PLANT FATTY ACIDS INFLUENCE BROOD DEVELOPMENT OF MOUNTAIN PINE BEETLE AND GROWTH OF ITS SYMBIOTIC FUNGUS: IMPLICATIONS TO HOST-RANGE EXPANSION OF AN HERBIVOROUS INSECT

by

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ABSTRACT

Nutritional composition of plants can affect the performance of insect herbivores and their associated microbial symbionts. Mountain pine beetle (*Dendroctonus ponderosae*) is an important bark beetle species that colonizes many species of *Pinus* within its historical range and encounter host species with different nutritional compositions. This insect has recently expanded its host range to jack pine (*P. banksiana*) forests, which is considered a novel host in terms of encounters with *D. ponderosae*. The nutritional aspects of selecting this new host are largely unknown. Here, I tested whether the recent host of *D. ponderosae* contains similar fatty acid concentrations as the beetle's historical hosts and whether such similarity influenced the host expansion of *D. ponderosae*. I demonstrated that historical (lodgepole pine, *P. contorta*) and novel hosts are distinguished from a non-host (*Populus tremuloides*) species of *D. ponderosae* by concentrations of phloem fatty acids, such as linoleic and alpha-linolenic acids. Furthermore, the resulting information provides new insights into the biological roles of plant fatty acids in the survival of *D. ponderosae* larvae and establishment of the beetle's symbiotic fungus, *Grosmannia clavigera*.

PREFACE

Fatty acids vary in terrestrial plants (Maguire *et al*. 2004; Habila *et al*. 2012) and might differently impact insect herbivores (Scheffrahn & Rust 1982; Mansson *et al*. 2005, 2006; Castillo *et al*. 2010) and their symbiotic microorganisms (Walters *et al*. 2004; Goodsman *et al*. 2012). Bark beetle-fungal symbiotic relationships indicate that beetles consume fungal hyphae to gain nutrition (Bentz & Six 2006; Bleiker & Six 2007) and are thought to be vital in helping beetles to overcome the tree defenses (Raffa & Berryman 1982). In turn, the beetles help with transferring the pathogen to a new host (Safranyik & Carroll 2006). Mountain pine beetle (*Dendroctonus ponderosae*), is a highly successful insect herbivore in pine forests (Cole & Amman 1980; Hiratsuka *et al*. 1981; McCambridge *et al*. 1982; Logan & Powell 2001; Lombardero *et al*. 2006; Wermelinger *et al*. 2008), and has a mutual relationship with the blue stain fungus, *Grosmannia clavigera* (Safranyik & Carroll 2006).

Fatty acids are abundant components of plants and might play an important role in plants (Smith 1998). Plant fatty acids play roles in different aspects of its physiology, for example during growth and development (Saranpää & Nyberg 1987), harsh weather conditions (Angelcheva *et al*. 2014), and under pathogen and insect attacks. However, there has been surprisingly little research on fatty acid composition of woody plants in North America that might impact the host range expansion of herbivorous insects, including *D. ponderosae*.

Most insect herbivores are not able to synthesize essential fatty acids and must acquire them from host plants (Dadd 1981; Otte *et al*. 2015). Fatty acids play key roles in different aspects of insect life cycle, including growth (Dadd 1985; Khani *et al*. 2007; and etc.), pheromone production (Vanderwel *et al*. 1992; Blomquist *et al*. 2010), and flight dispersal (Hodges & Barras 1974; Erbilgin *et al*. 2014; Evenden *et al*. 2014). Furthermore, insect symbiotic

microorganisms also play roles in detoxifying (DiGuistini *et al*. 2007) and digesting nutrients (Bentz & Six 2006; Goodsman *et al*. 2012), including fatty acids, for the beetle which are otherwise unattainable from host plants. A number of studies have explored how climate change and host secondary metabolites facilitated *D. ponderosae* to move northward and eastward in Canada and invade new host species, jack pine (*Pinus bansiakana*). However, few studies have focused on the role of host primary compounds in the host expansion of *D. ponderosae*.

In this thesis, I examine: (1) whether novel hosts with similar fatty acid composition as historical hosts are suitable to *D. ponderosae*; and (2) the effects of fatty acids on beetle larval survival and growth of its symbiotic fungi. Chapter 1 focuses on a literature review of fatty acids and their role in plants and insect herbivores; chapter 2 reports on the main findings of the experimental work. Chapter 3 discusses the research findings. Chapter 4 is an appendix with chromatogram analyses.

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LIST OF ABBREVIATIONS:

Fatty acids of saturated and unsaturated group:

Behenic acid - BA

Stearic acid - SA

Palmitic acid - PA

Levulinic acid - LvA

Pentadecanoic acid – PDA

Oleic acid - OA

Linoleic acid - LA

Alpha-linolenic acid - ALA

Gamma-linolenic acid - GLA

Arachidonic acid - ARA

Eicosadienoic acid – EDA

Gas Chromatography-Mass Spectrometry - GC/MS

GC-Flame Ionization Detector - GC/FID

Fatty acid methyl ester - FAME

Nonmetric multidimensional scale – NMDS

Canonical discriminant analysis - CANDISC

CHAPTER 1

INTRODUCTION

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionadae, Scolytinae) is the most destructive insect pest of lodgepole pine (*Pinus contorta*) forests in western North America (Safranyik *et al*. 2010). During the last decade, the range of beetles has expanded from lodgepole pine-dominated forests to lodgepole pine x jack pine hybrid forests in western Alberta and, more recently, it has invaded jack pine (*Pinus banksiana*) forests of the boreal system (Cullingham *et al*. 2011). Shifts in temperature from historically cold to relatively warmer regimes have promoted the invasion by influencing reproduction, development rate, and winter survival of beetles (Safranyik & Carroll 2006). In my MSc thesis, I investigated the impact of host nutrients, particularly fatty acids, on the biology of the mountain pine beetle and its associated fungus. I also determined whether the observed successful colonization of jack pine trees by beetles might be due to a similarity in fatty acid composition between the beetle's historical host, lodgepole pine and its novel host, jack pine.

Beetle biology

Mountain pine beetle completes one generation (egg, larva, pupa, and adult) in a year in western Canada (Safranyik *et al.* 2010). Pretty much all life stages occur underneath the bark of host trees, except when juveniles disperse to seek new suitable host trees. Female beetles initiate new attacks on trees sometime early in the summer (July-August) in Alberta, and release a highly potent aggregation pheromone, which attracts both sexes. Arriving males, release another aggregation pheromone to initiate mass attacks. If attacks successfully overcome tree defenses,

female beetles excavate galleries where they lay about 60-80 eggs per gallery (Safranyik *et al.* 2010). During this process, beetles introduce their symbiotic fungi, such as *Grosmannia clavigera*, into the host. Eggs hatch is followed by larval feeding. Larvae develop through four instars, construct galleries as they feed on phloem and fungal hyphae, and overwinter as late instars. Larval stage is the most active feeding stage, and thus at this stage host quality has the maximum impact on beetle biology and ecology. Pupation occurs in the spring, followed by development into teneral (sexually immature) adults. Teneral adults also feed on fungal spores prior to emerging from their hosts. Finally, beetles emerge from their natal hosts and seek a suitable tree to start a new colonization process (Safranyik & Carroll 2006).

Bark beetles are associated with a diversity of symbionts, including fungi, bacteria, mites, and nematodes (Bleiker & Six 2007; Zilber-Rosenberg & Rosenberg 2008; Cardoza *et al*. 2009; Hofstetter *et al*. 2014). Symbiotic fungi facilitate beetle nutrition, either directly by serving as a food source (Adams & Six 2007), or indirectly by digesting and concentrating host tree nutrients (Ayres *et al*. 2000; Bleiker & Six 2007; Goodsman *et al*. 2012). In addition, sterol produced by the mountain pine beetle symbiont *G. clavigera* may contribute to the beetle's cold-hardiness and survival at high elevations (Bentz & Six 2006). This fungus has also been shown to metabolize terpenes, suggesting that it may contribute to detoxifying tree defenses (DiGuistini *et al*. 2007).

Mountain pine beetle attacks most *Pinus* species in its natural range, including lodgepole pine (*Pinus contorta*) (Cole & Amman 1980), limber pine (*P. flexilis*) (Hiratsuka *et al*. 1981), ponderosa pine (*P. ponderosa*) (McCambridge *et al*. 1982), and whitebark pine (*P. abicaulis*) (Logan & Powell 2001). As beetles spread to jack pine boreal forests, Scots pine (*P. sylvestris*) (Wermelinger *et al*. 2008) and red pine (*P. resinosa*) (Lombardero *et al*. 2006) might also become potential hosts to the beetles. Mountain pine beetle also occasionally attacks species

other than pine, such as spruce, *Picea* (Huber *et al*. 2009). For example, earlier studies demonstrated that the mountain pine beetle can reproduce on white spruce (*Picea glauca*) (Unger 1993; Huber *et al*. 2009), which usually occur in mixed stands with lodgepole pine trees.

The role of fatty acids in plants

Fatty acids of lipids play essential roles in different physiological processes in plants although information about the functions of fatty acids in woody perennial plants is limited. For example, Angelcheva *et al*. (2014) reported increased concentrations of unsaturated fatty acids in the needles of Siberian spruce (*Picea obovata*) during winter months. Apparently, unsaturated fatty acids are used to maintain the fluidity and permeability in the membranes cell of needles in response to cold winter temperatures. Likewise, other studies provided similar results reporting that the ratio of unsaturated to saturated fatty acids increased under the low temperature conditions in other plant systems (Senser 1982; Öquist *et al*. 2001). Furthermore, changes in fatty acid compositions in sapwood and heartwood of Scots pine (*P. sylvestris*) were associated with heartwood development (Saranpää & Nyberg 1987). A number of studies have demonstrated the effects of fatty acids on plant physiology, including seasonal phenology (Fischer & Höll 1991), embryo development (Grigova *et al.* 2007), heartwood formation (Saranpää & Nyberg 1987), and stress responses following interactions between pathogens and insect attacks (Weber 2002). Recent studies demonstrated the following valuable information: host tree diameter is positively correlated with body size and lipid content of mountain pine beetle (Graf *et al*. 2012); crude fat content changes in lodgepole pine needles in different beetle-attack categories (unattacked, attacked, and red) (Jolly *et al*. 2012); and fungus inoculation negatively influences to the concentration of certain fatty acids in the sapwood of lodgepole pine

(Gao *et al*. 1994). Although this information, role of fatty acids in plant physiology, provides us with deep insights; the role of plant fatty acids, in mountain pine beetle brood survival and its symbiotic fungal growth is unclear.

The role of plant fatty acids in insect biology

Insects require specific fatty acids, called essential fatty acids, from their host plants (Dadd 1981; Otte *et al*. 2015) as most insects are not able to synthesize some of these essential fatty acids (Stanley-Samuelson *et al*. 1988). For example, some insects require unsaturated fatty acids such as linoleic (LA), alpha-linolenic (ALA), or arachidonic (ARA) acids from their diet normal growth (Dadd 1985; de Renobales *et al*. 1987). Likewise Khani *et al*. (2007) and others (Lambremont *et al*. 1964; Harwood & Takata 1964; Schaefer & Washino 1969; Valder *et al*. 1969) provided evidence of an increase in the ratio of unsaturated to saturated fatty acids in overwintering larvae during hibernation, suggesting that insects accumulate certain fatty acids to overcome the harsh winter conditions. Furthermore, a number of studies suggested that fatty acid precursors are crucial in pheromone production by insects (Stanley-Samuelson *et al*. 1988; Vanderwel *et al*. 1992; Blomquist *et al*. 2010). Finally, fatty acids as energy resource also take part in dispersal of insects to select and colonize their new hosts (Hodges & Barras 1974). Erbilgin *et al*. (2014) and Evenden *et al.* (2014) suggest that lipids play a major role during beetle flight, as lipid content of beetle is directly reduced with flight distance.

Thesis outline

In my thesis, I focus on the nutritional component of several tree species, lodgepole pine, limber pine, ponderosa pine, whitebark pine, jack pine, Scots pine, red pine, white spruce, and aspen

because: (1) nutritional quality of host trees can affect different aspects of beetle biology and ecology, and therefore should be integrated into models incorporating natural enemies, weather, species composition, and tree defenses; (2) host and range expansion of mountain pine beetle represents a particularly relevant biological invasion, in that it comprises a bridge into a new biome, and signifies an unprecedented climate change-induced epidemic; and (3) an understanding of host tree-beetle-fungus interactions will improve our ability to better understand the biology of the beetle invasion, which is the most important insect affecting North American coniferous forests.

I investigate fatty acids because fatty acid content varies among different plant species and only a few studies have ever investigated the role of plant fatty acids in herbivorous insects. In my thesis, I examine whether fatty acids play any role in the host expansion of the mountain pine beetle into the novel jack pine forests in western Canada. Although fatty acids can mediate insect-plant interactions (Stanley-Samuelson & Dadd 1984; Stanley-Samuelson 1994; Reddy & Guerrero 2004) and their compositions vary among plant species (Maguire *et al*. 2004; Habila *et al*. 2012), whether they also influence host selection by herbivorous insects in novel habitats is largely unknown. I hypothesize that fatty acid profiles and concentrations vary among perennial woody plant species and similarity of fatty acid compositions between historical hosts in the beetle's native range and the novel host, jack pine, has allowed beetles to expand their host range to jack pine forests. The following specific predictions were tested: (1) fatty acid profiles and concentrations are different among different tree species; (2) total fatty acid concentrations vary within tree species; (3) mountain pine beetle growth and the activity of the beetle's symbiotic fungus, *G. clavigera*, are differentially affected by the fatty acid concentrations of lodgepole pine, jack pine, and aspen tree species.

