at 0.3 mL/min. A 5 µL injection volume was used. The system began at 75% B for 1 min, then increasing to 85% B over 9 min, held for 2 min, then decreased to 75% B over 2 min, and held for 3 min. Total run time was 17 min.

Diterpene resin acids were quantified using analyte absorbance at wavelengths of 240, 268, and 282 nm, using methods adapted from Kersten et al. (2006). Standard curves used to quantify diterpene resin acids were calculated from dilutions prepared from analytical standards of pimaric acid (chemical purity 80%), abietic acid (95%), isopimaric acid (99%), neoabietic acid (99%), levopimaric acid (95%), sandarocopimaric acid (90%), dehydroabietic acid (99%), and palustric acid (92%) purchased from CanSynth (Toronto, ON, CAN). While lodgepole pine phloem can contain pimaric and isopimaric acids (Hall et al. 2013; Raffa et al. 2017), these compounds could not be quantified in our samples as, as indicated by analysis of analytical standards, they likely coeluted with abietic acid. Therefore, these three compounds were listed together.

2.4.2.3 Soluble sugar analysis

Soluble sugars (glucose, fructose, and sucrose) were extracted from 25 mg of lyophilized ground tissue. This tissue was placed in a 2 mL tube with 1.3 mL of ultra-pure water, and a marble was placed in the opening of each tube as adapted from Cale et al. (2019). Tubes were placed in a rack and enclosed in the steam above a pot of boiling water for 60 min. A 0.5 mL aliquot was collected and centrifuged, and 0.4 mL of the supernatant was transferred to a new 2 mL tube containing 1.0 mL of HPLC-grade methanol. Samples were incubated at room temperature for one h and then 0.5 mL of extracts were transferred to 2 mL amber glass GC vials and stored at −40 °C until analysis.

Soluble sugars were analyzed using the UHPLC system used to analyze diterpene resin acids, but fitted with an InfinityLab Poroshell 120 HILIC-Z column (2.1 x 100 mm 2.7μm; Agilent Tech.) and an Evaporative Light Scattering Detector (ELSD, 1290 ELSD II, Agilent Tech.) A gradient analysis was performed with a binary solvent system of ultra-pure water buffered with 0.034% v/v ammonium hydroxide (A) and acetonitrile (HPLC-grade) buffered with 0.034% v/v ammonium hydroxide (B) flowing at 0.2 mL/min. A 4 μL injection volume was used. The solvent system began at 90% B for 2 mins, then decreasing to 75% B over 3.5 min, held for 1 min, then increased to 90% B over 2 min, and held for 0.5 min. Total run time was 9 minutes. The ELSD settings were a nebulizer temperature of 25°C, evaporator tube temperature of 60°C, and gas flow of 1.40 SLM.

Quadratic standard curves used to quantify soluble sugars were calculated from dilutions prepared from analytical standards of glucose (chemical purity 99%), fructose (99%), and sucrose (99.5%).

2.4.2.4 Starch analysis

Starches were extracted from 25 mg of lyophilized ground tissue. A series of enzymatic digestions were used to convert starch into gluconate-6-phosphate as adapted from Cale et al. (2019). This procedure began with the steam bath procedure described for soluble sugars. Following the steam bath, samples were vortexed for 30sec, then a 0.4 mL aliquot was transferred to a new 2 mL tube containing 0.4 mL of alpha amylase solution [75 grams enzyme (Sigma-Aldrich) in 100 mL ultra-pure water]. Tubes were immediately vortexed for 30 sec and incubated in a 50° C bath for 16 h. Tubes were removed from the water bath, inverted twice,

centrifuged (18,213 rcf) for 15 min, and 0.4 mL of the supernatant was transferred to a new 2 mL tube containing 0.4 mL of amyloglucosidase solution [3 grams enzyme (Sigma-Aldrich) in 60 mL sodium acetate buffer (0.1M, pH 4.5)]. These tubes were incubated in a in a 50° C bath for 16 h. Tubes were removed from the water bath, inverted twice, centrifuged (18,213 rcf) for 15 min, and 0.4 mL of the final glucose extracts was transferred to 2 mL amber glass GC vials and stored at −40 °C until analysis. The use of alpha-amylase enzyme converts phloem tissues to maltose and similar sugars. The use of amyloclucosidase enzyme coverts maltose and similar sugars to glucose.

For analysis, 0.020 mL of a 2X dilution of glucose extract were pipetted into a 96-well plate. To each well, 0.2 mL of a solution of glucose hexokinase (glucose assay reagent Sigma Aldrich)-isomerase (phosphoglucose isomerase; Sigma Aldrich) was added to convert glucose into gluconate-6-phosphate. This plate was shaken on an orbital shaker at room temperature for 45 min, and the amount of gluconate-6-phosphate in each sample was measured using the Synergy Microplate Reader H1 (BioTek, Winooski, VT, USA) at an absorbance of 340 nm.

The concentration of starch in samples was quantified using two calibration curves in series: the first curve estimated glucose concentrations from the sample absorbance at 340 nm, and the second estimated starch concentration from glucose concentration. Following the first calibration curve, sample concentrations of total glucose (hexoses and from starch) were standardized by sample dry weight, and extraction volume. Next, for each sample, previously determined concentrations of hexoses (soluble glucose and fructose) were subtracted from the total concentration of glucose estimated from the absorbance measurements. Samples which had previously been quantified for soluble sugar concentration on the UHPLC were quantified on the microplate reader, and a conversion factor was calculated to account for potential quantification differences. In order to convert from this corrected glucose concentration to starch concentration, the second calibration curve was created. This was created using dilutions of pure potato starch standards which had been processed using the enzymatic digestions described above, and their component glucose concentration was quantified. Potential matrix effects were calculated and used to adjust final concentrations accordingly. For more information, please see Appendix 2.

2.5 Statistical analyses

All analyses were conducted in the R software environment version 3.4.4 (R Core Team 2018). Multivariate analyses were performed with functions provided in R packages "vegan" 2.4–6 (Oksanen et al. 2018) and "ecodist" version 2.0–1 (Goslee et al. 2007). To increase spread of the ordination datapoints, and explore role of climate, the datasets of the current project were appended with climate variables as derived from ClimateAB v3.21 (Wang et al. 2008). To explore potential mechanisms underlying patterns in the dataset, the potential role of various ecological "regulators" were explored (Alberta Agriculture and Forestry 2017, Chiu et al. 2017).

2.5.1 Elevation study vs. latitude study

I used descriptive statistics to explore relationships between predictor variables for both the elevation and latitude studies. Specifically, Pearson correlation values were determined, and the significance was tested. Additionally, I calculated the coefficients of variation to explore the variability of the predictor variables for both studies. Next, I created multivariate ordination plots for each study, to reduce the visual complexity of the datasets. Results were not pooled across the two studies, due to differences highlighted by the descriptive statistics. Specifically, plots were created based on dissimilarities between input predictor variables. In the current study, I used a Bray Curtis distance matrix due to non-normal distribution of predictor variables. I also tested Mahalanobis distance matrix, which showed similar patterns to the Bray Curtis. Ultimately, I selected Bray Curtis distance matrix for ordination, due to precedence in ecological studies. It is worth noting that Bray Curtis distance matrices have a possibility of over-representing variables in the dataset which are orders of magnitude larger than other variables. As such, I scaled all predictor variables by converting the units of measurement for each variable such that final values of all variables had similar orders of magnitude. I then compared the resultant "scaled" distance matrix of predictor variables to the distance matrix of the non-scaled variables, and the two matrices showed identical results. Ultimately, I selected the non-scaled variables for use in the distance matrix. Additionally, I selected the "direct" form to represent the study results, as this showed identical results to the "indirect" ordination. Finally, I compared the visual representation of the data using both non-metric multidimensional scaling (MetaNMDS) and principal coordinate (PCoA) ordinations. MetaNMDS and PCoA are both non-metric multidimensional scaling ordination techniques which produce low-dimensional graphical plots, where similarity between sites is implied by proximity of points. Results were identical between

the two ordination plot types. Therefore, I selected the PCoA plots for the current study, due to its superior statistical robustness.

2.5.2 Lodgepole pine chemical signatures

I calculated coefficients of variation to explore the variability of the response variables for both the elevation and latitude studies. Next, I generated multivariate ordination plots to display the results of the elevation study. Separate plots were created for (1) total compounds, (2) individual monoterpene compounds, and (3) individual diterpene resin acids. These plots were generated as described above; PCoA, direct analysis, Bray-Curtis distance matrix. Finally, I subsampled the elevation dataset for the 15% of sites that had the highest, and the lowest concentration of (1) monoterpenes and (2) diterpene resin acids, in order to showcase the trends for the chemical defense compounds. The concentrations of these compounds were then represented in bar graphs as a function of elevation.

3. RESULTS

3.1 Elevation study vs latitude study

There are inherent differences in the datasets of the two studies. First, the Pearson correlation values show that the patterns of correlation between predictor variables were different between the two studies (Tables S.1, S.2). In other words, two variables which were correlated to each other in one study, did not necessarily having the same r^2 values, or significance in the other study. Second, there was a higher range of variability sampled in the elevation study than in the latitude study (Table S.3). Third, there was a higher range of variability in the concentration of both monoterpene compounds and diterpene resin acids in the elevation study (Table S.4). Furthermore, there was a higher range of variability in the concentration of non-structural carbohydrates in the latitude study (Table S.4). Thus, these descriptive statistics indicate it would not be reasonable to pool the data across all sites into one analysis.

To enhance the predictive power of the datasets, ecological "regulators" were investigated. First, the dataset of the latitude study was appended with ecosite data as derived from the DEP Data Model (Alberta Agriculture and Forestry, 2017) Sites sampled for the latitude study crossed six natural subregions, 20 ecosite phases, five moisture regimes, and four

nutrient regimes (Figures S.1-S.4). These figures showed no spatial autocorrelation within the respective parameters. Similar analyses were not conducted for the elevation study, as no data was available. Second, the datasets of both studies were appended with climate variables as derived from ClimateAB v3.21 (Wang et al. 2008). Eighteen of the 19 climate variables tested were significantly correlated to the value of elevation in the elevation study (Table S.5). It is also worth noting that the correlation between elevation and six of the climate variables equaled or exceeded r= (±) 0.97. On the other hand, 14 of the 19 climate variables tested were significantly correlated to the value of latitude in the latitude study, with none exceeding r= (±) 0.97. Eighteen of the 19 climate variables contributed significantly in explaining variation in the elevation study ordination figures, whereas only 14 contributed significantly in explaining the variation in the latitude study ordination figures (Table S.6). Thus, these results suggest that the climate variables are more likely be correlated to the elevation study than the latitude study.

Between the two studies, much stronger statistical relationships were found in the elevation study. The comparison of Figures 1.3 and 1.4 show that there were five significant trends in response variables in the elevation study, whereas there were no significant trends in the latitude study. Thus, the results presented in the following sections will be based on the elevation study dataset only.

3.2 Lodgepole pine defense compounds

Trees occurring in higher elevation sites had significantly higher concentrations of both total monoterpenes and total diterpene resin acids (Figure 1.3). In the PCoA, the vectors for both total monoterpene (r=0.363, p=0.010**)**, and total diterpene resin acids (r=0.374, p=0.012) were significantly influenced by the differences between sites (Figure 1.3, Table S.7). Differences between sites refers to differences in predictor variables such as stand density, tree DBH (Table S.2, S.6). Next, I ordered by elevation, the 15% of trees with the highest and lowest of either monoterpene concentration, or diterpene resin acid concentrations (Figures S.5, S.6) These bar graphs re-enforced that trees at higher elevations generally had higher concentrations of either of the constitutive defense compounds, as compared to conspecifics in lower elevations.