In the second chapter, my objectives were to: (1) characterize and compare fatty acid compositions among tree species, including lodgepole pine, limber pine, ponderosa pine, whitebark pine, jack pine, Scots pine, red pine, white spruce, and aspen; (2) determine whether different concentrations of fatty acids from lodgepole pine, jack pine, and aspen affect mountain pine beetle development and its associated fungal activity. Phloem samples from nine tree species were collected across Canada (British Columbia, Alberta, Northwest Territories, Manitoba, and Ontario) and the USA (Montana). In the laboratory, the dried ground samples were analyzed using gas chromatography/flame ionization detection after a fatty acid extraction process. I identified fatty acid profiles and major fatty acids from these tree species were quantified. Furthermore, using species-specific fatty acid concentrations, I examined larval survival and fungal growth under different concentrations of fatty acids of lodgepole pine, jack pine, and aspen.

CHAPTER 2: Fatty acid composition of tree species and their role on the growth and development of mountain pine beetle (*Dendroctonus ponderosae*) and its associated fungi

INTRODUCTION

Herbivorous insects depend on the nutritional composition of their host plants and have developed several mechanisms to consume plant resources (e.g. nitrogen, proteins, water, minerals, and lipids) and tolerate plant defenses (Slansky & Scriber 1985; Futuyma & Levy 2001; Scriber 2010). Insect herbivore-host plant interactions in novel habitats are particularly interesting as the herbivore's ability to reproduce and invade may depend on host plant quality, characterized by primary (e.g. availability of nitrogen and carbon-based compounds, such as carbohydrates and lipids) and secondary (e.g., defensive compounds) compounds of naïve plants (Awmack & Leather 2002; Futuyma 2008; Ammunét *et al.* 2011). Although herbivores are not always successful in exploiting new host species (Lieutier 2006; Bertheau *et al.* 2010), they may successfully utilize novel plants and exploit them more effectively than the host species with which they have co-evolved (e.g., Mooney & Cleland 2001; Walther *et al.* 2009).

Several mechanisms have been suggested to explain the role of plant chemistry in host expansion of insect herbivores into novel habitats. Although these mechanisms are not mutually exclusive, they emphasize the importance of similarities in secondary chemical profiles, predicting that novel plants are more suitable hosts for colonizing insects if their chemical profiles are similar to those of historical host plants. Several studies have provided empirical evidence in support of these hypotheses (Mitter *et al.* 1991; Feeny 1995; Becerra 1997; Berenbaum 2001; Murphy & Feeny 2006; Erbilgin *et al.* 2014). In all cases, however, a mechanism revolves around insect responses to host secondary compounds, without explicit

reference to plant primary compounds despite their importance to the biology and ecology of insect herbivores (Awmack & Leather 2002; Joern *et al.* 2012; Roeder & Behmer 2014).

Here, I investigated the role of plant fatty acids in the host suitability for acceptance and colonization by bark beetle, mountain pine beetle (*Dendroctonus ponderosae*) (Coleoptera: Curculionidae, Scolytinae) of the novel jack pine (*Pinus banksiana*) forests in western Canada. Although fatty acids can be important in mediating insect-plant interactions (Stanley-Samuelson & Dadd 1984; Stanley-Samuelson 1994; Reddy & Guerrero 2004) and their composition varies among plant species (Maguire *et al.* 2004; Habila *et al.* 2012), their influence on host suitability and colonization by herbivorous insects in novel habitats is largely unknown.

Fatty acids in plants

Fatty acids are components of lipids primarily found in cell membranes (Senser 1982). Plants synthesize many fatty acid derivatives, notably jasmonic acids, which are biosynthesized from ALA precursor via the octadecanoid pathway (Blee 1998; Weber 2002). Jasmonic acid and its various metabolites regulate plant responses to abiotic and biotic stress (Pare & Tumlinson 1999; Weber 2002; Erbilgin *et al.* 2006) in addition to plant growth and development (Delker *et al.* 2006). Fatty acids are classified either as saturated or unsaturated, with each class performing different functional roles in plants (Senser 1982; Kodama *et al*. 1995; Murakami *et al*. 2000; Öquist *et al.* 2001; Khodakovskaya *et al*. 2006; Dominguez *et al*. 2010). For example, the unsaturated fatty acids, in particular LA, ALA, AGA, gamma-linolenic (GLA), and oleic (OA) acids in needles of Siberian spruce (*Picea obovata*) accumulated to maintain membrane fluidity during acclimation (Angelcheva *et al*. 2014). This suggests a potential role of fatty acids in sustaining plant performance across a range of environmental conditions.

Despite their importance, few studies have investigated the roles of fatty acids in trees. Saranpää & Nyberg (1987) compared fatty acids in the sapwood and heartwood of Scots pine (*Pinus sylvestris*) and reported changes in concentrations of individual fatty acids during heartwood formation. Likewise, studies have reported seasonal variation of fatty acids in pine (DeYoe & Brown 1979), spruce (Oequist 1982; Kyburz *et al*. 1991), and birch foliage (Martz *et al*. 2006). More recently, Gao *et al.* (1994) observed reduced fatty acid concentrations in lodgepole pine *Pinus contorta* sapwood after inoculation with the bark beetle – associated fungus *Ophiostoma piceae*, suggesting either tree utilization of some fatty acids in the production of fatty acid derivatives, such as jasmonic acid, or consumption of fatty acids by the fungus. Notably, Jolly *et al.* (2012) showed changes in crude lipid content of needles of *P. contorta* under different beetle attack categories (unattacked, 1-2 years, and 3-4 years – post attack), suggesting a possible allocation of lipids within the plant.

Roles of fatty acids in insect biology and ecology

Insect herbivores must acquire essential fatty acids from their diets (Dadd 1981; Stanley-Samuelson *et al.* 1988). In general, these fatty acids are critical for insect growth (Dadd 1985; de Renobales *et al.* 1987; Khani *et al.* 2007), pheromone production (Stanley-Samuelson *et al.* 1988; Vanderwel *et al.* 1992; Blomquist *et al.* 2010), and long distance dispersal (Hodges & Barras 1974). For example, most lepidopterans (butterflies and moths) acquire LA or ALA from plants to support their development (de Renobales *et al*. 1987). Likewise, the proportion of unsaturated to saturated fatty acids increased in overwintering larvae (Lambremont *et al.* 1964; Schaefer & Washino 1969). Interestingly, plant-derived LA, which is important in acclimating

Siberian spruce (Angelcheva *et al.* 2014), helps overwintering codling moth (*Cydia pomonella*) larvae maintain cellular membrane fluidity (Khani *et al.* 2007).

Fatty acids can also be important for insect pheromone production (Rule & Roelofs 1989; Blomquist *et al.* 2010). For example, from Arctiidae family, *Estigmene acrea* and *Phragmatobia fulinosa*, synthesize sex pheromones from ALA (Rule & Roelofs 1989). Notably, 1-octen-3-ol, which is synthesized from LA (Tressl *et al.* 1982) and is a volatile produced by the fungus *Aspergillus flavus* (Kaminski *et al.* 1972), can be repellent to a number of bark beetle species (Pureswaran & Borden 2004).

Furthermore, fatty acids are used in energy metabolism during insect dispersal flights (Atkins 1969). In fact, lipid content below a certain threshold inhibits any flight (Atkins 1966). Interestingly, concentrations of certain fatty acids vary by insect life history stage. For example, Hodges & Barras (1974) examined concentrations of several fatty acids between recently emerged southern pine beetles (*Dendroctonus frontalis*) and beetles that recently attacked trees and found that concentrations of LA and OA were higher in the latter group.

Mountain pine beetle biology, Dendroctonus ponderosae

Dendroctonus ponderosae is a native mortality agent of conifer forests of western North America (Safranyik *et al.* 2010). When new brood beetles emerge from hosts in which they developed and overwintered, they disperse and seek new host plants during summer. Female beetles enter a suitable host and release aggregation pheromones that attract conspecifics of both sexes. Following successful host colonization and mating, females excavate and oviposit eggs in maternal galleries. During this process, beetles introduce several microorganisms into the host tree, including the symbiotic fungus *Grosmannia clavigera* (Bleiker & Six 2007). Egg hatch is

followed by larval feeding. Larvae develop through four instars, construct galleries as they feed on phloem and symbiotic fungal hyphae, and overwinter as late instar larvae. Pupation occurs in the spring, followed by development into adults in early summer. There is commonly one generation per year.

This beetle colonizes many species of *Pinus* within its native range including lodgepole, ponderosa (*Pinus ponderosa*), whitebark (*P. albicaulis*), and limber (*P. flexilis*) pines (Wood 1982). Recent climatic shifts have facilitated a *D. ponderosae* outbreak of unprecedented scale in lodgepole pine forests of British Columbia (Cudmore *et al*. 2010). Rising temperatures have also allowed this insect to invade jack pine (*P. banksiana*) forests of northern Alberta (Erbilgin *et al*. 2014). Jack pine is a major component of the boreal forest, and extends from the Northwest Territories and Alberta to the Atlantic Provinces in eastern Canada, eventually overlapping with red pine (*P. resinosa*) in the Great Lakes and northeastern regions of the United States. Jack pine is considered a naïve host of *D. ponderosae* due to a lack of historical association between the two organisms.

In general, studies have focused on lipids in different life history stages of *D. ponderosae*, without any explicit reference to fatty acids. There is a reduction in beetle lipid content after flight, demonstrating the potential role of lipids in beetle dispersal (Erbilgin *et al*. 2014). Notably, consumption of lipid reserves differed between female and male beetles. Likewise, Evenden *et al*. (2014) reported a negative relationship between lipid content and flight distance of beetles on a flight mill. In addition, Graf *et al*. (2012) showed that the diameter of *P. contorta* is positively associated with body size and lipid content of beetles. The aggregation pheromone of male *D. ponderosae exo*-brevicomin is synthesized *de novo* likely from long chain-derived fatty acid precursors (Vanderwel *et al.* 1992; Blomquist *et al.* 2010).

Study goals

The goal of this study was to determine whether there is a similarity in the fatty acid composition between historical hosts and novel host, lodgepole pine and jack pine, respectively, allowed beetles to inhabit and colonize novel jack pine forests. This is the first study to evaluate the effects of fatty acids on the brood survival of any bark beetle species and growth of their symbiotic fungi. First, I characterized the fatty acid composition of beetle's historical (*P. contorta*, *P. flexilis*, *P. albicaulis*, *P. ponderosa*), potential (*P. banksiana*, *P. resinosa*, *P. sylvestris*) and occasional (*Picea glauca*) hosts and non-host (*Populus tremuloides*) tree species. *P. resinosa* and *P. sylvestris* were included as potential hosts because both species are commonly found in some portions of the jack pine-dominated boreal forest. Furthermore, some studies showed that *D. ponderosae* successfully reproduced in bolts of both (*P. contorta* and *P. banksiana*) species (Cerezke 1995). I selected *P. glauca* as an occasional host because *D. ponderosae* can produce broods in bolts of this species (Safranyik & Linton 1983). *Populus tremuloides* was selected as a non-host species because it commonly occurs in both the historical and expanded ranges of *D. ponderosae* in Canada and is never colonized by the beetle. I then tested the effects of fatty acids from *P. contorta*, *P. banksiana* and *P. tremuloides* on *D. ponderosae* brood development using a novel rearing technique consisting of tree phloem amended with fatty acid extracts and *G. clavigera*. The fungus was added to the nutrient-rich rearing medium inside a glass tube. Finally, I investigated the relationships between fatty acids and growth of *G. clavigera* in Petri dishes containing medium amended with lipid fractions and fatty acids of *P. contorta*, *P. banksiana* and *P. tremuloides*. Research objectives were to: (1) characterize the fatty acid composition of historical, potential, and occasional hosts as well as non-host tree species, and quantify major fatty acids present in these species; (2) determine the

relationship between fatty acid concentrations of *P. contorta*, *P. banksiana*, and *P. tremuloides* and brood development of *D. ponderosae* and growth of its symbiotic fungus, *G. clavigera*.

MATERIALS AND METHODS

2.1. Do fatty acid profiles and concentrations vary within and between trees?

Phloem sample collection

To determine fatty acid composition of nine tree species, *P. contorta*, *P. flexilis*, *P. albicaulis*, *P. ponderosa*, *P. banksiana*, *P. resinosa*, *P. sylvestris*, *P. glauca* and *P. tremuloides*, I collected phloem samples (5x5cm) from the stem of trees in July and August 2013. Sample locations are shown in Figure 5. Briefly, samples for *P. contorta* were collected from Alberta and southern and central British Columbia, *P. banksiana* from Alberta, Manitoba, Northwest Territories and Ontario, *P. resinosa* and *P. sylvestris* from Manitoba and Ontario, *P. flexilis*, *P. albicaulis*, *P. glauca*, and *P. tremuloides* from Alberta, and *P. ponderosa* from Montana (USA). Overall, we sampled 12 to 45 individual trees per species at each location with a total of 451 samples (see Supplementary Table 2.1.2 for details). In the field, samples were kept on dry ice and shipped to the University of Alberta where they were stored at -40°C until extraction and analysis.

Fatty acid extraction

All samples were freeze dried for 3 days (Labconco Corp. KS, USA), ground (Tissue Lyser II, QIAGEN Corp., Hilden, Germany) and stored at -40°C until extraction. I put 100 mg of dried ground sample into a 50 mL glass centrifuge tube for fatty acid extraction following the method described by Curtis *et al*. (2008). Briefly, ground samples were mixed with 1 mL of recovery

standard (M_{C19}), a 1 mg/g of trinonadecanoin solution in toluene (Nu-Chek Prep Inc., MN, USA) in the glass tube. A 2 mL of toluene and 6 mL of freshly prepared solution (5.4 mL methanol plus 0.6 mL acetyl chloride) were then added to the tube. This mixture was vortex mixed for 30 sec and placed into an 80°C water bath for 2 h. The tube was left to cool at room temperature for 15 min before being vortex mixed for another 30 sec, after which time 10 mL of 6 % sodium carbonate in deionized distilled water was added to the tube. To separate the toluene containing fatty acid methyl esters (FAMEs) from the mixture, the mixture was centrifuged at 1,100 rpm for 15 min and the separating organic layer was collected and transferred to 15 mL glass vial containing 1 g of sodium sulfate, which absorbed residual water in the solution. This solution was vortex mixed for 30 sec. I mixed 1 mL of the toluene extract with 0.2 mL of a standard solution (M_{C23}), containing 0.7 mg methyl tricosanoate (Nu-Chek Prep Inc.). The resulting solution was added to a 2 mL vial for chemical analyses.

Chemical analyses

Gas Chromatography-Mass Spectrometry (GC/MS) was used to identify FAMEs (see chromatogram analyses in the appendix). Additionally, to confirm GC/MS, a GC-Flame Ionization Detector (GC/FID) (Agilent 7890A, Agilent Tech., CA, USA) was used to identify fatty acid profiles and then quantify the amount of major fatty acids in each tree species tested using latter technique. A sample volume of 1 µL was injected in splitless mode into the GC equipped with a capillary column HP-80 (60 m length, 0.25 µm film and 250 µm I.D.) and with carrier gas of helium at 1.0 mL min^{-1} flow rate. The temperature program started at 75°C and increased at 40°C min^{-1} to 145°C, held for 2 min, then increased at 10°C min^{-1} to 205°C, held for 5 min, and then finally increased at 20°C min^{-1} to 250°C and held for 1 min.

The recovery coefficient was around 73% as per use of M_{C19} standard. Twenty-two FAMEs were identified from eight species (*P. ponderosa* was not included in the analysis) (see Supplementary Table 2.1.1 for the complete list of chemicals and an appendix). The most common eleven FAMEs in each tree species, around 95% of all fatty acid content (based on peak sizes of individual FAME), were quantified using standards. Unidentified FAMEs were minor relative to the eleven quantified fatty acids. The standards included: methyl pentadecanoate, methyl palmitate, methyl stearate, methyl oleate, methyl linolenate, methyl gamma linolenate, methyl 11,14 eicosadienoate, methyl arachidonate, methyl behenate, and methyl linoleate (Nu-Chek Prep Inc.), and methyl levulinate (Sigma-Aldrich, Oakville, CAN). These compounds had a purity of >99%.

Data analyses

I used nonmetric multidimensional scale (NMDS, package "ecodist" in R allowing to calculate Bray-Curtis distances) to characterize fatty acid profiles of trees. NMDS is an ordination technique allowing us to visualize the distribution of the data. The first two dimensions were performed in NMDS based on a scree-plot of stress values (axes X1 and X2) and the vectors are fatty acid concentrations indicating correlations between variables and tree species. A canonical discriminant analysis (CANDISC, package "candisc" in R showing a biplot) with group centroids and vectors representing fatty acid concentrations was used to test whether profiles of fatty acids differed among tree species (axes Can1 and Can2) or groups (historical hosts: *P. contorta*, *P. ponderosa*, *P. flexilis*, *P. abicaulis*; potential hosts: *P. banksiana*, *P. resinosa*, *P. sylvestris*; occasional host: *P. glauca*; non-host: *P. tremuloides*). Pie charts were used to report the proportions of total saturated and unsaturated fatty acids among nine tree species.

Furthermore, to visualize the concentrations of individual fatty acids between *P. contorta* and *P. banksiana* in their different locations, bar graphs were overlaid onto a map of Canada. CANDISC was applied to demonstrate fatty acid concentrations that differentiated among three tree species.

To test differences in total concentrations of fatty acids (μg/g of dry weight) within (*P. contorta*, *P. banksiana*, *P. sylvestris*, *P. resinosa*, *P. glauca*) and among tree species and in individual fatty acid concentrations among *P. contorta*, *P. banksiana*, and *P. tremuloides*, I conducted an Analysis of Variance (ANOVA) after a square root transformation to meet the assumptions of normality and homogeneity of variance to test significant differences of fatty acids among tree species. Tukey's Honestly Significant Difference (HSD) was performed to adjust for multiple comparisons.

2.2. Do fatty acid concentrations of *P. contorta*, *P. banksiana*, and *P. tremuloides* influence brood development of *D. ponderosae*?

Tree sampling and tissue processing

To test the biological significance of fatty acid concentrations observed in *P. contorta, P. banksiana,* and *P. tremuloides* on brood development by *D. ponderosae*, I conducted laboratory experiments with purified natural (extracted from fresh phloem tissue) and synthetic fatty acids. In July 2013, 3-4 trees (20-30 cm diameter at 1.4 m) per species were cut (*P. contorta*: Hinton, Alberta, 53°45.925'N, 118°22.298'W; *P. banksiana* and *P. tremuloides*: Lac La Biche, Alberta, 54°16.964'N, 111°37.251'W). In the field, both ends of the bolts were covered with paraffin wax to minimize moisture and secondary metabolite loss and then transported to the laboratory where they were kept at 4°C until use. The bark was carefully removed around each bolt and phloem

and sapwood with a depth of 1 cm were peeled off and placed in an oven at 70°C for 3 d. A Wiley Mill with 1 mm mesh screen was used to separately grind the dried phloem and sapwood samples. These ground samples (9:1 ratio of phloem to sapwood) were then mixed together. After lipid extraction from phloem and sapwood tissues (see *Lipid extraction* section below), the remaining spun pellet was presented to the beetle broods within the tubes.

Media preparation

I used a new rearing tube to determine if fatty acids affect growth of *D. ponderosae* from egg to adult as it allowed us to manipulate the content of the rearing environment for the beetle. This technique was developed by C Myrholm and D Langor at the Northern Forestry Centre in Edmonton. For medium, I used the solid phloem and sapwood tissue (ground medium) remaining after fat extraction (see *Fatty acid purification* section below). A 10 cm long glass tube (5 mm OD, 3.6 mm ID, Kimax Standard, Fischer Sci.) was filled with ground phloem and sapwood (9:1 ratio) amended with fatty acids and *G. clavigera*. Each tube was plugged on one end with an absorbent cotton wool, filled with lightly compressed 0.45 g (dry) ground medium using a sterile funnel, and plugged at the opposite end with cotton wool. A thin strip of Parafilm was wrapped around tube ends and a pipette filter tip (200 µL) lightly filled with glass wool was tightly fitted to minimize contamination while allowing air circulation inside the tube. Prior placing beetle's egg into the tube, this tube was autoclaved twice at 121°C for 30 min and kept at room temperature. Before they were used, the medium in each tube was rehydrated by removing the pipette tip and cotton from one end and adding approximately 0.6 ml of sterile distilled water, and replacing the pipette tip and cotton.

I used synthetic fatty acids (see *Fatty acids application* section below) associated with tree species: *P. contorta*, *P. banksiana*, and *P. tremuloides*. During rehydration, saturated or unsaturated fatty acids mixed with 10μL of Tween® 20 (Sigma-Aldrich, Inc.) were injected to the media tubes. During this process, a small of agar plug of cultured *G. clavigera* was also added to the medium (n=15). In our experiments, either *P. contorta* phloem was amended with saturated or unsaturated fatty acids of the above three tree species or phloem of the same three species was amended with the saturated or unsaturated fatty acids of *P. contorta*. Concentrations of saturated and unsaturated fatty acids injected into each tube were calculated based on the amount (μg/g) of dry weight of individual fatty acids of each tree species (see Supplementary Table 2.1.3 for specific concentrations added in each tube) that were identified by GC/FID from natural phloem samples of the tree species.

Fatty acid application

Based on our identification of major FAMEs of tree species (see *Chemical analyses* above), I purchased 11 synthetic fatty acids, including LA, ALA, GLA, ARA, OA, palmitic (PA), stearic (SA), pentadecanoic (PDA), behenic (BA), eicosadienoic (EDA), and levulinic (LvA) acids and determined their effects on brood development of *D. ponderosae* in rearing tubes and fungus culture. In our experiments, I grouped LvA as a saturated fatty acid, even though it is from the ketone acid group, because it is used in fatty acid synthesis. These compounds had purities of >99% (Nu-Chek Prep Inc., MN, USA and Sigma-Aldrich).

Rearing tube preparation

I placed a single egg of *D. ponderosae* in each tube. To obtain beetle eggs, I infested fresh *P. contorta* bolts with live pairs (male and female) of beetles caught in pheromone baited traps in Grande Prairie (Alberta). Five holes were made on the bark of each bolt using a cork borer (5 mm in diameter) and a female beetle was introduced into each hole followed by a male beetle. The infested bolts were kept at room temperature for 7 days. Eggs were removed from galleries after carefully removing the bark from each bolt and eggs were placed onto sterile, moistened filter paper in Petri dishes. Eggs surface were sterilized in Modified White's Solution (Barras 1972, Bleiker & Six 2007), and subsequently stored at 4°C for up to 7 days on sterile, moistened filter.

To place individual eggs in each tube, the filter tip and cotton plug were removed from one end of a hydrated tube and a small depression (5 mm deep) was made in the medium down one side of the tube using a sterile 200 µL pipette tip. Using a dissecting microscope, eggs were gently placed into the depression using a 200 µL pipette tip as close to the bottom as possible and resting on the medium. Eggs selected had no discoloration or evidence of deflation, and embryo development was evident by a clear area at one end of the egg (Reid & Gates 1970). Fungal propagules were added to the tube after egg placement by filling the same depression with fungal culture without putting pressure on the egg, and cotton plugs and pipette tips were replaced. Larval survival was quantified at the end of experiment after 70 days based on technique. All work was done under a laminar flow hood using sterile technique. Inoculated rearing tubes were placed into sterile covered trays and incubated at 24°C in the dark for 70 days.

Data analyses

The proportions of the larval survival in either *P. contorta* substrate, amended with saturated or unsaturated fatty acids of *P. contorta*, *P. banksiana*, and *P. tremuloides* or substrate of these tree species, amended with the saturated or unsaturated fatty acids of *P. contorta* were identified.

2.3. Do fatty acid concentrations of *P. contorta*, *P. banksiana*, and *P. tremuloides* influence growth of *Grosmannia clavigera*?

To determine if *G. clavigera* can grow in a liquid agar (medium) amended with different concentrations of fatty acids from *P. contorta*, *P. banksiana*, and *P. tremuloides*, fungus, which originally was collected from Fox Creek Alberta (Lusebrink *et al*. 2011), was inoculated into the medium, as fungal associates are essential for growth and survival of beetle larvae (Bleiker & Six 2007). Fatty acids from *P. contorta* were used as control treatment because *G. clavigera* inhabit *P. contorta*.

Lipid extraction

To extract lipids from phloem, I followed methods by Nelson and Dickson (1981) and Fischer and Höll (1991). Briefly, in a 50 mL centrifuge tube, 1.5 g of ground tissue sample was mixed in 40 mL of a solvent (chloroform/methanol, 2:1 v/v, at 99.9% purity, Sigma-Aldrich). The resulting solution was first mixed in a vortex for 30 sec and then left to rest for 3 min at room temperature. The tube was centrifuged for 5 min at 2,500 rpm and the resulting separate organic layer collected into a new centrifuge tube. This process was repeated twice. The third extraction was similar to the first two, but with a different solvent ratio (chloroform/methanol, 1:2 v/v). In all cases, after extraction and centrifugation, the resulting separated organic layers were combined. I added 4.5 mL of 3.6 mM $CaCl_2$ solution to the tube and then shook it for 5 sec. The

new solution was then centrifuged for 3 min at 3,000 rpm and the resulting layer containing chloroform (15 mL) with lipids was taken for further purification. After extraction, the remaining fat-free solid tissue samples were dried in the oven at 70°C for 3 days and used as a matrix for the rearing *D. ponderosae* brood in the ground medium (see *Media preparation* section above).

Fatty acid purification

A solid phase extraction cartridge (HF Mega BE-NH2, bed volume=10g, liquid volume=60mL, Agilent Tech.) was placed on the top of a vacuum Elute 20manifold (Agilent Tech.). I used 120 mL of hexane (Sigma-Aldrich) to condition the cartridge and then processed 150 mL of lipid extract from the phloem-sapwood mixture. I then used 150 mL of chloroform/propanol (2:1 v/v) in order to retain only free fatty acids and phospholipids in the matrix, then in order to elute free fatty acids, I used a 100 mL solution of 2% acetic acid in diethyl ether (both at 99.9% purity, Fischer Sci.). The eluent was collected from the cartridge into a glass vial and dried under a stream of N_2 gas. The evaporate was then weighed. The purified total 'natural' extracts were kept at -80°C until use.

Media preparation

I used fatty acids from two sources to amend with the medium. In one experiment, total purified lipid fractions, fatty acids, were prepared based on their natural concentrations (wet phloem) in each tree species: *P. contorta* (249.218 µg/ml), *P. banksiana* (48.305 µg/ml), and *P. tremuloides* (125.840 µg/ml). Twenty mL of sterilized distilled water, 10 µL of Tween® 20 and total purified lipid fractions were mixed together. To homogenize the mixture in the solution, it was sonicated for 20 min. For the medium preparation, 3 g of Bacto™ Agar (Becton Dickinson and Company

Sparks, MD, USA) was mixed with 180 mL of distilled water in a 1 L glass flask. This media was autoclaved at 121°C for 20 min. It was then cooled down for 35-40 min at room temperature and homogenized lipid fractions were added in the medium. Then the whole mixture was shaken for 1 min and poured into sterilized Petri dishes (100x15mm) (Fischer Sci.). These plates were ready for fungal inoculation 24 h later. A culture containing *G. clavigera* was inoculated into Petri dish using a cork borer (5 mm in diameter, one inoculum per Petri dish) (n=15) and the dishes were sealed with Parafilm. The dishes were kept for four weeks at room temperature.

In the second experiment, the same process for medium preparation and inoculations was repeated with the synthetic saturated and unsaturated fatty acids (see *Fatty acids application* section above). In addition, since I was interested in the role of individual fatty acids, I amended the fungal medium with individual fatty acids simulating the concentration of fatty acids in each tree species (see Supplementary Table 2.1.4 for specific concentrations added in each Petri dish). Fungal growth rate was measured to the nearest mm every 5 days. I determined the maximum growth rate of the fungus on each plate (mm^2 area covered by fungus) using digital imagery and fungal activity rate was compared with respect to the tree species among treatments.

Data analyses

To look at the relationship between concentration of individual fatty acids of *P. contorta*, *P. banksiana*, and *P. tremuloides* and *G. clavigera* activity, a leaner regression model was applied. For significant differences in the mean area of fungal growth, an ANOVA was used after square root transformation to meet the assumptions of normality and homogeneity of variance, and Tukey's HSD was performed for multiple comparisons.