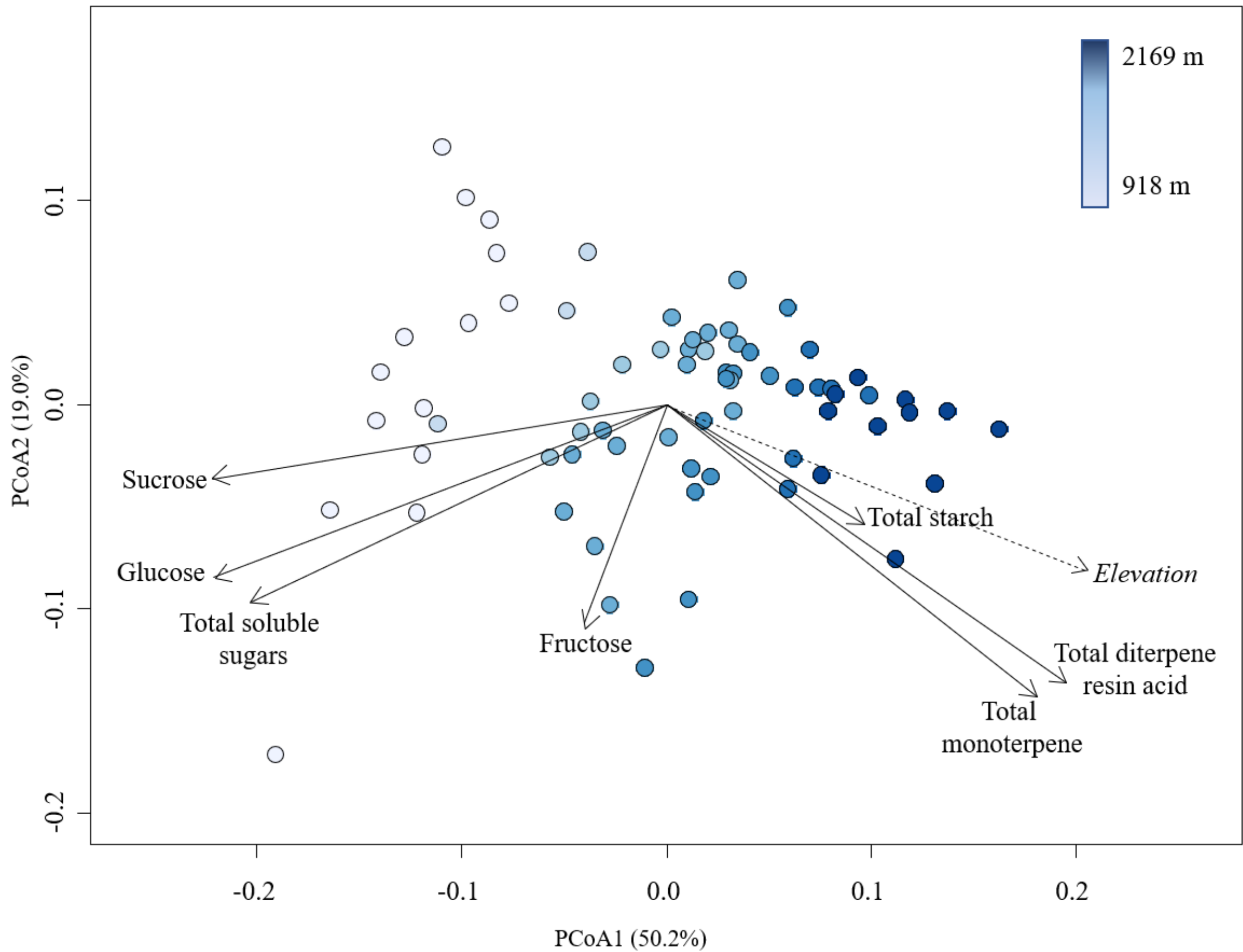

Figure 1.3: PCoA showing response variables for elevation study.

PCoA based on a direct, Bray-Curtis distance matrix. Point cloud coloured by elevation where darker blue indicates higher elevation. Dashed line vector for predictor variable of elevation. Solid line vector for response variables scaled by a factor of 2.8 (relative to elevation) for visual simplicity. Bolded p-values indicate sig. at $\alpha=0.05$: total monoterpene (r=0.363, **p=0.010**), total diterpene resin acid (r=0.374, **p=0.012**), total sugar (r=0.353, **p=0.011**), sucrose (r=0.353, p=**0.010**), glucose (r=0.372, **p=0.002**), fructose (r=0.179, p=0.349), total starch (r=0.177, p=0.348).

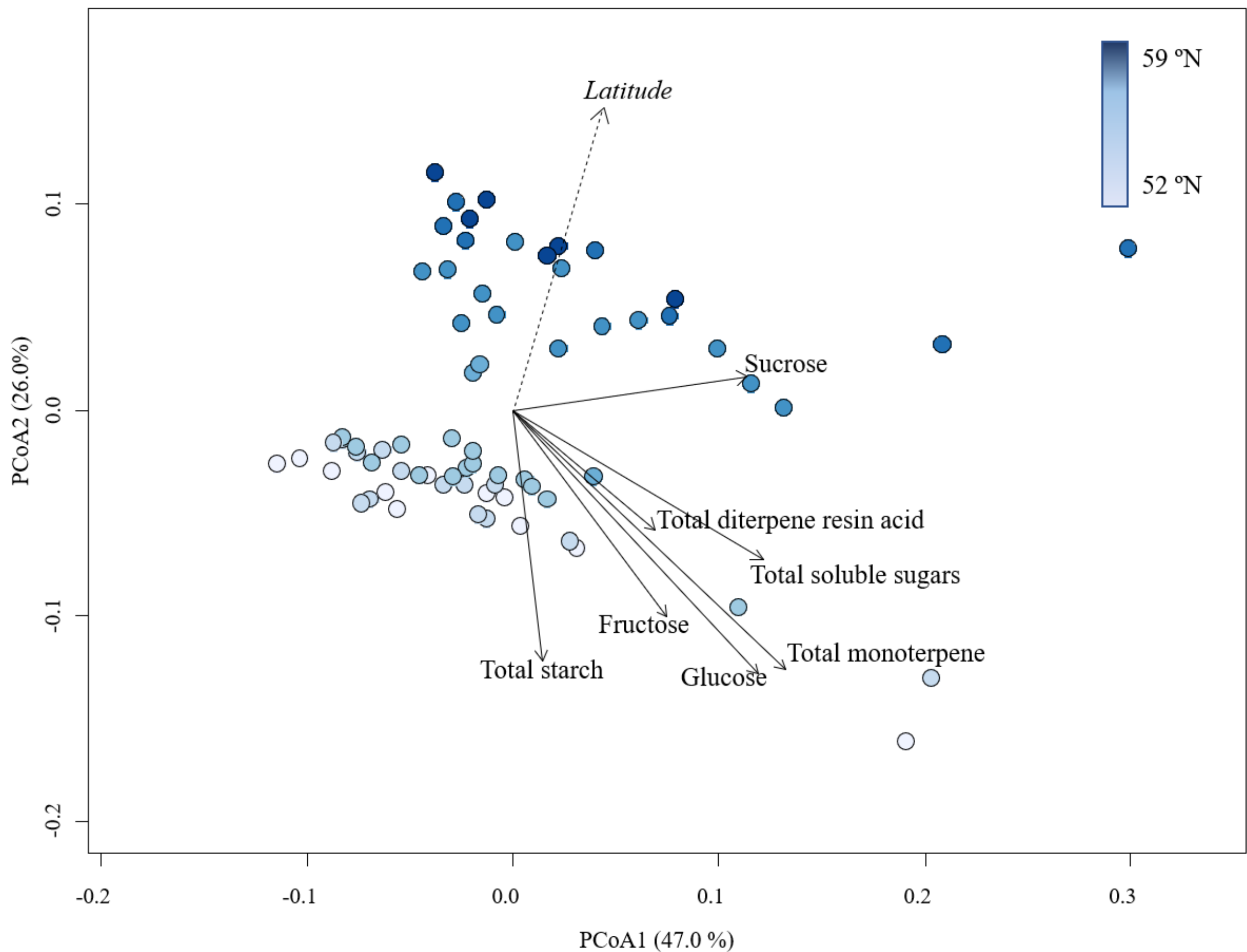

Figure 1.4: PCoA showing response variables for latitude study.

PCoA based on a direct, Bray-Curtis distance matrix. Point cloud coloured by latitude where darker blue indicates higher latitude. Dashed line vector for predictor variable of latitude. Solid line vector for response variables scaled by a factor of 3.8 (relative to latitude) for visual simplicity. No vectors are sig. at $\alpha=0.05$: total monoterpene ($r=0.287$, $p=0.073$), total diterpene resin acid ($r=0.143$, $p=0.512$), total sugar (0.223, $p=0.1921$), sucrose ($r=0.181$, $p=0.367$), glucose ($r=0.275$, $p=0.080$), fructose ($r=0.197$, $p=0.260$), total starch ($r=0.193$, $p=0.278$).

In parallel to the total monoterpene and diterpene resin acid concentrations, trees occurring in higher elevation sites also had significantly higher concentrations of some individual monoterpenes and diterpene resin acids (Figure S.7, S.8, Table S.7). For example, myrcene (r=0.402, p=0.008), (−)-α-pinene (r=0.318, r=0.040), levopimaric acid (r=0.425, p=0.006), and palustric acid (r=0.363, p=0.008) were significantly influenced by the differences between sites (Figure S.7, S.8, Table S.7). Specifically, this "significant influence" likely manifested in an increase of constitutive defense compounds at higher elevation sites.

To further investigate the result that there were significant increases in some individual monoterpene compounds with elevation, the median lethal dose (LC_{50}) for six of the most common monoterpenes were calculated based on Chiu et al. (2017). ArcGIS software was used to query the sites which had greater than or equal to the derived LC_{50} concentration of monoterpenes. The distribution of trees at or exceeding the derived LC_{50} is correlated neither with elevation nor with latitude (Figure S.9).

With regards to variability of the chemical defense compounds in the elevation study, there were three noteworthy patterns. First, the concentration of diterpene resin acids had higher variation in the elevation study than in the latitude study (Table S.4). The coefficients of variation were higher in the elevation study for both total diterpenes, as well as for each of the individual diterpenes. Second, the coefficient of variation of total diterpenes was 6.6% higher than that of total monoterpenes (CV_{TD} = 72.5%, CV_{TM} = 68.6%) (Table S.4). This higher variation in total diterpenes was also noticed when comparing Figures 2.5 and 2.6. Third, the variation in concentration of individual monoterpene compounds was study-specific (Table S.4). The coefficients of variation were higher in the elevation study for total monoterpenes, as well as for the individual monoterpenoids bornyl acetate, (−)-β-pinene, (+) -α-pinene, p-cymene, thujone, 4-allylanisole, (+)-limonene, (±)-camphor, and myrcene. The coefficients of variation were higher in the latitude study for (−)-limonene, (+)-3-carene, terpinolene, (+)-camphene, (−)-α-pinene, γ-terpinene, and β-phellandrene.

3.3 Lodgepole pine non-structural carbohydrates

Trees occurring in higher elevation sites had significantly lower concentrations of some non-structural carbohydrates, as compared to trees in low elevation sites (Figure 1.3). In the PCoA, the vectors for glucose (r=0.372, p=0.002), sucrose (r=0.353, p=0.010), and total sugars

(r=0.353, p=0.011) were significantly influenced by the differences between sites (Figure 1.3, Table S.7). On the other hand, though the vector for fructose pointed orthogonal to the direction of elevation, and the vector for total starch pointed in the same direction as elevation, these were non-significant at α=0.05. Further, there is a marginally significant negative Pearson correlation value between elevation and values of total sugar, sucrose, and glucose, but no relationship with between elevation and values of fructose or starch (Table S.7). These correlations re-enforced the result from the PCoA that trees at higher elevations generally had decreased concentrations of some of the non-structural carbohydrates.

4. DISCUSSION

The overarching objective for the current study was to initiate research which would lead to the identification of geographic areas where lodgepole pine trees in Alberta may be more vulnerable to attack by mountain pine beetle. In the current study, I found that differences in chemical profile of trees responded to changes in elevation, but not latitude. I found trees at higher elevations had higher concentration of total monoterpenes and total diterpene resin acids, decreased concentration of total sugars, and no differences in concentration of total starch. On a more refined scale, individual defense compounds also generally increased with elevation, while two of the three soluble sugars decreased with elevation.