RESULTS

In general, I identified 22 different fatty acid methyl esters from eight tree species (*P. ponderosa* not included) (Supplementary Table 2.1.1). Eleven of these fatty acids: LA, ALA, GLA, ARA, OA, SA, PA, PDA, BA, LvA, and EDA were found among all species, while some others were unique to one or two tree species. For example, hepta-2,4-dienoic acid was only found in *P. tremuloides* while heptacosylic acid was only found in *P. albicaulis*. The common fatty acids were also the most abundant, accounting for 95% of all fatty acid content of all species based on peak sizes of individual fatty acids. Since I focused on whether concentrations of different fatty acids affect brood survival by *D. ponderosae* and growth of its symbiotic fungus, *G. clavigera*, I used these 11 fatty acids in most experiments although in some cases I used total purified lipid fractions.

Fatty acids in tree species

Concentrations of major fatty acids in phloem showed variation within and among tree species although differences among species were more pronounced (Fig. 2.1). Within species, *P. contorta* sampled from southern British Columbia had the lowest association with most fatty acid profiles compared to those from central British Columbia and Alberta. Likewise, *P. banksiana* from the Northwest Territories and Ontario, which clustered together, had higher association with fatty acid profiles than those from Alberta and Manitoba. There were also differences in fatty acid profiles of *P. sylvestris* from Manitoba and Ontario. There was no location effect on the fatty acid profiles of *P. resinosa* from Manitoba and Ontario and for *P. glauca* between Alberta and the Northwest Territories. Among species, *P. albicaulis* was highly associated with most of the fatty acid profiles as compared to other tree species (Fig. 2.1). In contrast, *P.*

ponderosa, *P. glauca*, *P. tremuloides* had weak associations with most of the fatty acid profiles compared to other trees (Fig. 2.1, Supplementary Table 2.1.2).

I also compared total fatty acid concentrations within (Fig. 2.2a) and among (Fig. 2.2b) tree species (Supplementary Table 2.1.2). For *P. contorta*, trees from southern British Columbia had lower concentrations of total fatty acids than trees from central British Columbia and Alberta ($F_{2,87} = 43.52$, $P = 0.001$; Fig. 2.2a). In addition, concentrations of total fatty acids in the phloem of *P. banksiana* from Alberta and Manitoba were lower than those from the Northwest Territories and Ontario ($F_{3,118} = 13.8$, $P = 0.001$). The only other species that showed geographical separation was *P. sylvestris* for which total fatty acid concentrations were higher in Ontario than in Manitoba ($F_{1,48} = 12.84$, $P = 0.001$). For *P. resinosa* and *P. glauca*, there was no location effect on fatty acid concentration (*P. resionosa*: $F_{1,47} = 1.10$, $P = 0.31$; *P. glauca*: $F_{1,39} = 0.29$, $P = 0.6$). Among tree species, fatty acid concentrations exhibited some differences ($F_{8,214} = 82.93$, $P = 0.001$; Fig. 2.2b). *P. albicaulis* and *P. ponderosa* had the highest and lowest amount of total fatty acids, respectively. However, fatty acid concentrations of *P. contorta*, *P. banksiana*, *P. sylvestris*, *P. flexilis* and *P. tremuloides* were intermediate and similar. In addition, *P. resinosa* and *P. glauca* had similar fatty acid concentrations.

Canonical discriminant analysis (CANDISC) indicated a correspondence of fatty acid profiles with tree species based on concentrations of individual fatty acids (Fig. 2.3). When all nine species were included in the analysis, *P. tremuloides* separated from the other eight (Can1 axis described 44.1% of the correspondence of fatty acid profiles in *P. tremuloides*) and showed a positive correlation with LA and ALA but a negative correlation with OA and ARA (Fig. 2.3a). Other tree species were clustered together and Can 2 explained 29.4% of the correspondence of fatty acid profiles among these species (Fig. 2.3a). Notably, *P. sylvestris* with LvA and *P.*

albicaulis with GLA, SA, and PDA showed positive correlations (Fig. 2.3a). When I removed *P. tremuloides* from the CANDISC analysis, the Can1 axis describes 54.2 % of fatty acid profile correspondences in *P. sylvestris* and *P. albicaulis* species; the latter species positively correlated again with GLA, SA, and PDA and *P. sylvestris* only with LvA (Fig. 2.3b).

I have also applied CANDISC analysis to determine if fatty acid profiles differentiate historical (*P. contorta*, *P. ponderosa*, *P. flexilis*, *P. albicaulis*), potential (*P. banksiana*, *P. resinosa*, *P. sylvestris*), and occasional (*P. glauca*) hosts of *D. ponderosae* and non-host (*P. tremuloides*) species (Fig. 2.4). Can1 axis explained 80.5% of the fatty acid profiles corresponding in non-host tree, which was strikingly isolated from the rest of groups and had high correlation with LA and ALA and low correlation with EDA (Fig. 2.4a). Other tree groups were clustered together. When I removed the non-host group from CANDISC analysis, even though Can1 axis described 75.9% of the correspondence of fatty acid profiles within the hosts there was no noticeable differentiation among the remaining groups by fatty acid profiles (Fig. 2.4b).

I also tested for variation in the tree species in terms of their saturated and unsaturated fatty acid ratios (Fig. 2.5). In all locations, all species contained more unsaturated than saturated fatty acids, ranging from 64.57 % to 79.42 % of total fatty acids. Notably, specimens (*P. contorta* and *P. banksiana*) sampled in northern latitudes contained more unsaturated fatty acids compare to the specimens in southern latitudes (Fig. 2.6), including LA and OA.

Finally, I conducted CANDISC analysis to look at the pattern of fatty acid profiles of *P. contorta*, *P. banksiana*, and *P. tremuloides* (Fig. 2.7). Can1 axis explained 91% of the correspondence of LA and ALA with *P. tremuloides* and separated from the other two species. Additionally, I compared mean concentrations of individual fatty acids among *P. contorta*, *P.*

banksiana and *P. tremuloides* using ANOVA (Table 2.1). Out of 11 fatty acids, five did not vary between *P. contorta* and *P. banksiana*, including the most abundant fatty acid, LA. In contrast, 10 of 11 fatty acids differed between *P. contorta* and *P. tremuloides*, including LA and OA, and 7 of 11 were different between *P. banksiana* and *P. tremuloides*. Notably, *P. tremuloides* contained the highest concentrations of several unsaturated fatty acids, including LA.

Beetle brood survival

When *P. contorta* substrate was amended with the total saturated or unsaturated fatty acids of *P. contorta* and *P. banksiana*, these two species did not interfere with larval survival (Fig. 2.8a). In contrast, no larvae survived in substrates amended with total saturated or unsaturated fatty acids from *P. tremuloides*. Notably, mean larval survival was similar between *P. contorta* and *P. banksiana* when phloem has amended with unsaturated fatty acids ($F_{2,42}=3.1$, $P = 0.59$), but the former had more surviving larvae when phloem was amended with saturated fatty acids ($F_{2,42}=33.03$, $P = 0.001$).

When substrates of *P. contorta*, *P. banksiana*, or *P. tremuloides* were amended with the total saturated or unsaturated fatty acids of *P. contorta*, with the exception of *P. tremuloides* substrate amended with unsaturated fatty acids, all were suitable for larval survival. The highest larval survival was associated with the host substrate of *P. contorta* amended with saturated fatty acids and *P. banksiana* substrate amended with the unsaturated fatty acids (Fig. 2.8b).

Fungal growth

Overall, total purified lipid fractions of *P. banksiana* were suitable for *G. clavigera* activity while the other two host species (*P. contorta* and *P. tremuloides*) reduced the fungal growth (Fig.

9a). When I amended the medium with saturated or unsaturated fatty acids from one of the three tree species, *G. clavigera* grew only in the medium amended with total saturated fatty acids of *P. banksiana* (Fig. 9b). These results demonstrate that some fatty acids in *P. contorta* phloem were reducing fungal growth. Thus, I investigated the effects of mimicking the concentration of individual fatty acid of *P. contorta*, *P. banksiana*, and *P. tremuloides* on fungal activities (Fig. 2.10). I observed interesting patterns as the individual fatty acids can have negative or positive effects on fungal growth depending on their concentrations. Increasing concentrations of saturated BA (*P=0.01*, Fig. 2.10a) and PA (*P=0.009*, Fig. 2.10e) and unsaturated ARA (*P=0.001*, Fig. 2.10k) increased the growth of *G. clavigera* and in all cases *P. contorta* had the highest concentration of all three fatty acids. For BA and ARA, *P. contorta* and *P. banksiana* had similar fungal growth while these two species had different impacts on fungal growth than PA. In contrast, increasing concentrations of OA (*P=0.002*, Fig. 2.10f), ALA (*P=0.007*, Fig. 2.10h), GLA (*P=0.02*, Fig. 2.10i) and EDA (*P=0.02*, Fig. 2.10j) (all unsaturated) reduced the growth of *G. clavigera*. ALA was high in *P. tremuloides* among three species and reduced fungal growth (Fig. 2.10h). LA (unsaturated) applied at any of the tested concentrationsdid not yield any fungal growth (Fig. 2.10g). Notably, LA was the most abundant fatty acids in all three species (70% of *P. tremuloides* vs. 40% of *P. contorta*, 42% of *P. banksiana*) and *P. tremuloides* had the highest concentration. Only LvA (saturated) from *P. banksiana* (Fig. 2.10c) yielded fungal growth. Although increasing concentrations of SA (Fig. 2.10b) (saturated) reduced fungal growth, it was only moderately significant (*P=0.07*).

DISCUSSION

Fatty acid concentrations differed within and among tree species, however, dissimilarities were more pronounced among species. While plant fatty acids have been shown to affect insect

herbivores (Bird *et al*. 1987; Honda & Bowers 1996; Mansson *et al*. 2005, 2006; Castillo *et al*. 2010; Santana *et al*. 2012), I present here the first direct evidence that fatty acids can influence bark beetle brood development and growth of their fungal symbionts. This study provides three pieces of evidence that, in addition to their known role in plant secondary compounds effects on insect host suitability (e.g. Erbilgin *et al*. 2014). Plant fatty acids, particularly their concentrations, can also be important in elucidating the host suitability of insects: (1) host and non-host trees were separated by individual fatty acids in their phloem; (2) the effect of fatty acid concentration on beetle larval survival varied by tree species; (3) the fungal symbionts of beetles responded differently to the lipid fractions and fatty acid concentrations of different tree species. Below, I discuss these pieces of evidence to support the hypothesis that fatty acids should be considered in insect-plant co-evolution and host suitability of insects. I argue that fatty acids may have positive or negative effects on herbivore performance depending on their concentrations.

First, I observed a clear separation among tree species based on fatty acid concentrations in tree phloem. Such variation is not uncommon considering the phylogenetic differences among the examined tree species (Senser 1982; Kyburz *et al*. 1991; Martz *et al*. 2006; Le Guédard *et al*. 2012). What is novel in our findings is the clear distinction between the historical hosts (*P. contorta*, *P. flexilis*, *P. albicaulis*, *P. ponderosa*) and non-host (*P. tremuloides*) species of *D. ponderosae*. With the exception of *P. albicaulis*, all historical hosts clustered together and there was little or no difference in the fatty acid profiles among them. Furthermore, when I grouped the tree species as historical, potential (*P. banksiana*, *P. resinosa*, *P. sylvestris*) and occasional (*Picea glauca*) hosts and non-hosts, the dissimilarity between non-hosts and the other hosts sharply increased, confirming that concentrations of individual fatty acids can be used to differentiate host from the non-host species. In particular, the non-host was highly correlated

with two major unsaturated (LA and ALA) fatty acids. High concentrations of these two fatty acids in *P. tremuloides* are not unexpected as other *Populus* species also contain high proportions of these fatty acids (Kyburz *et al*. 1991; Le Guédard *et al*. 2012). I hypothesize that high concentrations of LA and ALA may mediate rejection of *P. tremuloides* as a host by *D. ponderosae* because they have demonstrated antifeedant activity against a number of other insect herbivores (Smith 1998; Shepherd *et al*. 1999; Puterka *et al*. 2003; Castillo *et al*. 2010).

Secondly, tree fatty acid concentrations affected *D. ponderosae* brood development. Among various combinations of *P. contorta* substrate with saturated or unsaturated fatty acids from one of three species (*P. contorta, P. banksiana*, *P. tremuloides*), only the fatty acids of *P. tremuloides* did show larval survival. To demonstrate the importance of fatty acids in the biology of these beetles, I further amended substrates from the same three species with saturated or unsaturated fatty acids of *P. contorta* and found that larvae were able to survive in all three substrates mixed with saturated fatty acids. A combination of *P. tremuloides* substrate with unsaturated fatty acids of *P. contorta*, however was not suitable. This could be due to either the antifeedant (or deterrent) role of unsaturated fatty acids or the unsuitability of *P. tremuloides* substrate. Since beetles sustained their development in *P. tremuloides* substrate amended with saturated fatty acids of *P. contorta*, any influence of substrate on beetle larvae seems unlikely or negligible. Because unsaturated fatty acids are ubiquitous components of plant epicuticular waxes (Muller & Riederer 2005) and are quite abundant in many plant species (Eigenbrode & Espelie 1995), it is not surprising, therefore, that they provide some ecological value to the plant (Smith 1998). In fact, unsaturated "free" fatty acids, such as LA and ALA, are known to be antifeedants effective against a number of insects, including aphids (Castillo *et al*. 2010; Santana *et al*. 2012), termites (Scheffrahn & Rust 1982) and beetles (Bird *et al*. 1987; Honda & Bowers 1996; Mansson *et al*.

2005, 2006). Unsaturated fatty acids, being deterrents, probably indicate the unsuitability of a host insect for oviposition or feeding (Sherwood *et al*. 1981; Herrbach 1987; Eigenbrode & Espelie 1995; Smith 1998; Shepherd *et al*. 1999; Puterka *et al*. 2003; Mansson *et al*. 2005, 2006; Castillo *et al*. 2010). Notably, some fatty acids provide a feeding and oviposition stimulus at certain concentrations (Knight & Corbet 1991). For example, low doses of a given fatty acid can enhance oviposition by the female spruce budworm (*Choristoneura fumiferana*) while high doses act as a deterrent (Grant *et al*. 2000).

Finally, plant lipids and fatty acids influenced the growth of *G. clavigera*, a fungal symbiont of *D. ponderosae*. I demonstrated this effect using two bioassays. Firstly, I amended the fungal culture medium separately with the total lipids of *P. contorta*, *P. tremuloides*, or *P. banksiana* and found that the former two species exhibited *G. clavigera* inactivity while the fungus sustained growth on a *P. banksiana* amended medium. I further amended the medium with saturated or unsaturated fatty acids of the same three species and again only saturated fatty acids of *P. banksiana* were suitable for the fungus. Secondly, I measured fungal growth at three concentrations of 11 fatty acids, simulating their relative amounts in each of three species (*P. contorta*, *P. tremuloides*, *P. banksiana*). I showed that increasing concentrations of two saturated (BA and PA) and one unsaturated (ARA) fatty acid increased fungal growth and *P. tremuloides* had the lowest concentration of BA and ARA. Conversely, increasing concentrations of three unsaturated fatty acids (OA, GLA, and EDA) had negative effects on the fungus and *P. contorta* had the highest concentration of OA, GLA, and EDA. In addition, there was no fungal activity at any concentration of LA and at the highest concentration of ALA (in both cases, *P. tremuloides* had the highest concentration). These results indicate that *P. tremuloides* not only lacks some fatty acids that enhance fungal growth, but also contains higher concentrations of antifungal fatty

acids. Antifungal properties of unsaturated fatty acids, including LA and ALA, have been previously demonstrated (Walters *et al*. 2004).

Although it is unknown how fatty acid–fungal symbiont interactions affect the biology and host selection of *D. ponderosae*, symbiotic fungi are likely involved in fatty acid breakdown and synthesis (Kamisaka *et al*. 1990; Gao *et al*. 1994), which may benefit host beetles (Goldhammer *et al*. 1990; Six & Paine 1998; Six 2012). In fact, in preliminary trials, I could not rear beetles in tubes without *G. clavigera*. In general, symbiotic fungi can facilitate beetle nutrition, either directly by serving as a food source (Adams & Six 2007), or indirectly by digesting and concentrating host tree nutrients (Ayres *et al*. 2000; Bleiker & Six 2007; Goodsman *et al*. 2012). Moreover, sterol produced by *G. clavigera* may contribute to cold-hardiness and survival of *D. ponderosae* at high elevations (Bentz & Six 2006). This fungus may also help detoxify tree defenses (DiGuistini *et al*. 2007), and symbiotic fungi have helped expand the capacity of bark beetles to use nutrient-poor plant resources (Marvaldi *et al*. 2002). Considering the importance of fungal symbionts in bark beetle survival and the significance of the host plant chemistry and nutritional quality in fungal growth (Lieutier 2004), I hypothesize that the utilization of certain plant species may be linked to the formation of associations with fungi that can grow therein, and may influence host selection and discrimination by bark beetles (Six 2012).

In conclusion, plant fatty acids can have cascading impacts on insects, and there appear to be at least three mechanisms by which bark beetles can be influenced by host fatty acids. First, dietary fatty acids, in particular LA and ALA, are required by insects to sustain their development (Hodges & Barras 1974; de Renobales *et al*. 1987) as they are unable to synthesize them (Dadd 1981; Blomquist *et al*. 1991). Second, at high concentrations some fatty acids such as LA and ALA may act as feeding deterrent of bark beetles (Knight & Corbet 1991; Shepherd *et*

al. 1999; Grant *et al*. 2000). Third, fatty acids may indirectly affect beetles through their antifungal properties against a beetle's fungal symbionts (Walters *et al*. 2004; Pohl *et al*. 2011).

I argue that similarities in the concentration of individual fatty acids between the beetle's historical and novel hosts may have contributed to the host suitability of *D. ponderosae*. These results extend the recognized role of plant secondary compounds in insect host expansion (Ehrlich & Raven 1964; Jermy 1984; Erbilgin *et al*. 2014), and that plant fatty acids also should be considered in elucidating host and range expansion of herbivorous insects. Despite much literature examining the diversity of fatty acids in plants, few of these compounds have been investigated in regards to insect biology and ecology, nor for their role in the evolution of host range in insect–plant interactions.

Environmental conditions might effect on fatty acids of tree species (Baradat and Yazdani 1988). The results showed that fatty acids of phloem tissue from jack pine in Alberta and Manitoba were lower comparing to those in Ontario and Northwest Territories. Lodgepole pine phloem fatty acid concentrations in Alberta and Central British Columbia were higher comparing to those in Southern British Columbia. Previous studies suggested that secondary compounds of trees might vary with environmental conditions (Baradat & Yazdani 1988; Wallis *et al.* 2011) which might be related to our results and variation of individual fatty acid concentrations within tree species most likely depend on abiotic (e.g. temperature) (Murakami *et al.* 2000; Iba 2006) and biotic (e.g. insect herbivorous and microorganisms) factors. These results suggest further studies on whether variation of fatty acids in lodgepole pine and jack pine across their different stands is due to environmental factors.

Table 2.1. Mean (± SE) concentration (µg/g of dry weight of phloem) of individual fatty acids from *Pinus contorta*, *P. banksiana* and *Populus tremuloides*, sampled in Alberta. The abbreviated letters denote saturated (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid) and unsaturated (LA=linoleic acid, OA=oleic acid GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid) fatty acids. An ANOVA was applied for statistical analyses of individual fatty acid effects in tree species. The letters after means indicate significant difference among tree species.

Tree Species	**Saturated Fatty Acids**					**Unsaturated Fatty Acids**					
	PA	BA	LvA	PDA	SA	LA	OA	GLA	ALA	EDA	ARA
P. contorta	8.18 a (0.56)	6.65 a (0.46)	4.89 a (0.35)	0.86 a (0.11)	0.78 a (0.06)	31.98 a (2.61)	18.02 a (1.53)	2.95 a (0.47)	2.34 (0.17)	1.74 a (0.11)	0.95 a (0.17)
P. banksiana	6.88 b (0.20)	4.52 b (0.17)	7.49 b (0.43)	0.29 b (0.01)	0.74 a (0.04)	30.00 a (1.11)	13.87 b (0.48)	1.38 b (0.20)	3.40 (1.61)	1.61 a (0.07)	0.93 a (0.14)
P. tremuloides	6.26 b (0.18)	1.09 c (0.10)	5.35 b (0.33)	0.26 b (0.01)	1.07 b (0.06)	48.12 b (2.03)	1.02 c (0.05)	0.03 c (0.004)	4.69 (0.20)	0.81 b (0.03)	0.03 b (0.02)
$F_{2,72}$	7.45	94.11	13.99	26.90	11.31	24.44	91.72	24.81	1.56	44.35	16.84
P	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.22	<0.001	<0.001

Supplementary Table 2.1.1. Fatty acid methyl ester (ME) profiles identified from the phloem of *Pinus contorta* (*Pc*), *P. banksiana* (*Pb*), *P. resinosa* (*Pr*), *P. sylvestris* (*Ps*), *P. flexilis* (*Pf*), *P. albicaulis* (*Pa*), *Picea glauca* (*Pg*), and *Populus tremuloides* (*Pt*). *C*, *D*, and *n-x* indicate the number of carbon atoms, double bonds in the fatty acids, and name of individual compound respectively. (+) and (−) denotes presence and absence respectively.

Compound name	**Common name**	**C:D(n-x)**	***Pc***	***Pb***	***Pr***	***Ps***	***Pf***	***Pa***	***Pg***	***Pt***
Pentanoic acid , *4-oxo*, ME	Levulinic acid	5:0	+	+	+	+	+	+	+	+
Cyclopentanetridecanoic acid ME	Cyclopentane Tridecanoic acid		+	+	+	+	+	−	−	+
Heptacosanoic acid ME	Heptacosylic acid		−	−	−	−	−	+	−	−
Hepta-*2*,*4*-dienoic acid ME	Hepta-2,4-dienoic acid		−	−	−	−	−	−	−	+
Nonanoic acid ME	Pelargonic acid		−	−	+	−	−	−	−	+
Decanoic acid ME	Capric acid		+	+	−	−	−	−	−	−
Pentadecanoic acid ME	Pentadecanoic acid	15:0	+	+	+	+	+	+	+	+
Hexadecanoic acid ME	Palmitic acid	16:0	+	+	+	+	+	+	+	+
Hexadecanoic acid,14-methyl-, ME	14-Hexadecanoic acid		+	−	−	−	+	+	+	−
Heptadecanoic acid, *16*-methyl-, ME	16-Heptadecanoic acid		−	+	−	−	−	+	−	−
Octadecanoic acid ME	Stearic acid	18:0	+	+	−	−	+	+	+	+
9-Octadecenoic (*Z*) ME	Oleic acid	18:1 (n-9)	+	+	+	+	+	+	+	+
9,*12*-Octadecadienoic acid (*ZZ*) ME	Linoleic acid	18:2 (n-6)	+	+	+	+	+	+	+	+
9,*12*,*15*-Octadecatrienoic (*ZZZ*) ME	Alpha-linolenic acid	18:3 (n-3)	+	+	+	+	+	+	+	+
13,*16*-Octadecadienoic acid ME	Docosadienoic acid		+	+	+	+	+	+	+	−
5,*9*,*12*-Octadecatrienoic acid (ZZZ) ME	Gamma-linolenic acid	18:3 (n-6)	+	+	+	+	+	+	+	+
8,*11*-Octadecadienoic acid ME	8,11-octadecadienoic acid		+	−	+	−	+	+	+	−
Eicosanoic acid ME	Arachidic acid		+	+	−	+	+	+	−	−
11,*14*-Eicosadienoic acid (*ZZ*) ME	Eicosadienoic acid	20:2 (n-6)	+	+	+	+	+	+	+	+
5,*8*,*11*,*14*-Eicosatetraenoic (all *Z*) ME	Arachidonic acid	20:4 (n-6)	+	+	+	+	+	+	+	+

11,*14*,*17*-Eicosatetraenoic acid ME	Eicosatrienoic acid		+	+	−	+	+	+	−	−
Docosanoic acid ME	Behenic acid	22:0	+	+	+	+	+	+	+	+

Supplementary Table 2.1.2. Mean (± SE) concentration of major fatty acids (µg/g of dry weight of phloem) from *Pinus contorta*, *P. banksiana*, *P. resinosa*, *P. sylvestris, P. albicaulis*, *P. ponderosa*, *P. flexilis*, *Picea glauca*, and *Populus tremuloides* sampled in various locations in North America. Abbreviated letters denote individual fatty acids: LA=linoleic acid, OA=oleic acid, PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, ARA=arachidonic acid, PDA=pentadecanoic acid, and SA=stearic acid. (-) denotes absence.

Tree species	**Location (n)**	LA	OA	PA	BA	LvA	GLA	ALA	EDA	ARA	PDA	SA	Total
P. contorta	Alberta (**25**)	31.98 (2.61)	18.02 (1.53)	8.18 (0.56)	6.65 (0.46)	4.89 (0.35)	2.95 (0.47)	2.34 (0.17)	1.74 (0.11)	0.95 (0.17)	0.86 (0.11)	0.78 (0.06)	79.34 (5.91)
	Central BC (**30**)	24.99 (1.75)	13.37 (1.00)	5.62 (0.43)	4.65 (0.40)	7.37 (0.72)	5.02 (0.58)	2.55 (0.20)	1.73 (0.14)	1.06 (0.15)	1.22 (0.13)	0.67 (0.08)	68.26 (4.63)
	Southern BC (**35**)	11.41 (0.67)	5.67 (0.37)	3.01 (0.15)	2.48 (0.14)	5.82 (0.40)	1.96 (0.25)	1.19 (0.07)	1.07 (0.05)	0.54 (0.08)	0.38 (0.04)	0.10 (0.03)	33.63 (1.55)
P. banksiana	Alberta (**25**)	30.00 (1.11)	13.87 (0.48)	6.88 (0.20)	4.52 (0.17)	7.49 (0.43)	1.38 (0.20)	3.40 (1.61)	1.61 (0.07)	0.93 (0.14)	0.29 (0.01)	0.74 (0.04)	71.12 (2.54)
	Northwest Territories (**12**)	47.76 (4.25)	21.37 (1.76)	10.14 (0.69)	8.30 (0.69)	18.01 (1.66)	1.01 (0.20)	2.38 (0.22)	2.79 (0.27)	1.49 (0.34)	0.45 (0.04)	1.33 (0.14)	115.03 (8.99)
	Manitoba (**40**)	27.03 (1.01)	13.15 (0.67)	6.19 (0.24)	5.42 (0.19)	10.62 (0.92)	1.03 (0.11)	1.84 (0.07)	1.63 (0.08)	0.78 (0.10)	0.26 (0.01)	0.78 (0.04)	68.74 (2.63)
	Ontario (**45**)	43.00 (2.37)	21.10 (1.27)	8.69 (0.49)	8.33 (0.45)	7.72 (0.48)	1.22 (0.11)	2.58 (0.15)	2.75 (0.16)	1.52 (0.19)	0.31 (0.02)	1.15 (0.07)	98.36 (5.34)
P. resinosa	Manitoba (**24**)	17.58 (0.96)	10.64 (0.69)	1.81 (0.09)	4.09 (0.23)	8.43 (0.48)	0.19 (0.04)	1.77 (0.10)	1.26 (0.07)	0.67 (0.09)	0.07 (0.02)	- -	46.53 (2.40)
	Ontario (**25**)	16.31 (0.72)	9.03 (0.50)	1.92 (0.08)	3.88 (0.15)	8.36 (0.49)	0.16 (0.01)	1.76 (0.08)	1.22 (0.07)	0.37 (0.07)	0.05 (0.01)	0.05 (0.03)	43.10 (1.81)