4.1 Elevation vs. Latitude

The most notable difference between the elevation and latitude studies is the lack of significant trends of response variables in the latter. This result may be due to non-significant differences in the predictor variables. However, for both climate-change proxy gradients, there were significant correlations among predictor variables, and a notable variation upon which to associate a response, consistent with the space-for-time sampling framework. In addition, as mentioned earlier, within Alberta, lodgepole pine trees can occur from 550 m to 2,100 m elevation, and from 49ºN to 60ºN (Little 1971, Archibald et al. 1996). In this study, I sampled 80.6% of the species' elevational range (918 m to 2,169 m), of which included the highest possible elevations. Comparatively, I sampled 63.6% of the species' latitudinal range (52 ºN to 59 ºN), of which the most northern possible latitudes were not sampled. I appended climate data to the datasets of

both studies. For the elevation and latitude studies, these climate variables greatly assisted in explaining variation among sites. In other words, while the range sampled for the latitude study was proportionally smaller than that of elevation study, the range sampled for the former did indeed reflect a climate change gradient.

Alternatively, I explored the idea that perhaps the non-significant differences in response variables in the latitude study could be due to some unmeasured predictor variables which might have had more influence in the latitude study than in the elevation study. For example, I did not incorporate genetic information in the current study. Pine chemotypes are known to be controlled by genetic and/or environmental factors. Due to the larger geographic area covered in the latitude study (relative to the elevation study), it is possible that trees sampled may have had more diverse chemotypes than those in the elevation study (Forrest 1980, 1981, 1987, Taft et al. 2015). There is agreement that lodgepole pine monoterpenes are heritable, and that certain monoterpene compounds are more heritable than others (e.g., Birks and Kanowski 1986, Ott et al. 2011). For example, in a recent study, the two most heritable monoterpene compounds in lodgepole pine were 3-carene, and limonene (Ott et al. 2011). In the latitude study, (+)-3-carene and (−)-limonene were more variable in concentration, lending support to the idea that a wider variety of chemotypes could have been sampled as compared to the elevation study. This idea should be verified in future studies. For example, provenance trials could be used to test the relative influence imposed by environmental condition and genetics, over the patterns observed in defense compounds and non-structural carbohydrates.

The analysis of the ecological differences between the elevation and latitude studies lead to the conclusion that for the current project, the elevation study displayed clearer differences in chemical profile of lodgepole pine populations than the latitude study**.** Thus, future research should favor modeling climate change gradients as a function of elevation rather than latitude, as the former are much more "compact" studies. If impossible (or inappropriate) to sample on an elevational range, latitudinal experiments should consider measures of climate and continentality, as well as measures of genetic variation.

4.2 Project results and proposed future directions

To be clear, greater concentrations of defense compounds were only marginally correlated to the value of elevation itself. What is more likely is that the changes in defense compounds are driven

by the combination of climate and environmental factors correlated to increases in elevation. In fact, I found that the variable of elevation was significantly correlated to phloem thickness, phloem mass, tree height, and stand density. Stand density in turn, was significantly correlated to variables such as tree diameter, as well as stand basal area. In addition, the variable of elevation is significantly correlated to almost all climate variables tested. These patterns highlight that the growth relationships which are characteristic of the high elevation sites, are influenced by both climate and physical parameters of sites. Thus, the interpretation of the relationship between elevation and any of the response variables must remain a multivariate one.

Greater constitutive monoterpene and diterpene resin acid concentration at the higher elevation sites does not necessarily imply an increased defense capacity to the mountain pine beetle. First, one must consider that the allocation of resources to the production of terpenoids is energetically costly; in order to invest heavily in defense, other biological pathways such as growth, maintenance, respiration, or reproduction may receive less photosynthates (Stamp 2003, Keeling and Bohlmann 2006, Moreira et al. 2014). In the current study, no correlation was found between the higher concentrations of defense compounds (both monoterpenes and diterpene resin acids) and lower concentrations of soluble total sugars, suggesting that the relationship between the production of defense chemicals and non-structural carbohydrates is nonlinear. It is worth acknowledging the multiple theories surrounding general plant resource allocation strategies. For example, studies have reported that resource allocation strategies are influenced by resource availability (abundant resource vs. resource limitation), evolutionary history with biotic agents (co-evolved vs. novel interactions), and physiology (stressed vs. healthy, young vs. old) (e.g., Wiley and Helliker 2012, Martinez-Vilalta et al. 2016, Roth et al. 2017, Cale et al. 2019). Future research may be warranted to determine how various environmental and climatic variables influence resource-allocation patterns of lodgepole pine populations.

The second reason defense capacity cannot be implied from the results of the current study is related to the concept of time. The current study determined that the constitutive defense compounds of the pines change with elevation. Thus, future research is needed to investigate if, and how, elevation and perhaps latitude changes the induced chemical profile of the same trees (Karban and Myers 1989).

I developed a conceptual diagram “hypothesized defense capacities of high elevation trees” that incorporates both the results of current study and hypothesized results of an induction

study along elevation gradient (Figure 1.5). Panel (a) shows the best-case scenario; with increasing elevation, there is an increase in constitutive concentration of defense compounds, and an increase in the photosynthates available for mobilization and uptake during a beetle attack. Trees are most likely to survive such an attack due to availability of internal resources. In comparison, Panel (c) shows the statistically significant trends of the current study. The significant investment in constitutive defense allows for notable pre-attack preparations, which is consistent with Coley et al. (1985)'s resource availability hypothesis. However, the decrease in internal reserves may leave the tree incapable of being able to support continued production of defense compounds for a prolonged period (Moreira et al. 2014, Roth et al. 2017). This would especially be the case, should the primary line of defense be depleted. Ultimately, a depletion of both the constitutive defense compounds and internal storage pools would likely lead to tree mortality (Panel d). On the other hand, should the attack situation deplete the constitutive defense, but not deplete the storage pools, then the trees may be capable of supporting continued production of defense compounds for a longer period (Panel b).

Evidently, there are more possible outcomes to an induction study than those presented above (Clark et al. 2010). The point to be made between the hypothesized outcomes is that comparing the chemical signatures of constitutive and induced responses would provide more insight into the ability of the tree to mobilize resources, as well as whether trees in higher elevations may be resource stressed. Consequently, the defense capacity of high elevation trees would be more apparent.

The need for an induction study also highlights the need to conduct additional research regarding the host preference and colonization behaviours by the mountain pine beetle and its vectored fungal symbionts. For example, it is known that individual monoterpene compounds have different effects on mountain pine beetle biology; these roles range from pheromone precursors and synergists, to aggregation inhibitor and antifeedants (Erbilgin et al. 2017b, Erbilgin 2019). In the current study, I found that regardless of functional classification, individual monoterpene compounds were generally at greater concentrations at higher elevations.

Thus, it is unclear if the mountain pine beetles will be naturally attracted or repelled from high elevation areas. It is consequently also unclear if the beetles would be able to successfully colonize the trees. This proposed research would help reduce uncertainty of bark beetle

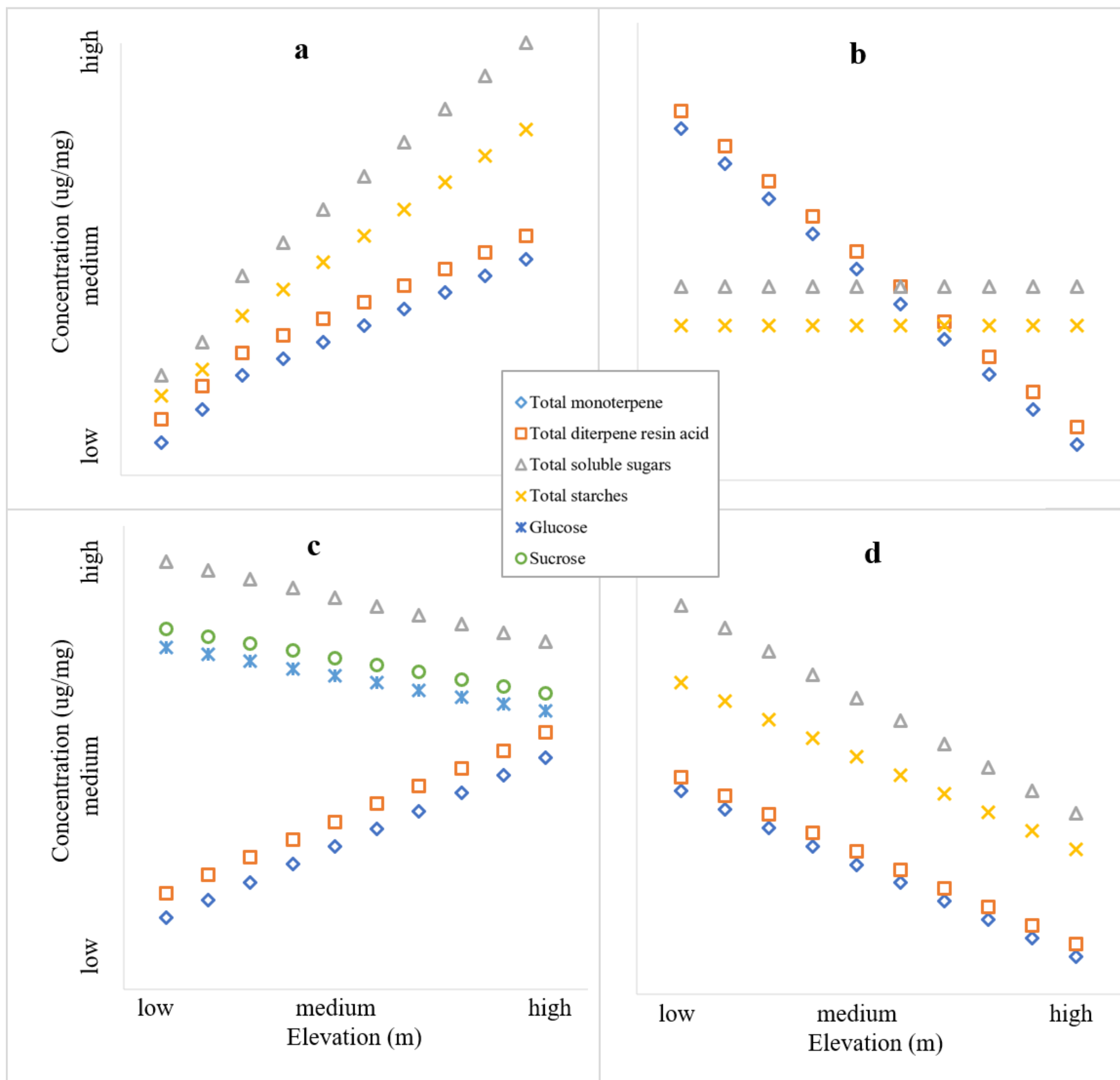

Figure 1.5: Hypothesized defense capacities of high elevation trees

Panel (a) shows the (highly unlikely) best-case scenario, (b) shows project hypothesis (a middle-ground scenario) (c) shows statistically significant trends from current study, and (d) shows the worst-case scenario for high elevation trees.

behaviour in high elevation pines.

4.3 Project implications for high-elevation pines

Combining the results of the current study and the various proposed studies, would allow for a much clearer identification of geographic areas which may be more vulnerable to attack by mountain pine beetle. The identification of such areas is timely, given the current context of climate-changed induced range expansion by mountain pine beetle. The possibility for these bark beetles to succeed in colonizing high elevation areas has critical ecological implications. For example, within Jasper National Park, high-elevation sites are occupied by both lodgepole and whitebark pines (*Pinus albicaulis)*. Whitebark pine is listed as "endangered" and as Schedule 1 under the "species at risk act" (COSEWIC 2010). It has been recently found that whitebark pines have less thoroughly integrated defenses against mountain pine beetle, as compared to lodgepole pines (Raffa et al. 2017). Furthermore, mountain pine beetle can attack both species in mixed lodgepole-whitebark pine stands (Raffa et a. 2013). Though it is unclear if mountain pine beetles would succeed in host selection and colonization, the presence of bark beetles in high elevation stands poses a significant ecological risk (Logan et al. 2010, Raffa et al. 2017).