P. sylvestris	Manitoba (**25**)	32.86 (2.04)	16.23 (1.25)	3.79 (0.21)	5.94 (0.37)	15.69 (1.26)	0.96 (0.13)	2.08 (0.13)	1.68 (0.10)	0.98 (0.20)	0.19 (0.02)	- -	80.40 (4.38)
	Ontario (**25**)	46.73 (2.22)	23.37 (1.47)	4.58 (0.18)	7.07 (0.26)	13.35 (0.65)	0.80 (0.09)	2.98 (0.15)	2.16 (0.08)	0.96 (0.19)	0.16 (0.03)	- -	102.15 (3.91)
P. albicaulis	Alberta (**21**)	58.71 (2.82)	26.79 (1.66)	11.60 (0.58)	11.65 (0.41)	3.58 (0.28)	26.89 (3.14)	6.10 (0.30)	3.63 (0.14)	1.53 (0.35)	7.13 (0.71)	1.90 (0.11)	159.50 (7.13)
P. flexilis	Alberta (**22**)	23.79 (2.46)	10.17 (91.13)	6.86 (0.70)	5.28 (0.54)	2.56 (0.39)	2.79 (0.82)	1.66 (0.20)	2.05 (0.20)	0.33 (0.09)	0.86 (0.16)	0.88 (0.10)	57.23 (5.85)
P. ponderosa	Montana (**31**)	3.29 (1.00)	1.90 (0.53)	1.50 (0.21)	1.86 (0.35)	0.38 (0.14)	3.96 (1.70)	0.52 (0.08)	2.27 (0.38)	1.45 (0.45)	0.56 (0.16)	0.13 (0.04)	16.24 (2.45)
P. glauca	Alberta (**25**)	16.12 (1.04)	5.16 (0.60)	3.38 (0.23)	3.12 (0.26)	1.02 (0.18)	2.63 (0.20)	1.74 (0.09)	1.77 (0.10)	0.41 (0.12)	0.33 (0.05)	0.03 (0.02)	35.70 (2.36)
	Northwest Territories (**25**)	14.38 (0.85)	3.70 (0.26)	2.30 (0.18)	3.44 (0.23)	1.40 (0.10)	3.20 (0.48)	1.33 (0.06)	2.46 (0.19)	1.00 (0.29)	0.42 (0.07)	- -	33.64 (1.43)
P. tremuloides	Alberta (**25**)	48.12 (2.03)	1.02 (0.05)	6.26 (0.18)	1.09 (0.10)	5.35 (0.33)	0.03 (0.004)	4.69 (0.20)	0.81 (0.03)	0.03 (0.02)	0.26 (0.01)	1.07 (0.06)	68.73 (2.59)

Supplementary Table 2.1.3. Mean concentration (µg/g of dry weight of phloem) of individual fatty acids from *Pinus contorta*, *P. banksiana*, and *Populus tremuloides* added in each tube to observe *Dendroctonus ponderosae* development in Figure 2.8. (a,b). Abbreviated letters denote individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid).

Tree Species	**Saturated Fatty Acids**					**Unsaturated Fatty Acids**					
	PA	BA	LvA	PDA	SA	LA	OA	GLA	ALA	EDA	ARA
P. contorta	2.46	2.00	1.47	0.26	0.23	9.61	5.42	0.89	0.70	0.52	0.28
P. banksiana	2.07	1.36	2.25	0.09	0.22	9.02	4.17	0.42	1.02	0.48	0.28
P. tremuloides	1.88	0.33	1.61	0.08	0.32	14.46	0.31	0.01	1.41	0.24	0.01

Supplementary Table 2.1.4. Mean concentration (µg/ml of wet weight of phloem) of individual fatty acids from *Pinus contorta, P. banksiana,* and *Populus tremuloides* added in each Petri dish plate to observe growth of a symbiotic fungus *Grosmannia clavigera* associated with *Dendroctonus ponderosae* in Figures 2.9. (b) and 2.10. (a-k). Abbreviated letters denote individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid).

Tree Species	**Saturated Fatty Acids**					**Unsaturated Fatty Acids**					
	PA	BA	LvA	PDA	SA	LA	OA	GLA	ALA	EDA	ARA
P. contorta	78.06	63.48	46.69	8.23	7.41	305.07	171.92	28.13	22.36	16.59	9.04
P. banksiana	46.57	30.56	50.67	1.99	5.04	203.01	93.87	9.37	23.02	10.87	6.30
P. tremuloides	57.89	10.07	49.48	2.39	9.91	445.04	9.442	0.26	43.40	7.52	0.24

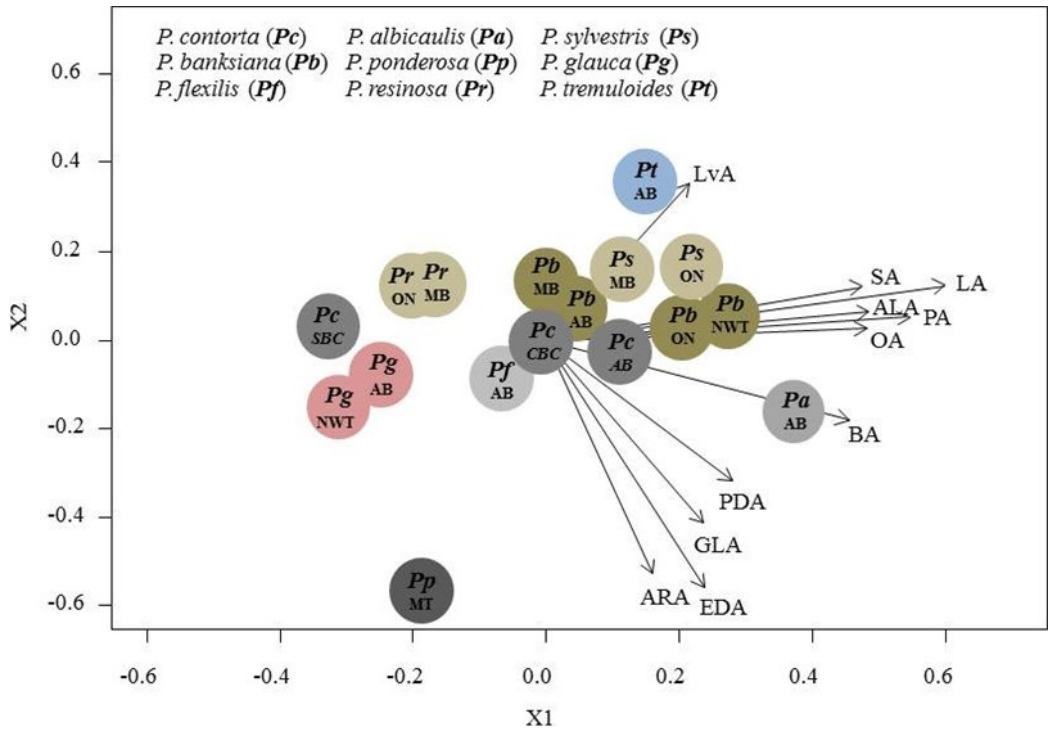


Figure 2.1. Non-metric multidimensional scale biplot, X1 and X2 axes display average concentration (µg/g of dry weight of phloem) of fatty acid profiles with various tree species in different locations. Vectors are individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid). Colored circles with abbreviations representing tree species (*Pinus contorta* (*Pc*), *P. banksiana* (*Pb*), *P. flexilis* (*Pf*), *P. albicaulis* (*Pa*), *P. ponderosa (Pp)*, *P. resinosa* (*Pr*), *P. sylvestris* (*Ps*), *Picea glauca* (*Pg*), and *Populus tremuloides* (*Pt*) and locations of tree sampling (ON=Ontario, MB=Manitoba, NWT=Northwest Territories, AB=Alberta, CBC and SBC are central and southern British Columbia, MT=Montana (USA)). The overlay of vectors indicates how the individual fatty acids are correlated with the tree species in each location. The number of trees varied per species and location from 12 to 45, total 451.

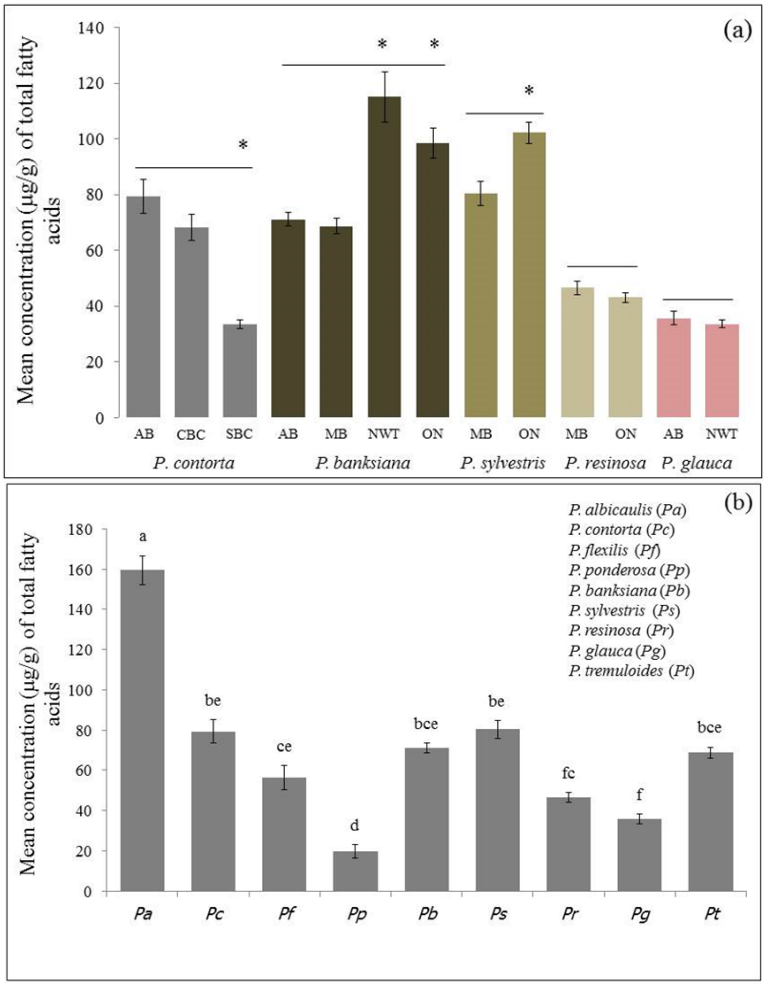


Figure 2.2. Comparison of mean concentrations of total fatty acids (µg/g of dry weight of phloem) among various tree species. (a) Colored bars represent relative mean concentrations of total fatty acids within a species in different locations: *Pinus contorta* from Alberta (AB), central and southern British Columbia (CBC and SBC); *P. banksina* from AB, Manitoba (MB), Northwest Territories (NWT), and Ontario (ON), *P. sylvestris* and *P. resinosa* from MB and ON, and *Picea glauca* from AB and NWT. Stars indicate statistical differences within a species. (b) Bars represent the pooled mean concentrations of total fatty acids for each tree species indicated in (Figure 2a). ANOVA and Tukey's HSD were applied for statistical comparisons. Letters represent differences among tree species at α=0.05 and error bars represent (±SE). Sample size varied between 12 - 45.

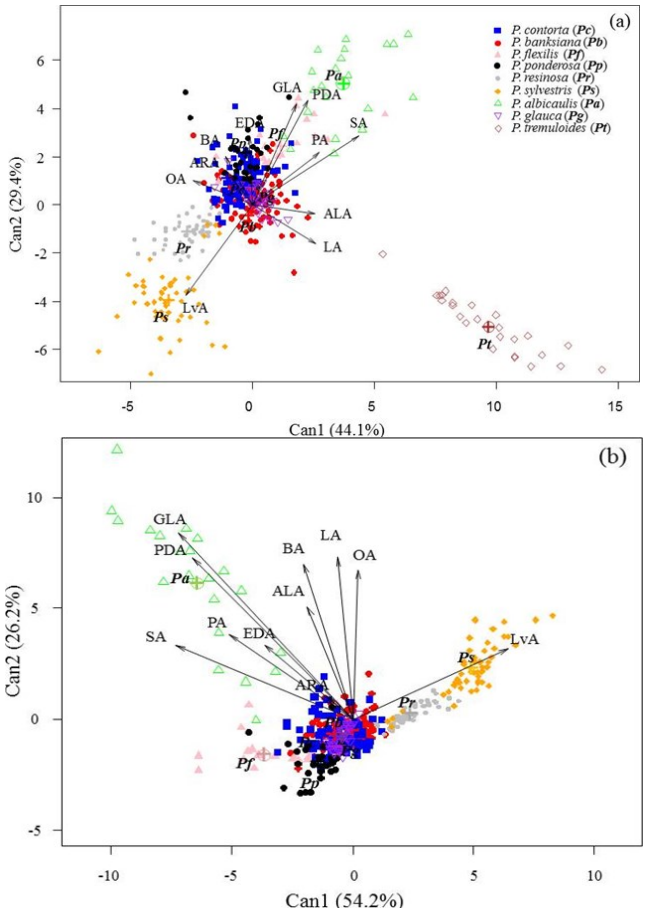


Figure 2.3. Canonical discriminant analysis, biplot with Can1 and Can2 axes, demonstrates a correspondence of fatty acid profiles and tree species with individual centroids (+) for each tree species based on concentrations of individual fatty acid profiles (µg/g of dry weight of phloem). Each point characterizes one tree, including (*Pinus contorta* (*Pc*), *P. banksiana* (*Pb*), *P. flexilis* (*Pf*), *P. ponderosa (Pp)*, *P. resinosa* (*Pr*), *P. sylvestris* (*Ps*), *P. albicaulis* (*Pa*), *Picea glauca* (*Pg*), and *Populus tremuloides* (*Pt*). Vectors represent individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid). (a) All nine tree species were included in the analysis. (b) Only conifers were included in the analysis.