THESIS SUPPLIMENTAL TABLES, FIGURES, AND APPENDICES

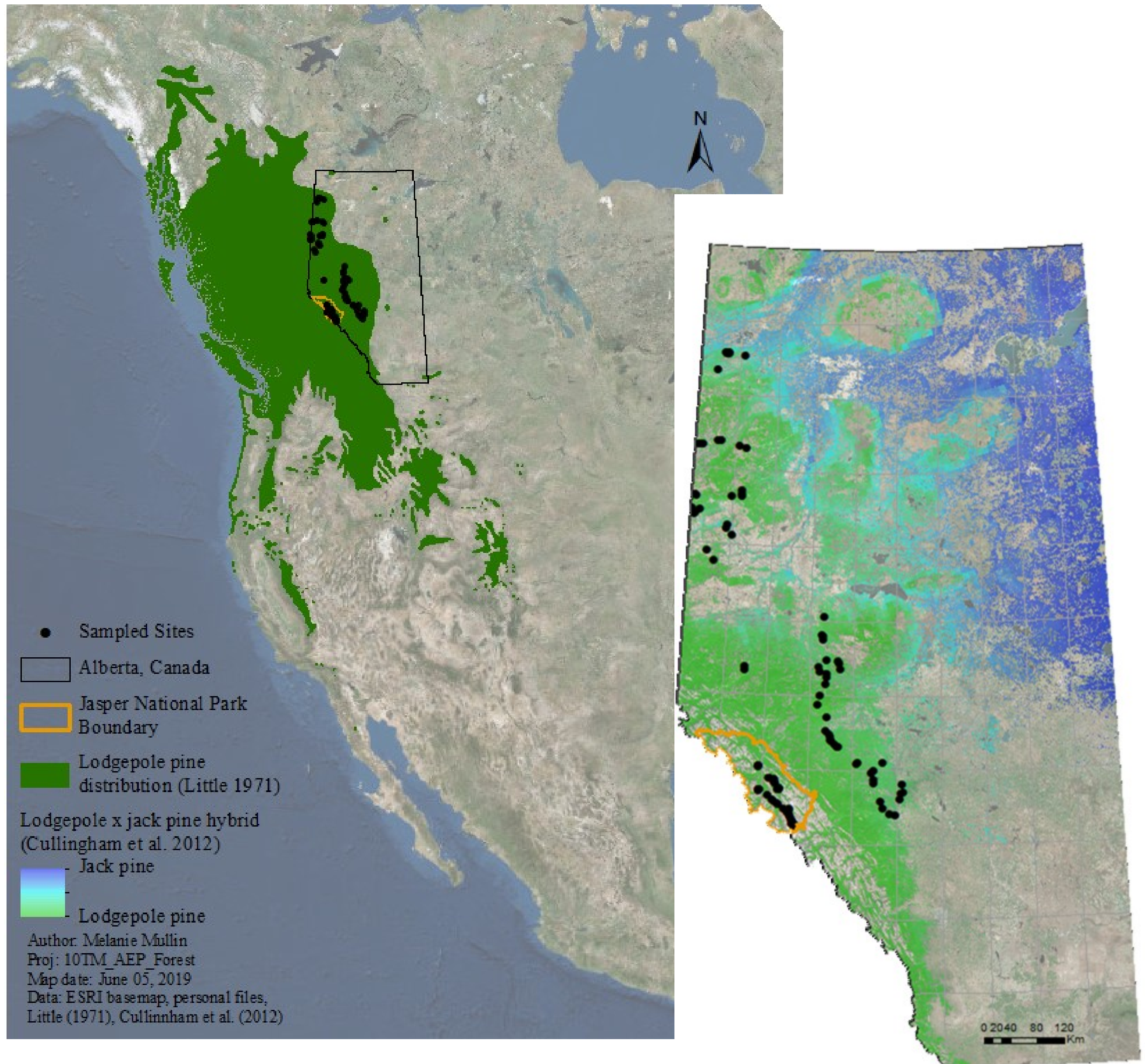

Figure S.1: Distribution of study sites relative to *Pinus contorta*'s North American distribution. Extent of sampled sites as compared to extent of distribution of lodgepole pine trees in north America. Logepole pine presence shapefile (dark green) from Little (1971) is comprised of three sub-species; *P. contorta* var. *murrayana, P. contorta var. latifolia,* and *P. contorta var. contorta*

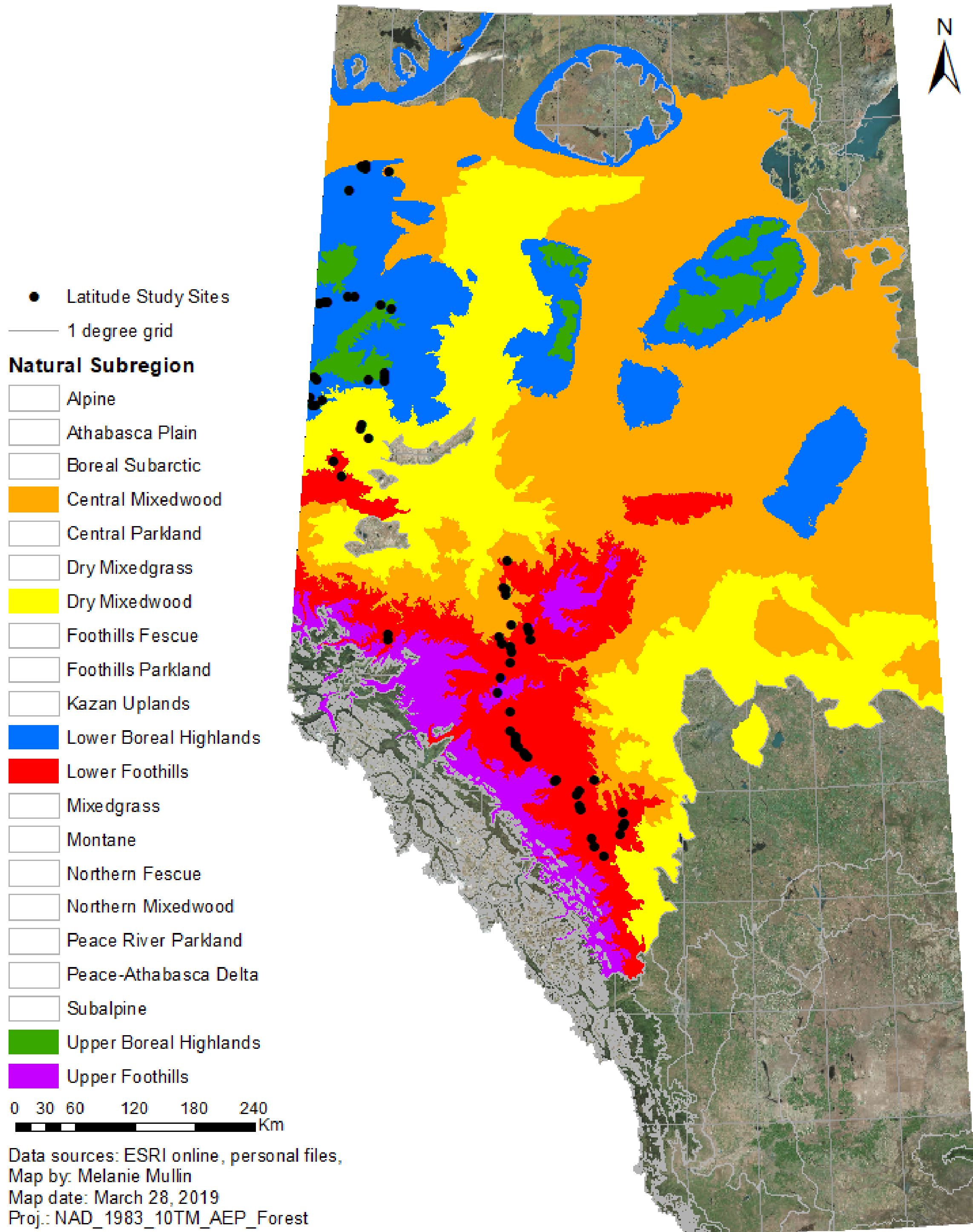

Figure S.2: Natural subregions sampled in latitude experiment
Extent of sampled sites as compared to extent of Alberta's natural subregions. Natural subregions derived from Alberta Agriculture and Forestry open-access Derived Ecosite Phase model (2017).

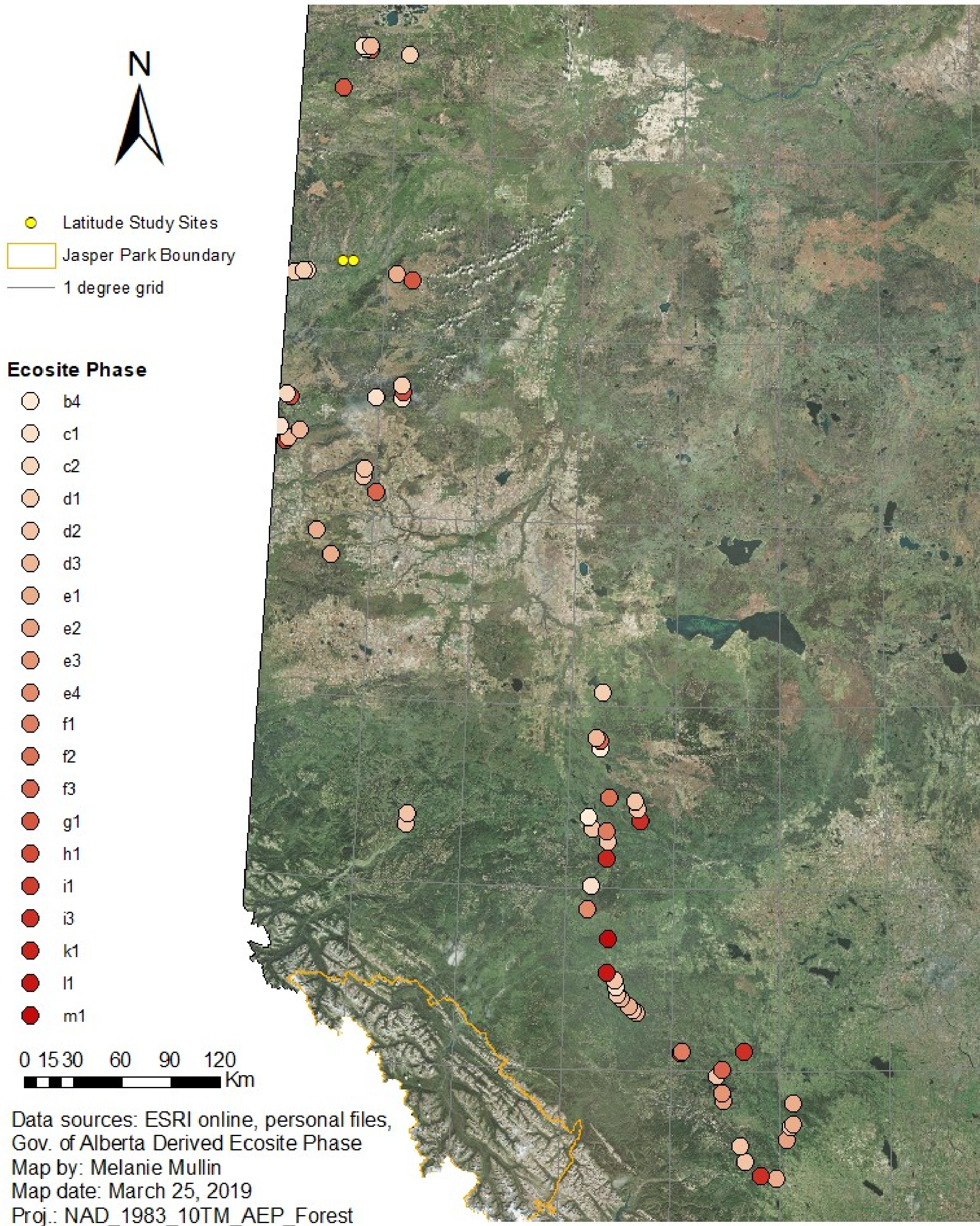

Figure S.3: Ecosite phases sampled in latitude experiment

Extent of sampled sites as categorized by Alberta's ecosite phases. Ecosite classification derived from Alberta Agriculture and Forestry open-access Derived Ecosite Phase model (2017). Note that the 20 ecosite phases sampled are well distributes across the study, with no spatial autocorrelation occurring by ecosite phase. Yellow circles are sites for which no data was available.

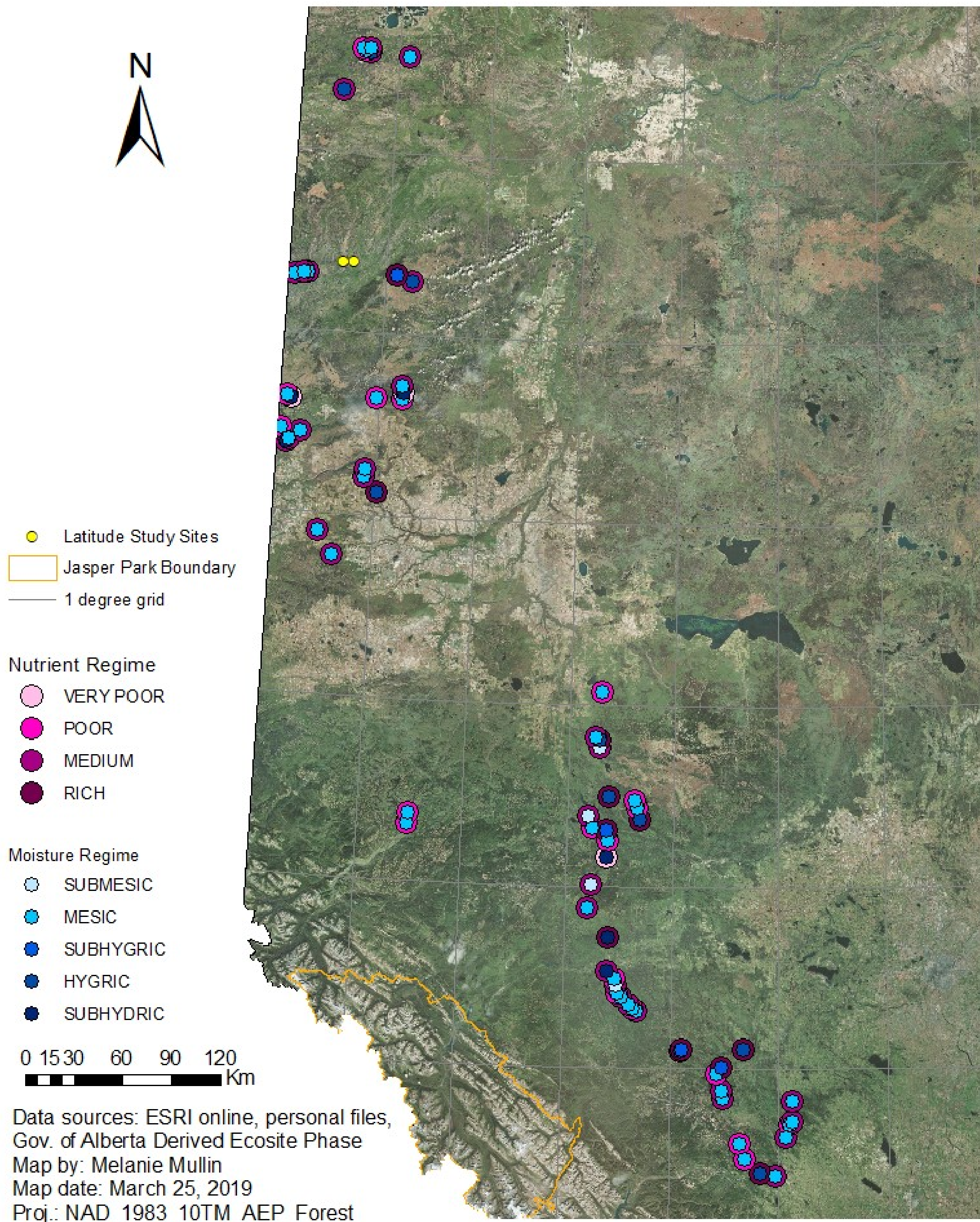

Figure S.4: Soil nutrient and moisture regimes sampled in latitude experiment
Extent of sampled sites as categorized by soil nutrient and moisture regimes. Soil information derived from Alberta Agriculture and Forestry open-access Derived Ecosite Phase model (2017). Yellow circles are sites for which no data was available. Blue circles represent the moisture regime for a sampled side, where the darker points are the wetter sites. Pink rings represent the nutrient regime for a sampled site, where the darker the ring, the more nutrient-rich the site.

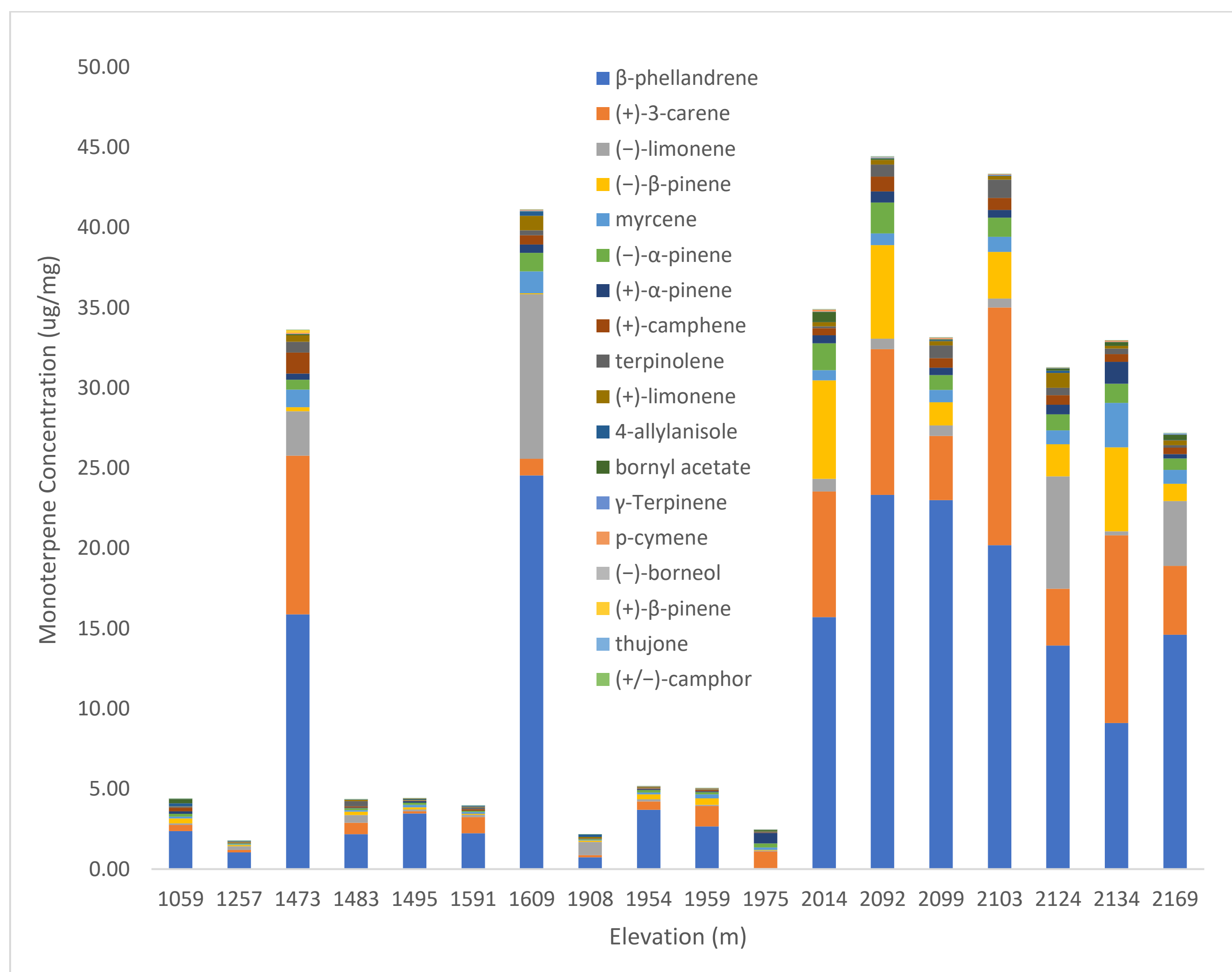

Figure S.5: Sub-setting the elevation dataset to show trends in monoterpenes

Bar graph ordered by elevation (x-axis) showing trees (n=9, 15% of dataset) with the highest and trees (n=9, 15% of dataset) with the lowest concentration of monoterpenes. Note that results are consistent with patterns presented in figures 1.3 and 2.5.

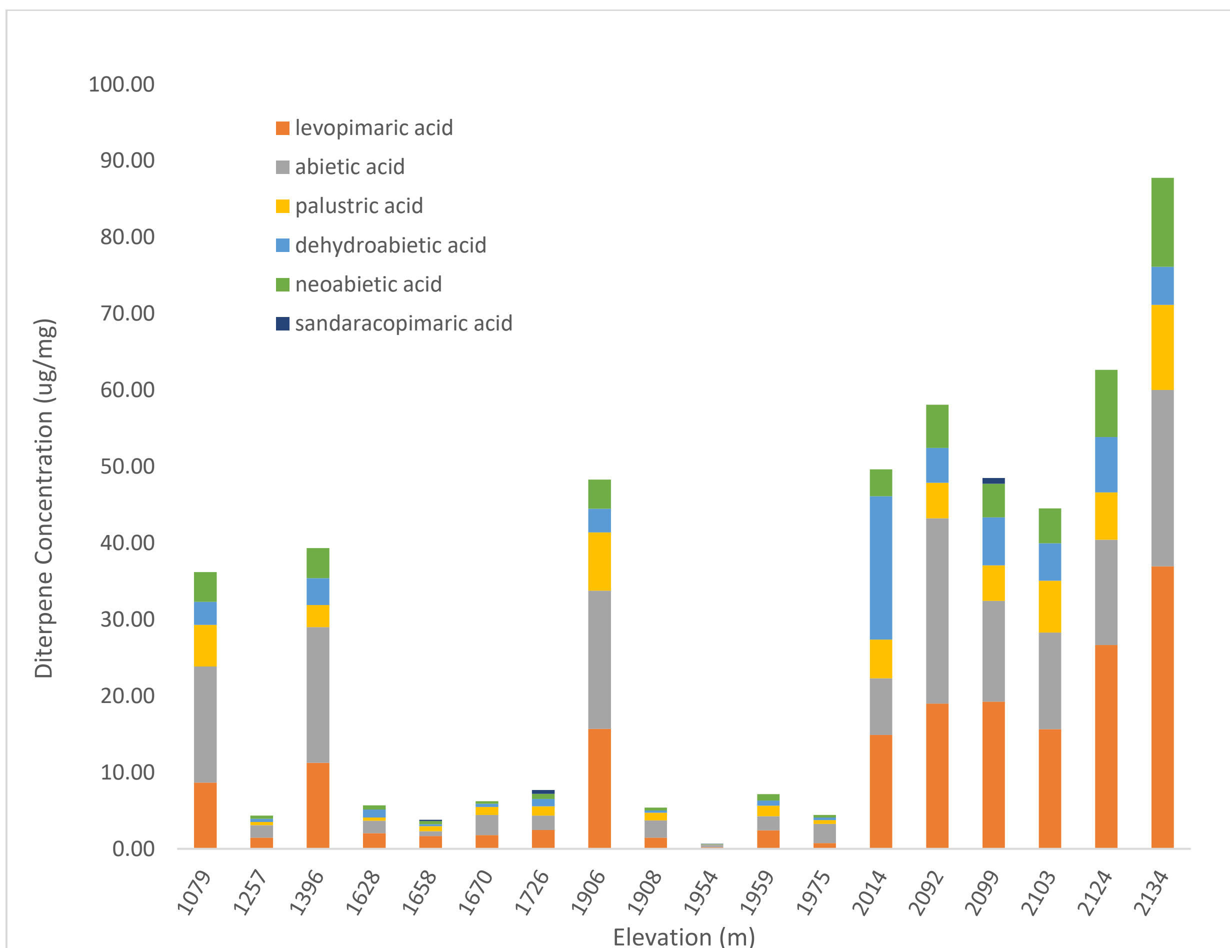

Figure S.6: Sub-setting the elevation dataset to show trends in diterpenes
Bar graph ordered by elevation (x-axis) showing trees (n=9, 15% of dataset) with the highest and trees (n=9, 15% of dataset) with the lowest concentration of diterpene resin acids. Note that results are consistent with patterns presented in figures 1.3 and 2.8.