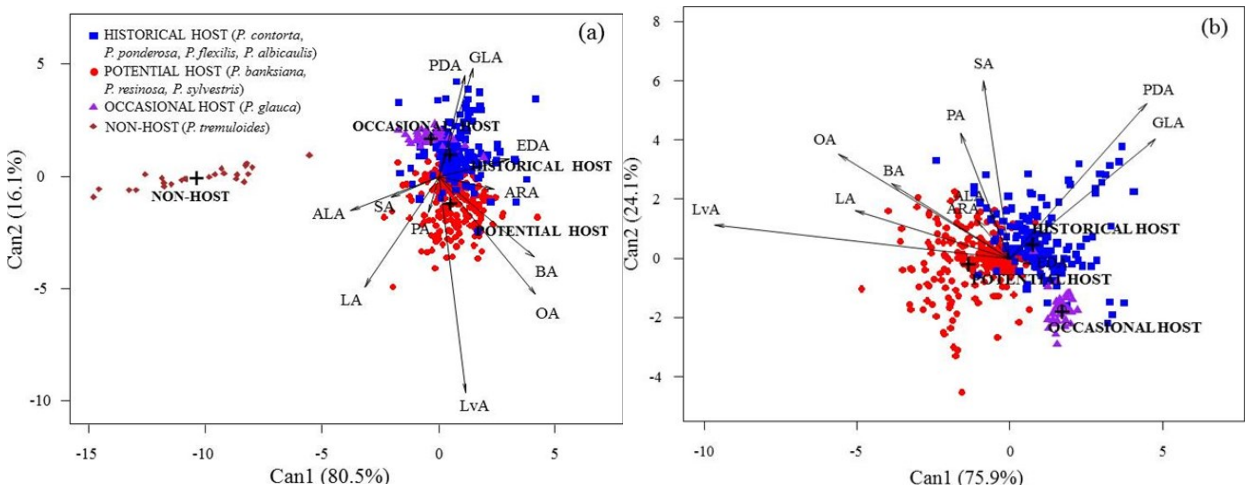


Figure 2.4. Canonical discriminant analysis, biplot with Can1 and Can2 axes, demonstrates a correspondence of fatty acid profiles and historical, occasional, potential hosts, and non-host group of centroids (+) based on concentration of fatty acids (µg/g of dry weight of phloem). Tree species reported in Figure 3 were grouped based on their interaction with the mountain pine beetle, *Dendroctonus ponderosae* as: historical hosts (*Pinus contorta,* n=90, *P. ponderosa*, n=31, *P. flexilis*, n=22, and *P. albicaulis*, n=21); potential hosts (*P. banksiana*, n=122, *P. resinosa*, n=49, *P. sylvestris*, n=50), occasional host (*Picea glauca*, n=41), and non-host (*Populus tremuloides*, n=25). Points characterize number of trees in each group. Vectors denote individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid). (a) All four groups (historical, potential, occasional hosts and non-host) were included in the analysis. (b) Only historical, potential, occasional hosts were included in the analysis.

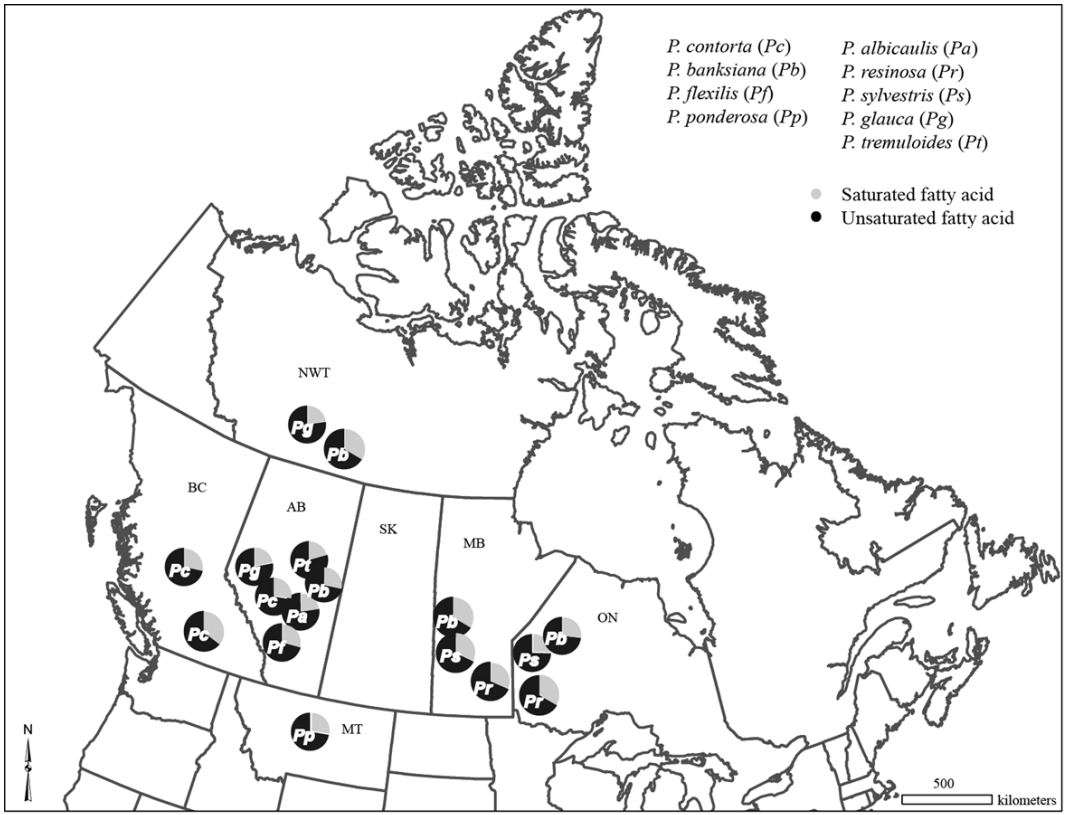


Figure 2.5. Mean proportion (%) of total saturated and unsaturated fatty acids (dry weight of phloem) from tree species in different locations. Abbreviated letters denote sample locations: AB=Alberta, BC=British Columbia, MB=Manitoba, ON=Ontario, NWT=Northwest Territories, MT=Montana. In each circle, proportions of saturated and unsaturated fatty acids are shown and abbreviations denote tree species, including *Pinus contorta* (*Pc*), *P. banksiana* (*Pb*), *P. flexilis* (*Pf*), *P. ponderosa (Pp)*, *P. albicaulis* (*Pa*), *P. resinosa* (*Pr*), *P. sylvestris* (*Ps*), *Picea glauca* (*Pg*), and *Populus tremuloides* (*Pt*).

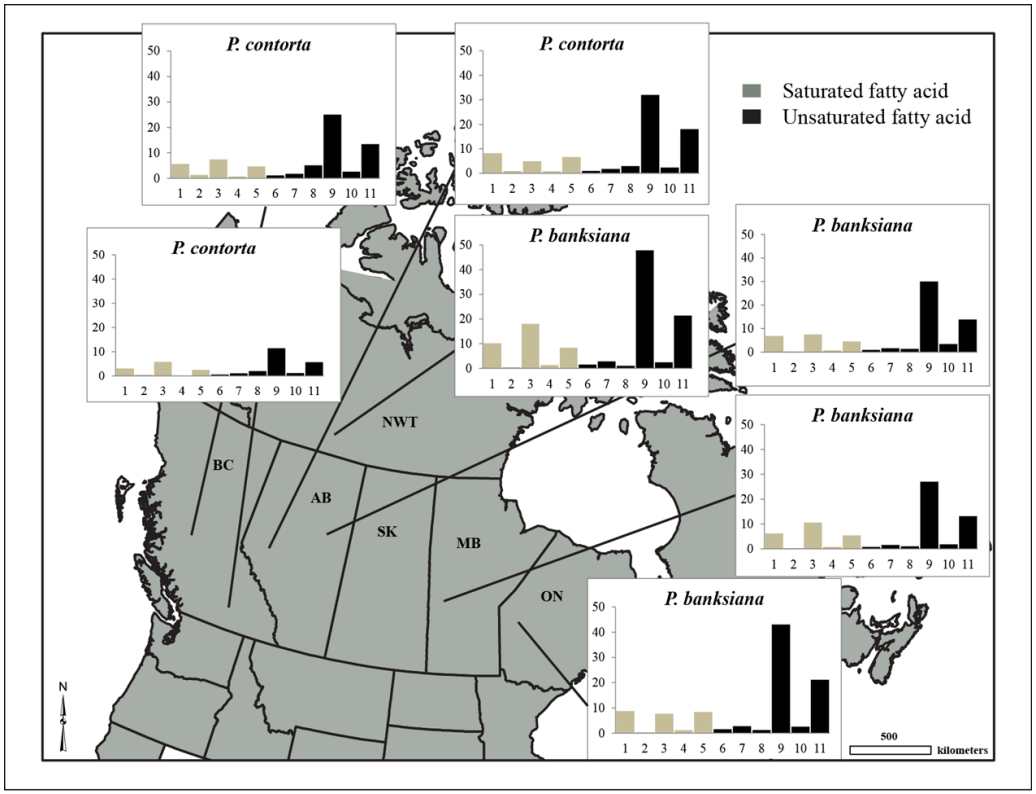


Figure 2.6. Mean concentration (µg/g of dry weight of phloem) of individual saturated and unsaturated fatty acids from *Pinus contorta* and *P. banksiana* in different locations. Bars with different colors indicate individual fatty acids (1-5; 1=palmitic acid, 2=pentadecanoic acid, 3=levunilic acid, 4=stearic acid, 5=behenic acid, 6-11; 6=arachidonic acid, 7=eicosadienoic acid, 8=gamma-linolenic acid, 9=linoleic acid, 10=alpha-linolenic acid, 11=oleic acid). Abbreviated letters denote sampled locations: AB=Alberta, BC=British Columbia, MB=Manitoba, ON=Ontario and NWT=Northwest Territories.

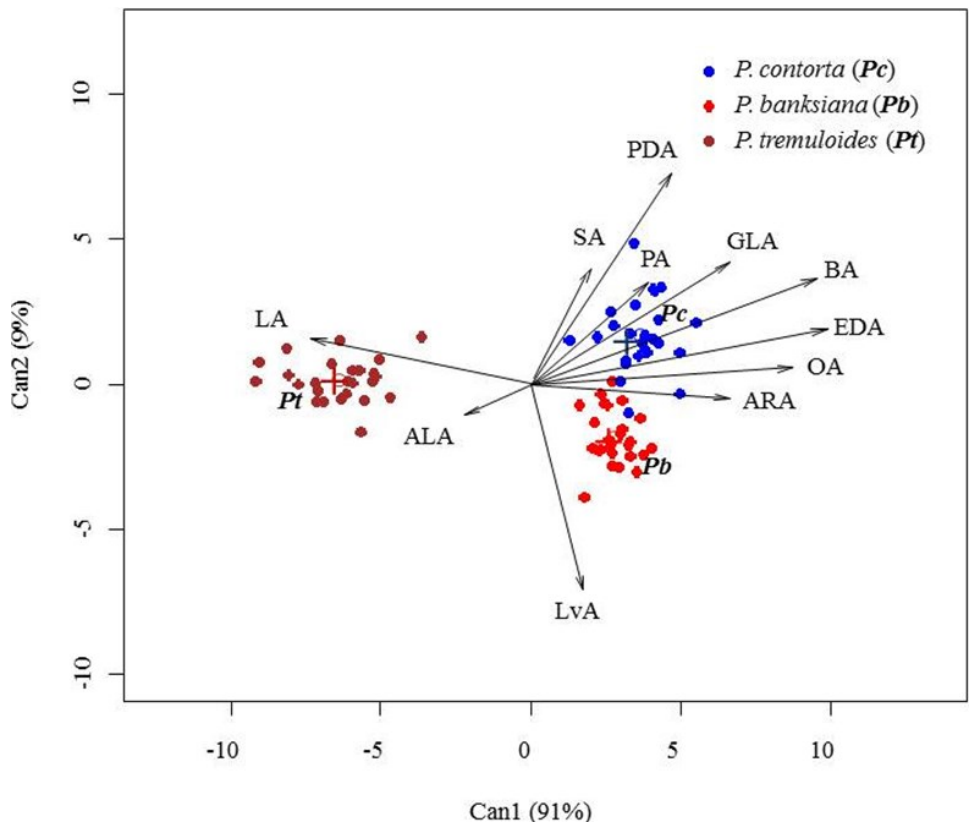


Figure 2.7. Canonical discriminant analysis, biplot with Can1 and Can2 axes, demonstrates a correspondence of fatty acid profiles and tree species group of centroids (+) based on concentration of fatty acids (µg/g of dry weight of phloem). Tree species: *Pinus contorta, P. banksiana*, and *Populus tremuloides*, n=25. Points characterize the number of trees in each species. Vectors denote individual fatty acids (PA=palmitic acid, BA=behenic acid, LvA=levulinic acid, PDA=pentadecanoic acid, SA=stearic acid, LA=linoleic acid, OA=oleic acid, GLA=gamma-linolenic acid, ALA=alpha-linolenic acid, EDA=eicosadienoic acid, and ARA=arachidonic acid).