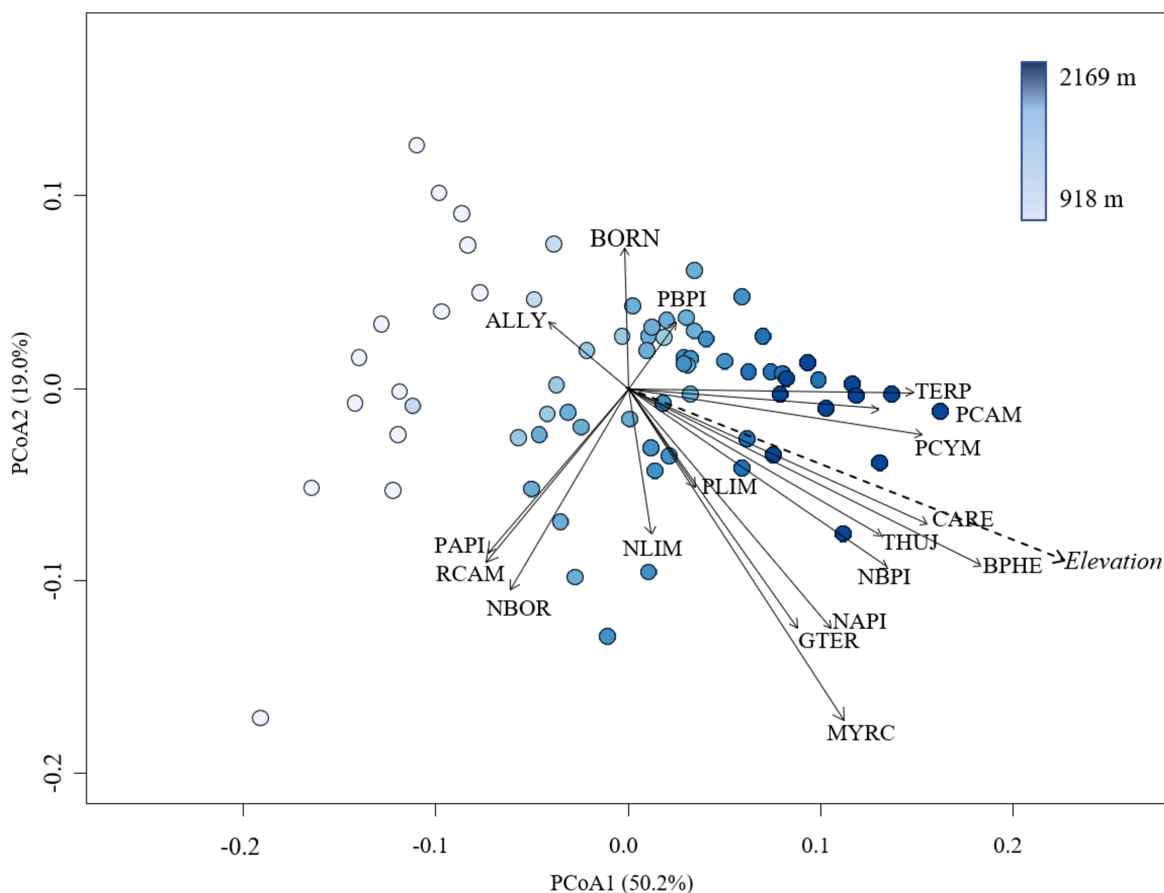

Figure S.7: PCoA showing results for individual monoterpene compounds for elevation study. PCoA based on a direct, Bray-Curtis distance matrix. Point cloud coloured by elevation where darker blue indicates higher elevation. Dashed line vector for predictor variable of elevation. Solid line vectors for monoterpene compounds scaled by a factor of 2.1 (relative to elevation) for visual simplicity. Vector codes as per table below. Author's classification of monoterpene function based on Erbilgin et al. (2017). Bolded p-values in table indicate sig. at α=0.05

Anti-feedant or aggregation inhibitor	**Unknown effect on beetle**
BPHE β-phellandrene (r=0.397, **p=0.002**)	NBPI (−)-β-pinene (r=0.317, **p=0.032**)
CARE (+)-3-carene (r=0.329, **p=0.031**)	GTER γ-terpinene (r=297, **p=0.040**)
NAPI (−)-α-pinene (r=0.318, **p=0.040)**	THUJ thujone (r=0.293, **p=0.047**)
NLIM (−)-limonene (r=0.147, p=0.492)	PCYM p-cymene (r=0.302, **p=0.048**)
PLIM (+)-limonene (r=0.121, p=0.615)	CAMP (+)-camphene (r=0.255, p=0.103)
ALLY 4-allylanisole (r=0.104, p=0.688)	NBOR (−)-borneol (r=0.236, p=0.135)
Pheromone precursor or synergist	RCAM (+/−)-camphor (r=0.227, p=0.160)
MYRC myrcene (r=0.402, **p=0.008**)	BORN bornyl acetate (r=0.143, p=0.477)
TERP terpinolene (r=0.288, p=0.05)	PBPI (+)-β-pinene (r=0.081, p=0.802)
PAPI (+)-α-pinene (r=0.219, p=0.181)	

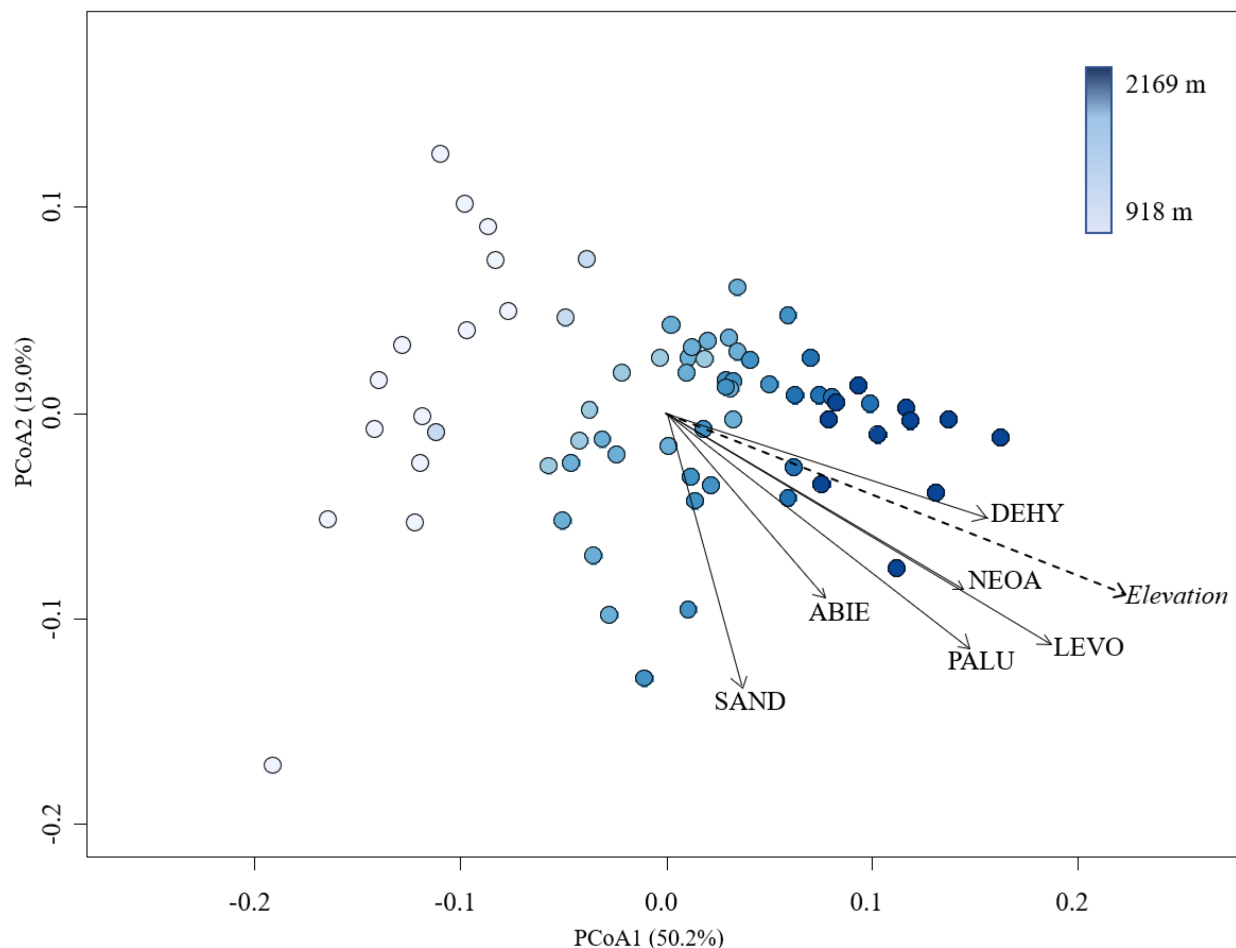

Figure S.8: PCoA showing results for individual diterpene resin acids for elevation study.
PCoA based on a direct, Bray-Curtis distance matrix. Point cloud coloured by elevation where darker blue indicates higher elevation. Dashed line vector for predictor variable of elevation. Solid line vectors for response variables scaled by a factor of 2.1 (relative to elevation) for visual simplicity. Vector codes as per table below. Bolded p-values in table indicate sig. at α=0.05

LEVO	levopimaric acid (r=0.425, **p=0.006**)
PALU	palustric acid (r=0.363, **p=0.008**)
DEHY	dehydroabietic acid (r=0.319, **p=0.035**)
NEOA	neoabietic acid (r=0.328, p=**0.030**)
SAND	sandarocopimaric acid (r=0.267, p=0.083)
ABIE	abietic + pimaric + isopimaric acids (r=0.23, p=0.144)

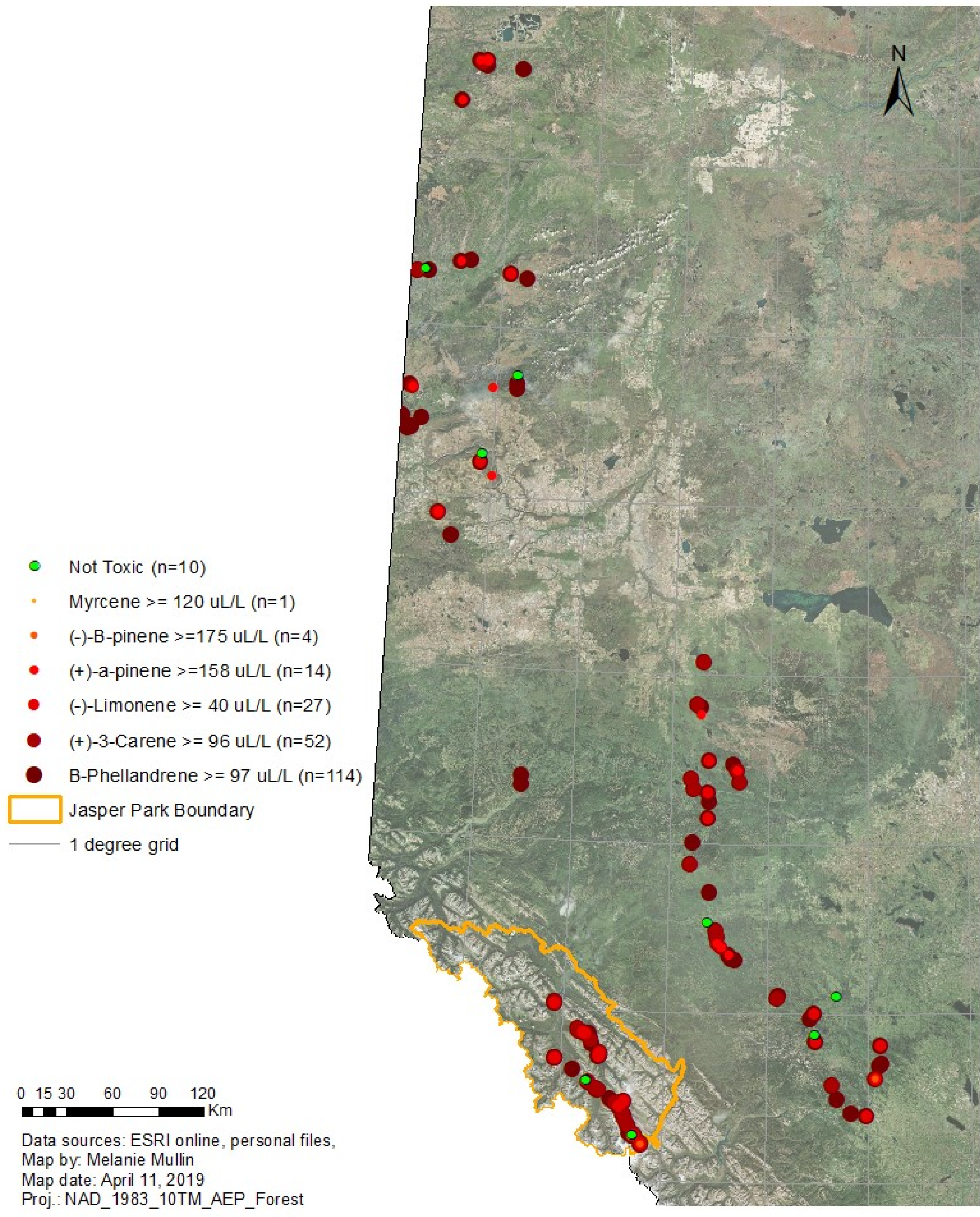

Figure S.9: Distribution of sites which could be lethal to mountain pine beetle
Extent of sampled sites as categorized by lethal concentration of 6 monoterpene compounds. Author derived LC_{50} vales from data presented in Chiu et al. (2017). Values used as thresholds are presented in map legend. Note that distribution of trees with compounds at or exceeding LC_{50} for any tested monoterpene is not confined to a given latitude or elevation.

Latitude Study	Days Since Start	Latitude	Longitude	Elevation	Aspect	Slope Degree	Diameter at Breast Height	Phloem Thickness	Phloem mass	Tree height	Stand Basal Area	Stand Density	Tree Age	Growth in 10 years	Basal Area Index
Days Since Start		**0.00**	**0.00**	**0.00**	0.357	0.343	0.76	**0.03**	0.10	**0.00**	0.82	0.11	0.60	**0.01**	**0.01**
Latitude	0.90		**0.00**	**0.00**	0.202	0.438	0.86	0.05	0.08	**0.00**	0.76	0.26	0.48	**0.01**	**0.01**
Longitude	-0.70	-0.90		**0.00**	0.325	0.536	0.81	0.26	0.12	**0.00**	0.61	0.37	0.52	**0.01**	**0.01**
Elevation	-0.70	-0.80	0.70		0.95	0.46	0.95	**0.00**	**0.02**	**0.00**	0.92	0.80	0.74	**0.02**	**0.02**
Aspect	0.10	0.20	-0.10	0.00		0.55	0.69	0.90	0.57	0.10	0.88	0.23	0.62	0.98	0.96
Slope Degree	-0.10	-0.10	0.10	0.10	-0.10		0.62	0.56	0.44	0.13	0.89	0.19	0.65	0.42	0.49
Diameter at Breast Height	0.00	0.00	0.00	0.00	0.00	-0.10		0.84	0.28	0.29	0.87	0.45	**0.00**	**0.04**	0.27
Phloem Thickness	-0.30	-0.20	0.10	0.40	0.00	0.10	0.00		**0.00**	0.85	0.37	0.08	0.07	**0.00**	**0.00**
Phloem mass	-0.20	-0.20	0.20	0.30	0.10	-0.10	-0.10	0.80		0.93	0.79	**0.02**	**0.01**	**0.00**	**0.00**
Tree height	-0.80	-0.60	0.60	0.40	-0.20	0.20	0.10	0.00	0.00		0.47	**0.03**	0.97	0.12	0.10
Stand Basal Area	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.10	0.00	-0.10		**0.00**	0.45	0.45	0.61
Stand Density	0.20	0.10	-0.10	0.00	0.10	-0.20	-0.10	0.20	0.30	-0.30	0.40		0.05	0.24	0.24
Tree Age	0.10	0.10	-0.10	0.00	0.10	0.10	0.30	-0.20	-0.30	0.00	-0.10	-0.20		**0.00**	**0.00**
Growth in 10 years	-0.30	-0.30	0.30	0.30	0.00	0.10	-0.30	0.60	0.60	0.20	-0.10	0.10	-0.50		**0.00**
Basal Area Index	-0.30	-0.30	0.30	0.30	0.00	0.10	-0.10	0.60	0.60	0.20	-0.10	0.10	-0.50	1.00	

Table S.1: Pearson correlation values for predictor variables in latitude study

Pearson correlation values (bottom half of table), as well as the degrees of significance (p-value) for predictor variables used in the latitude experiment. The darker the orange, the closer the r value is to either -1 or 1. Bolded p-values indicate significance at α=0.05.

Elevation study	Days Since Start	Latitude	Longitude	Elevation	Aspect	Slope Degree	Diameter at Breast Height	Phloem Thickness	Phloem mass	Tree height	Stand Basal Area	Stand Density	Tree Age	Growth in 10 years	Basal Area Index
Days Since Start		0.07	0.40	0.28	0.45	0.06	0.402	0.829	0.61	**0.00**	0.59	1.00	**0.03**	0.62	0.49
Latitude	-0.22		0.60	**0.00**	0.19	0.44	0.66	0.16	0.08	0.16	0.27	0.70	0.11	0.80	0.89
Longitude	-0.10	-0.06		**0.00**	**0.00**	0.80	0.29	0.54	0.97	**0.00**	**0.01**	0.07	**0.01**	**0.01**	**0.02**
Elevation	0.13	-0.51	-0.49		**0.02**	0.17	0.40	**0.00**	**0.00**	**0.00**	0.12	**0.01**	0.15	0.90	0.86
Aspect	0.09	-0.16	-0.41	0.28		0.56	0.67	0.48	0.44	**0.00**	0.44	0.57	0.14	0.81	0.88
Slope Degree	-0.22	0.09	-0.03	-0.17	0.07		0.28	0.76	0.84	0.11	0.92	0.62	0.89	0.52	0.76
Diameter at Breast Height	-0.10	0.05	-0.13	0.10	-0.05	0.13		0.60	0.53	0.97	0.27	**0.00**	0.10	0.27	0.74
Phloem Thickness	0.03	-0.17	-0.07	0.52	0.09	-0.04	0.06		**0.00**	**0.00**	**0.00**	0.34	**0.01**	**0.00**	**0.00**
Phloem mass	-0.06	-0.21	0.00	0.44	0.09	-0.03	0.08	0.94		**0.01**	**0.02**	0.62	**0.01**	**0.00**	**0.00**
Tree height	-0.36	0.17	0.55	-0.48	-0.45	0.19	0.00	-0.37	-0.31		**0.00**	0.34	0.22	0.78	0.62
Stand Basal Area	0.06	-0.13	0.33	-0.19	-0.09	-0.01	-0.13	-0.35	-0.28	0.43		**0.00**	0.16	**0.01**	**0.00**
Stand Density	0.00	0.05	0.22	-0.31	-0.07	-0.06	-0.36	-0.12	-0.06	0.12	0.43		0.12	0.24	0.55
Tree Age	0.25	-0.19	-0.29	0.18	0.18	0.02	0.20	-0.29	-0.30	-0.15	0.17	-0.19		0.00	**0.00**
Growth in 10 years	-0.06	-0.03	0.33	-0.02	-0.03	-0.08	-0.13	0.55	0.58	-0.03	-0.31	0.14	-0.56		**0.00**
Basal Area Index	-0.08	-0.02	0.28	0.02	-0.02	-0.04	0.04	0.61	0.64	-0.06	-0.35	0.07	-0.55	0.97	

Table S.2: Pearson correlation values for predictor variables in elevation study

Pearson correlation values (bottom half of table), as well as the degrees of significance (p-value) for predictor variables used in the elevation experiment. The darker the blue, the closer the r value is to either -1 or 1. Bolded p-values indicate significance at α=0.05.

Predictor Variables		Coefficient of Variation (%)	
		Elevation	Latitude
Site Physical	slope degree	86.9	10.0
	elevation	17.7	17.6
	slope aspect (ºN)	16.6	13.7
	latitude (degrees ºN)	0.5	3.2
	longitude (degrees W)	0.2	1.3
Site Growth	stand density (trees/ha)	83.1	108.1
	Basal Area Index (BAI)	90.0	70.0
	stand basal area (m^2/ha)	38.7	35.9
Tree growth	10 year radial growth (mm)	88.3	70.1
	tree age	54.7	38.3
	phloem thickness (mm)	42.1	27.3
	phloem fresh weight (mg)	38.1	27.9
	height (ft)	21.2	18.7
	Diameter (DBH in cm)	14.0	9.3

Table S.3: Predictor variable coefficients of variation for elevation and latitude studies

Coefficients of variation expressed as percentages for the predictor variables of both the elevation and latitude experiments. Note that for a given row, the cell coloured orange is the cell with the higher coefficient of variation. Overall, elevation study had higher degree of variability for most of the predictor variables.

Group	Response Variables (µg/mg)	Coefficient of Variation (%) Elevation	Latitude
Monoterpenes	bornyl acetate	195.4	171.0
	(−)-limonene	178.5	183.0
	(−)-β-pinene	137.4	112.2
	(+)-3-carene	103.8	137.1
	(+) -α-pinene	131.2	118.0
	p-cymene	125.1	117.2
	terpinolene	101.6	120.2
	thujone	118.9	80.8
	4-allylanisole	112.9	105.9
	(+)-limonene	108.4	101.9
	(+/−)-camphor	105.7	75.7
	myrcene	105.0	73.6
	(+)-camphene	93.5	104.8
	(−)-α-pinene	85.2	95.8
	γ-terpinene	65.3	94.3
	β-phellandrene	70.0	79.6
	total	68.6	59.1
Diterpene resin acids	sandaracopimaric acid	222.6	192.5
	dehydroabietic acid	123.0	73.7
	neoabietic acid	90.7	62.7
	levopimaric acid	89.1	61.3
	total	75.2	58.5
	abietic acid	72.7	59.2
	palustric acid	69.9	61.5
Non-Structural Carbohydrates	total starches	48.7	60.7
	glucose	34.5	39.6
	sucrose	19.6	23.3
	total sugars	18.5	23.2
	fructose	21.9	23.2

Table S.4: Response variable coefficients of variation for elevation and latitude studies
Coefficients of variation expressed as percentages for the response variables of both the elevation and latitude experiments. Note that for a given row, the cell coloured orange is the cell with the higher coefficient of variation. Overall, elevation study had higher degree of variability for most of the response variables, except for the non-structural carbohydrates, which had higher coefficients of variation in the latitude study.

		1. Elevation		2. Latitude	
		r	p	r	p
Site Physical	slope degree	-0.17	0.166	-0.09	0.438
	Elevation (m)			-0.76	**0.000**
	slope aspect (°N)	0.28	**0.018**	0.16	0.202
	latitude (degrees N)	-0.51	**0.000**		
	longitude (degrees W)	-0.49	**0.000**	-0.91	**0.000**
Site Growth	stand density (trees/ha)	-0.31	**0.010**	0.14	0.263
	basal area index BAI	0.02	0.863	-0.33	**0.005**
	stand basal area (m^2/ha)	-0.19	0.125	-0.04	0.757
Tree growth	10-year radial growth (mm)	-0.02	0.900	-0.33	**0.005**
	tree age (yr)	0.18	0.147	0.09	0.480
	phloem thickness (mm)	0.52	**0.000**	-0.23	0.053
	phloem fresh weight (mg)	0.44	**0.000**	-0.21	0.080
	height (ft)	-0.48	**0.000**	-0.65	**0.000**
	diameter at breast height DBH (cm)	0.10	0.399	-0.02	0.860
Climate AB v3.2.1	mean annual temperature MAT (°C)	-0.99	**0.000**	-0.93	**0.000**
	mean warmest month temperature MWMT (°C)	-0.99	**0.000**	0.02	0.860
	mean coldest month temperature MCMT (°C)	-0.42	**0.000**	-0.96	**0.000**
	temperature difference between MWMT and MCMT, or continentality (°C)	-0.97	**0.000**	0.94	**0.000**
	mean annual precipitation MAP (mm)	0.90	**0.000**	-0.80	**0.000**
	mean annual summer (May to Sept.) precipitation (mm)	0.16	0.178	-0.84	**0.000**
	annual heat:moisture index (MAT+10)/(MAP/1000))	-0.96	**0.000**	-0.27	**0.025**
	summer heat:moisture index ((MWMT)/(MSP/1000))	-0.86	**0.000**	0.78	**0.000**
	degree-days below 0°C, chilling degree-days	0.96	**0.000**	0.95	**0.000**
	degree-days above 5°C, growing degree-days DD>5	-0.99	**0.000**	-0.29	**0.016**
	the Julian date on which DD>5 reaches 100, the date of budburst for most plants	1.00	**0.000**	0.28	**0.018**
	degree-days below 18°C, heating degree-days	0.99	**0.000**	0.93	**0.000**
	degree-days above 18°C, cooling degree-days	-0.86	**0.000**	0.06	0.630
	the number of frost-free days	-0.93	**0.000**	-0.37	**0.002**
	frost-free period FFP	-0.91	**0.000**	0.13	0.293
	the Julian date on which FFP begins	0.95	**0.000**	-0.25	0.598
	the Julian date on which FFP ends	-0.81	**0.000**	-0.04	0.764
	precipitation as snow (mm)	0.94	**0.000**	0.73	**0.000**
	extreme minimum temperature over 30 years	0.47	**0.000**	-0.90	**0.000**

Table S.5: Correlation values between climate change proxy gradients and predictor variables
Pearson correlation values as well as the degrees of significance for climate change proxy variable and all other variables in respective study. Bolded p values are significant at α=0.05. Darker coloured r-value cells are closer to either 1 or -1. Climate variables determined from ClimateAB v3.21 (Wang et al. 2008) based on annual climate data from 1991-2000. Note that results presented herein are consistent with tables 2.1 and 2.2.

		1. Elevation		2. Latitude	
		r	p	r	p
Site Physical	slope degree	0.17	0.363	0.20	0.289
	elevation (m)	0.98	**0.001**	0.72	**0.001**
	slope aspect (°N)	0.27	0.088	0.25	0.121
	date	0.12	0.670	0.80	**0.001**
	latitude (degrees N)	0.56	**0.001**	0.94	**0.001**
	longitude (degrees W)	0.45	**0.002**	0.87	**0.001**
Site Growth	stand density (trees/ha)	0.95	**0.001**	0.96	**0.001**
	basal area index BAI	0.21	0.211	0.47	**0.001**
	stand basal area (m^2/ha)	0.42	**0.001**	0.41	**0.002**
Tree growth	10-year radial growth (mm)	0.26	0.109	0.46	**0.001**
	tree age (yr)	0.26	0.099	0.32	**0.033**
	phloem thickness (mm)	0.61	**0.001**	0.54	**0.001**
	phloem fresh weight (mg)	0.58	**0.001**	0.61	**0.001**
	height (ft)	0.47	**0.001**	0.61	**0.001**
	diameter at breast height DBH (cm)	0.40	**0.003**	0.12	0.616
Climate AB v3.2.1	mean annual temperature MAT (°C)	0.98	**0.001**	0.95	**0.001**
	mean warmest month temperature MWMT (°C)	0.98	**0.001**	0.25	0.137
	mean coldest month temperature MCMT (°C)	0.47	**0.001**	0.97	**0.001**
	temperature difference between MWMT and MCMT, or continentality (°C)	0.94	**0.001**	0.95	**0.001**
	mean annual precipitation MAP (mm)	0.90	**0.001**	0.83	**0.001**
	mean annual summer (May to Sept.) precipitation (mm)	0.18	0.305	0.86	**0.001**
	annual heat:moisture index (MAT+10)/(MAP/1000))	0.96	**0.001**	0.31	**0.030**
	summer heat:moisture index ((MWMT)/(MSP/1000))	0.86	**0.001**	0.79	**0.001**
	degree-days below 0°C, chilling degree-days	0.96	**0.001**	0.97	**0.001**
	degree-days above 5°C, growing degree-days DD>5	0.97	**0.001**	0.39	**0.008**
	the Julian date on which DD>5 reaches 100, the date of budburst for most plants	0.98	**0.001**	0.39	**0.006**
	degree-days below 18°C, heating degree-days	0.98	**0.001**	0.95	**0.001**
	degree-days above 18°C, cooling degree-days	0.85	**0.001**	0.22	0.198
	the number of frost-free days	0.91	**0.001**	0.45	**0.002**
	frost-free period FFP	0.90	**0.001**	0.07	0.853
	the Julian date on which FFP begins	0.93	**0.001**	0.16	0.408
	the Julian date on which FFP ends	0.80	**0.001**	0.09	0.749
	precipitation as snow (mm)	0.94	**0.001**	0.73	**0.001**
	extreme minimum temperature over 30 years	0.44	**0.002**	0.94	**0.001**

Table S.6: Correlation values for predictor variables in both studies in PCoA ordinations

Pearson correlation values as well as the degrees of significance for all variables used in the distance matrices of PCoA ordinations. Bolded p values are significant at α=0.05. Darker coloured r-value cells are closer to either 1 or -1. Climate variables determined from ClimateAB v3.21 (Wang et al. 2008) based on annual climate data from 1991-2000

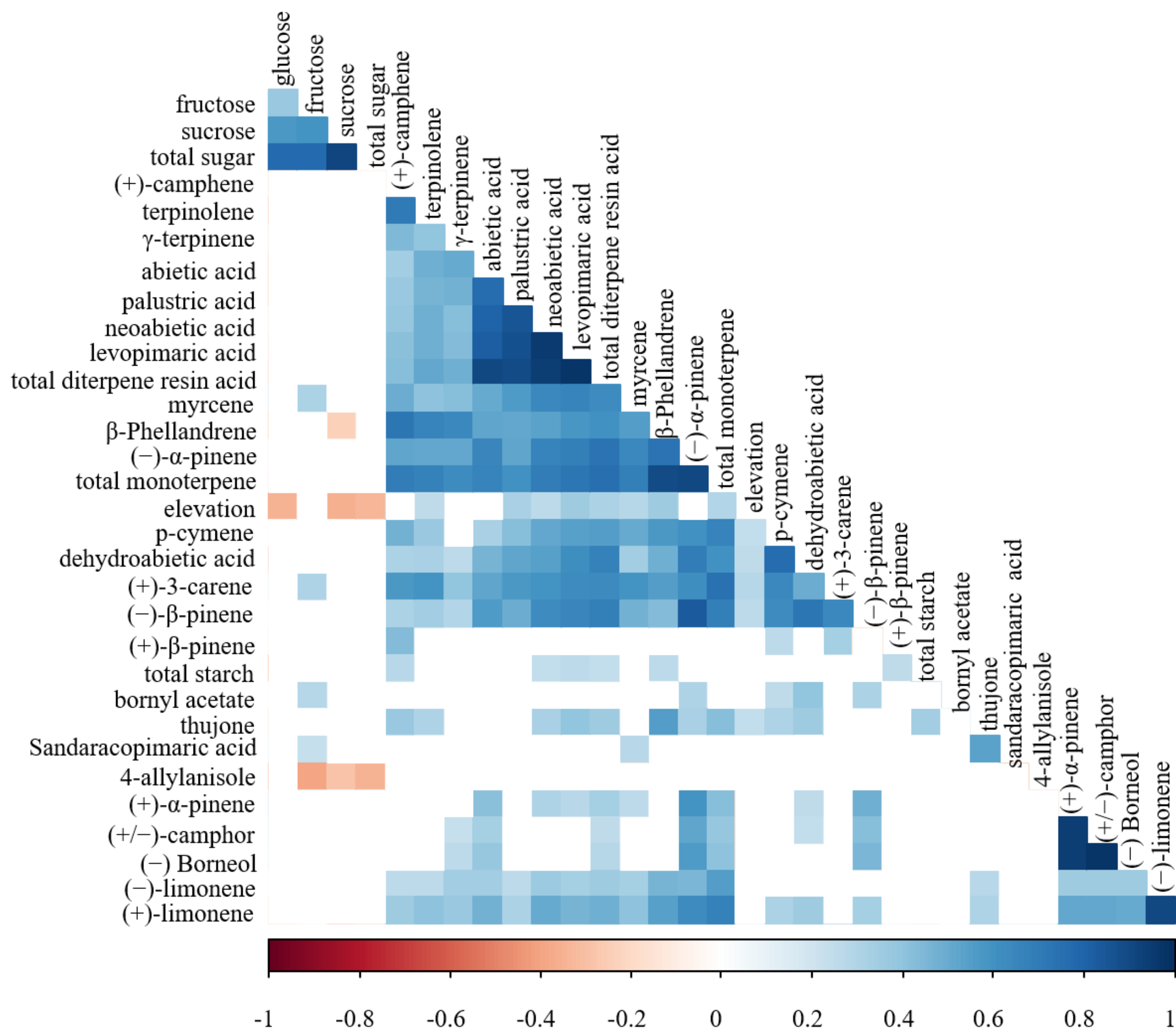

Table S.7: Correlation values for response variables in elevation study PCoA ordinations

Significant Pearson correlation values for the value of raw elevation and all response variables of the elevation study. Table is reordered according to correlation coefficient. White cells are where correlation p>0.05 (insignificant)

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Appendix 1: How to report monoterpenes in terms of dry weight

Monoterpene extractions were performed on fresh tissues. However, diterpene resin acid, sugar, and starch extractions were all performed on dry tissue. Thus, in order to compare between these four groups of compounds, I wanted all compounds to be standardized on the same measure: dry tissue. As such, a conversion was performed in order to standardize monoterpenes by dry weight. This conversion was performed as follows:

W1: Sample fresh weight used for extractions

W3: Sample fresh weight used for modelling dry weight

W6: Sample weight dry batch 1

The data from the 130 sites was used in a linear regression of (W3~W6). The objective was test if it would be appropriate to use one conversion factor for all sites, to predict dry weight from fresh weight. However, the model failed to meet the assumptions of linearity. In addition, when the model was run, the adjusted R-square value was only 0.25. It was deemed overall inappropriate to use only one conversion factor for all sites.

If the differences from fresh to dry weight across sites was so vast that an adjusted R squared value could not come out to greater than 0.25, then it must be the case that the difference in water content of the phloem of sampled trees was important to account for in the dataset. In order to account for this difference, a conversion factor was calculated for each site, and these values were used to standardize monoterpene results. These conversion factors were calculated for each site as follows:

$$(\text{eq } 1) = (W1) / (W6/W3)$$

The author recognizes that the statistical significance of these conversion factors would have been increased, if more than one sample was used to model the fresh-to-dry weight relationship. In the case of the current study, there was insufficient fresh tissue with which to acquire more data points for the model. Future students may which to set aside a greater amount of fresh tissue, with which to more accurately model the relationship between fresh and dry tissues.

Appendix 2: How to calculate concentrations of soluble sugars, and starches

Presented below are the equations used to quantify soluble sugar concentration, as well as starch concentration.

SU_F	Quantified fructose in μg /mL from sugar extracts
SU_G	Quantified glucose in μg/mL from sugar extracts
SU_S	Quantified sucrose in μg/mL from sugar extracts
SU_Extr	Extraction volume used for sugar extraction
SU_m	Phloem dry weight used in from sugar extraction
D_1	Dilution factor resulting from methanol treatment (D_1=3.5)

Soluble sugars in μg/mg

(eq 1) *=(((SU_F)*(SU_Extr)*(D_1))/(SU_m)) * (compound purity of fructose)*

(eq 2) *=(((SU_G)*(SU_Extr)*(D_1))/(SU_m)) * (compound purity of glucose)*

(eq 3) *=(((SU_S)*(SU_Extr)*(D_1))/(SU_m)) * (compound purity of sucrose)*

Total glucose (hexoses and from starch) concentration in μg /mL

(eq 4) *=1135 * (absorbance at 340 nm)*

Total glucose (hexoses and from starch) concentration (in μg/mg)

(eq 5) *=(eq 4) / (total dilutions accounting for all aliquots + enzymatic reactions)*

Total soluble sugars to be subtracted in μg/mL

(eq 6) *=(eq 1) + (eq 2)*

Hexoses from starch in μg/mL

(eq 7) *=(eq 4) – ((eq 6)* 1.6)*

Starch concentration in μg/mL

(eq 8) *=(eq 7)* 0.746*

Final starch values used in analysis in μg/mg

(eq 9) *=(eq 8) / 0.92 / (total dilutions accounting for all aliquots + enzymatic reactions)*