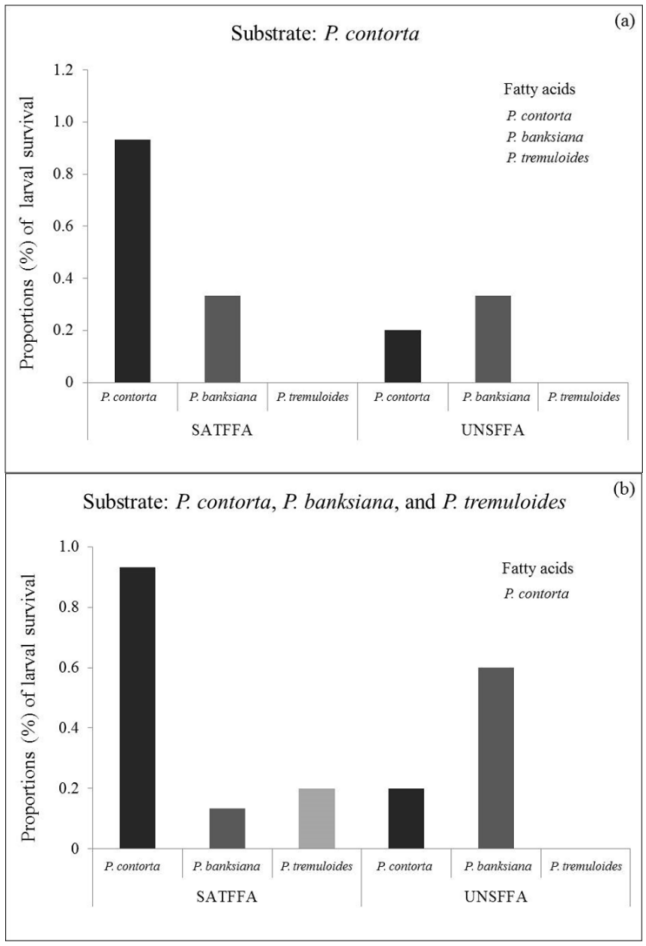


Figure 2.8. The proportion (%) of *Dendroctonus ponderosae* larval survival in rearing tubes with different treatments (one egg per tube). (a) Substrate (phloem: sapwood, ratio 9:1) (*Pinus contorta*) was amended with saturated or unsaturated fatty acids from one of three tree species, including *Pinus contorta*, *P. banksiana*, and *Populus tremuloides*. (b) Saturated or unsaturated fatty acids from *P. contorta* mixed with substrates from one of three tree species, including *P. contorta*, *P. banksiana*, and *P. tremuloides.* In both experiments, tubes were also inoculated with *Grosmannia clavigera*, a fungal associate of *D. ponderosae*. Mean concentration added for each tube were reported in Supplementary Table 2.1.3. SATFFA indicates saturated fatty acid plus fungus and UNSFFA unsaturated fatty acid plus fungus. Proportions were determined by dividing the larval survival by the sum of all tubes per treatment (n=15).

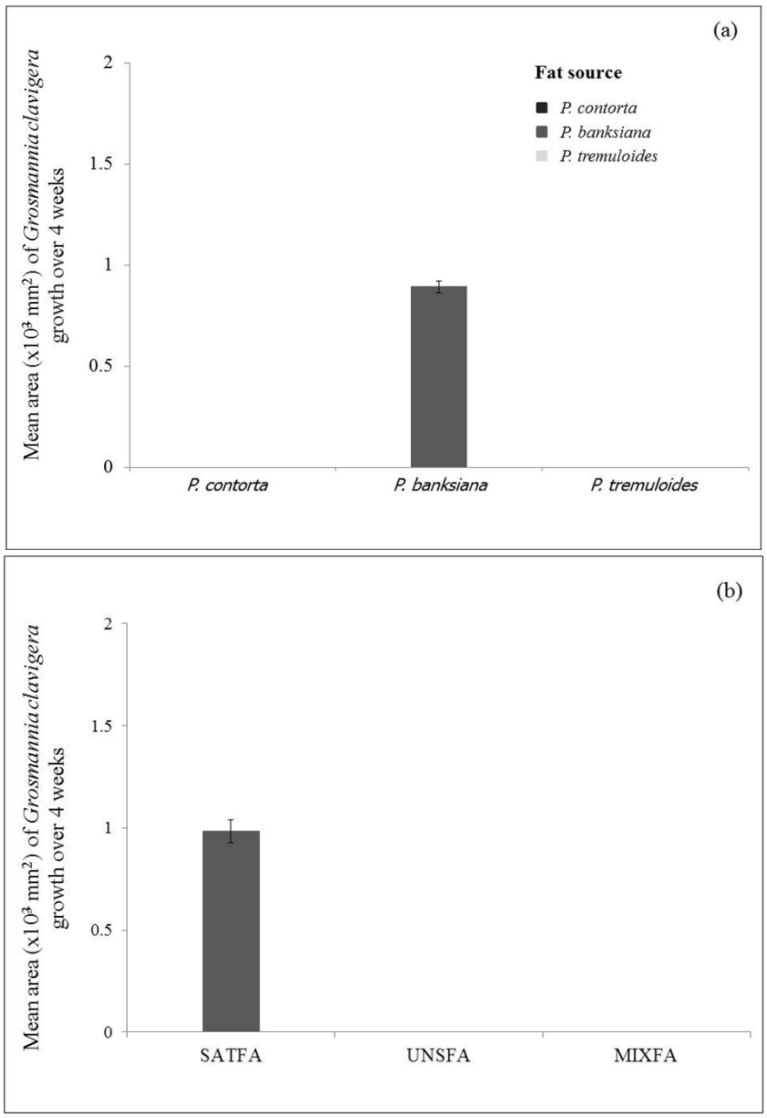


Figure 2.9. Mean area (x 10^3 mm^2) of *Grosmannia clavigera* growth on medium amended with fatty acid concentrations of different host species. (a) Total fatty acids from one of three tree species, *Pinus contorta*, *P. bansksiana*, and *Populus tremuloides*, were added to the medium. (b) Saturated (SATFA), unsaturated (UNSFA), or total (MIXFA) fatty acids simulated from each tree species, *P. contorta*, *P. bansksiana*, and *P. tremuloides*, were added to the medium, n=15 for each treatment. Total growth of fungus was measured after four weeks in each Petri dish. Mean concentration added for each Petri dish were reported in Supplementary Table 2.1.4. Bars represent standard error.

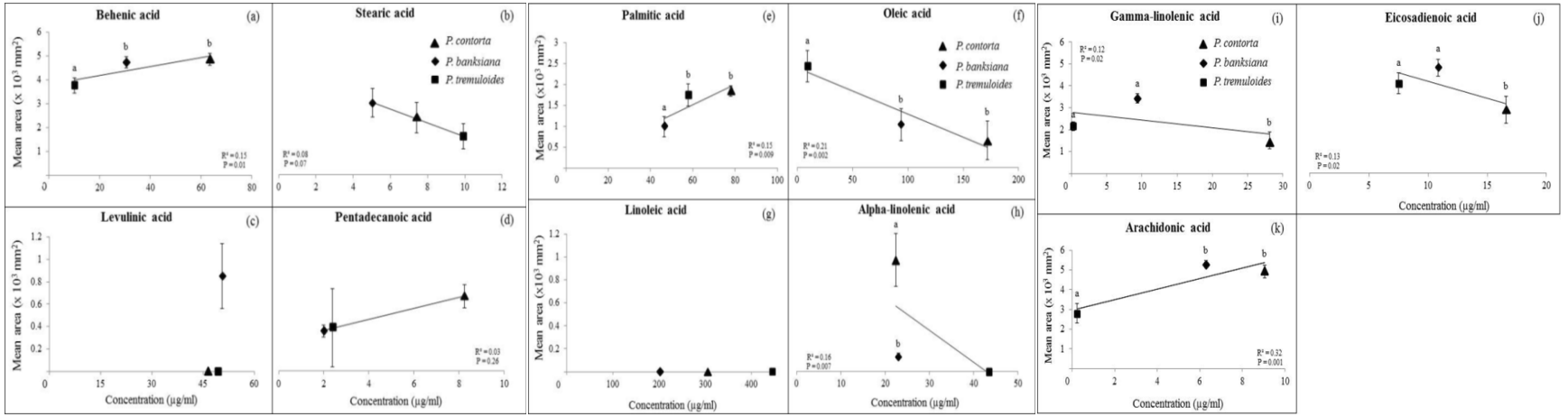


Figure 2.10. Mean area (x 10^3 mm^2) of *G. clavigera* growth on medium amended with individual fatty acids at different concentrations. Each concentration represents a particular tree species and concentrations added for each Petri dish were reported in Supplementary Table 2.1.4. Total growth of fungus was measured after four weeks in each Petri dish, n=15 per concentration treatment. Lines indicate relationships between dependent variable (y axis) and explanatory variables (x axis) and symbols denote tree species. Comparisons among mean were done using ANOVA and lines were generated using regression analysis, except for LvA and LA (c and g). Letters represent differences between tree species at α=0.05 and error bars represent (±SE).

CHAPTER 3

THESIS DISCUSSION

Overall, our studies show that fatty acid composition varied within and among tree species and more importantly between hosts and non-host tree species. In addition, unlike non-host species (aspen, *Populus tremuloides*), fatty acid concentrations of both historical (lodgepole pine, *Pinus contorta*) and potential (jack pine, *P. banksiana*) hosts were suitable for mountain pine beetle (*Dendroctonus ponderosae*) larval survival. Furthermore, historical and potential hosts, and non-host influenced the growth of beetle's associated fungus differently depending on their fatty acid concentrations.

Fatty acid composition of different perennial woody trees

Tree species were different in terms of their fatty acid concentrations. Interestingly, historical hosts and potential hosts (jack pine, red pine, *P. resionosa*, Scots pine, *P. sylvestris*) of *D. ponderosae* were grouped closely together, suggesting that potential hosts may be suitable for beetles. This suggestion is in agreement with earlier observations that jack pine, red pine, and Scots pine are all susceptible to mountain pine beetle. For example, during a mountain pine beetle epidemic in Colorado, they attacked and killed Scots pine (Leatherman & Cranshaw 1996; Ono 2003). Likewise, Taft *et al*. (unpublished record) showed that mountain pine can reproduce in bolts of red pine.

I also found that lodgepole pine (40%), jack pine (42%), and aspen (70%) had high concentrations of LA in their phloem, aspen had the highest. Although it was not within the scope of this thesis to investigate why aspen trees had a high of LA concentration compared to

the other species, perhaps differences in plant traits (fast growth vs. slow growth, winter dormancy vs. no dormancy) may explain the difference among species (Saranpää & Nyberg 1987; Schoettle & Fahey 1994; Angelcheva *et al*. 2014). Likewise, environment and genetics may also influence concentrations of fatty acids (Mattson & Haack 1987; Schoettle & Fahey 1994; Routaboul *et al.* 2000; McCaughey & Tomback 2001; Black & Colbert 2008; Angelcheva *et al*. 2014).

Fatty acid concentrations and survival of beetle larvae and its associated fungal growth

I found that effects of fatty acid concentrations on the larval survival were mediated by host species. Particularly, I did not observe any larval survival in the media amended with fatty acids of aspen, relative to other species. These results indicate that larval survival depends on fatty acids at certain concentrations. This is the first study demonstrating the role of total fatty acids in any bark beetle species. Even though most insects require some essential fatty acids, including unsaturated fatty acids, in their diet from their hosts (Dadd 1985) apparently the same fatty acids at high concentrations might not be suitable for larval growth. Although there is no particular work on how fatty acids affect insect development in other systems, it is generally accepted that nutritional quality of plants influence host preference of insect herbivores (Mattson 1980; Awmack & Leather 2002; Behmer 2009), suggesting that fatty acids can also be important in host selection by herbivores.

Furthermore, I found differences in growth of mountain pine beetle's associated fungus in media amended with fatty acids from historical and potential hosts and non-host species. Interestingly, total purified lipids and saturated fatty acids of jack pine yielded fungal growth, in contrast to the fatty acid concentrations of lodgepole pine and aspen, which reduced fungal

growth. In particular, I found noticeably positive correlations between fungal growth and concentrations of saturated (BA, PA, PDA) and unsaturated (ARA) fatty acids, in all cases lodgepole pine had higher concentrations of these compounds than aspen. Likewise, unsaturated fatty acids, such as OA, GLA, and EDA reduced fungal growth and the concentrations of these compounds were higher in lodgepole pine, suggesting anti-fungal properties of these fatty acids. For LvA (saturated), only concentration of jack pine yielded fungal growth and the other two species, lodgepole pine and aspen, reduced fungal growth. Aspen had the highest concentration of ALA (unsaturated) relative to lodgepole pine and jack pine, and reduced fungal growth. Notably, any concentration of LA (unsaturated) reduced fungal growth, indicating anti-fungal properties of this fatty acid. Although earlier studies reported changes in fatty acid concentrations in lodgepole pine sapwood in response to fungal inoculations (Gao *et al*. 1994) and anti-fungal properties of individual fatty acids (Walters *et al*. 2004), my study is the first comprehensive work demonstrating how fatty acids affect to both a bark beetle species and its symbiotic microbial associates.

Management implications

In this thesis, I focused on fatty acid profiles and concentrations of various tree species and their effects on growth and development of mountain pine beetle and its associated fungus. Apparently, host and non-host tree species were separated based on the profiles and concentrations of their fatty acids. I speculate that close correlation with both LA and ALA and highest concentrations of total unsaturated fatty acid in aspen, relative to the lodgepole and jack pines explain in part why beetles do not colonize aspen even though it is highly abundant in its native range. Additionally, similarity between fatty acid profiles of potential hosts of mountain

pine beetle, jack pine, red pine, and Scots pine, and beetle's historical hosts might indicative suitability of jack pine as well as others to mountain pine beetle in the new extended habitat. Furthermore, fatty acid concentrations strongly affected brood development of mountain pine beetle and growth of its symbiotic fungus. Overall, these results strongly demonstrate that fatty acid composition of trees can be important in host and range expansion of mountain pine beetle and should be considered in management of beetles in jack pine forests. For example, such information might be useful in modeling studies integrating abiotic (i.e. weather) and biotic (i.e. natural enemies, species composition) factors in determining suitability of native trees of the boreal forests. These results are also important for determining suitability of jack pine as a host to mountain pine beetle as both host secondary compounds and fatty acids likely play essential roles in suitability of jack pine trees.

Implications to future studies

I found that both profiles and concentrations of fatty acids varied within and among tree species, suggesting possible roles of environment and genetics in fatty acid composition of plants. Since, earlier studies reported that fatty acid compositions of trees, particularly the ratio of unsaturated to saturated fatty acids, change in response to seasonal temperature fluctuations (Angelcheva *et al*. 2014), and in this thesis, I found that unsaturated fatty acids appear to have defensive properties against mountain pine beetle and its symbiotic fungus, future studies should investigate whether susceptibility of jack pine as a host varies depending on its unsaturated fatty acid concentrations. Likewise, one should investigate the changes between the unsaturated and saturated fatty acid concentrations of trees before, during, and after insect attacks.

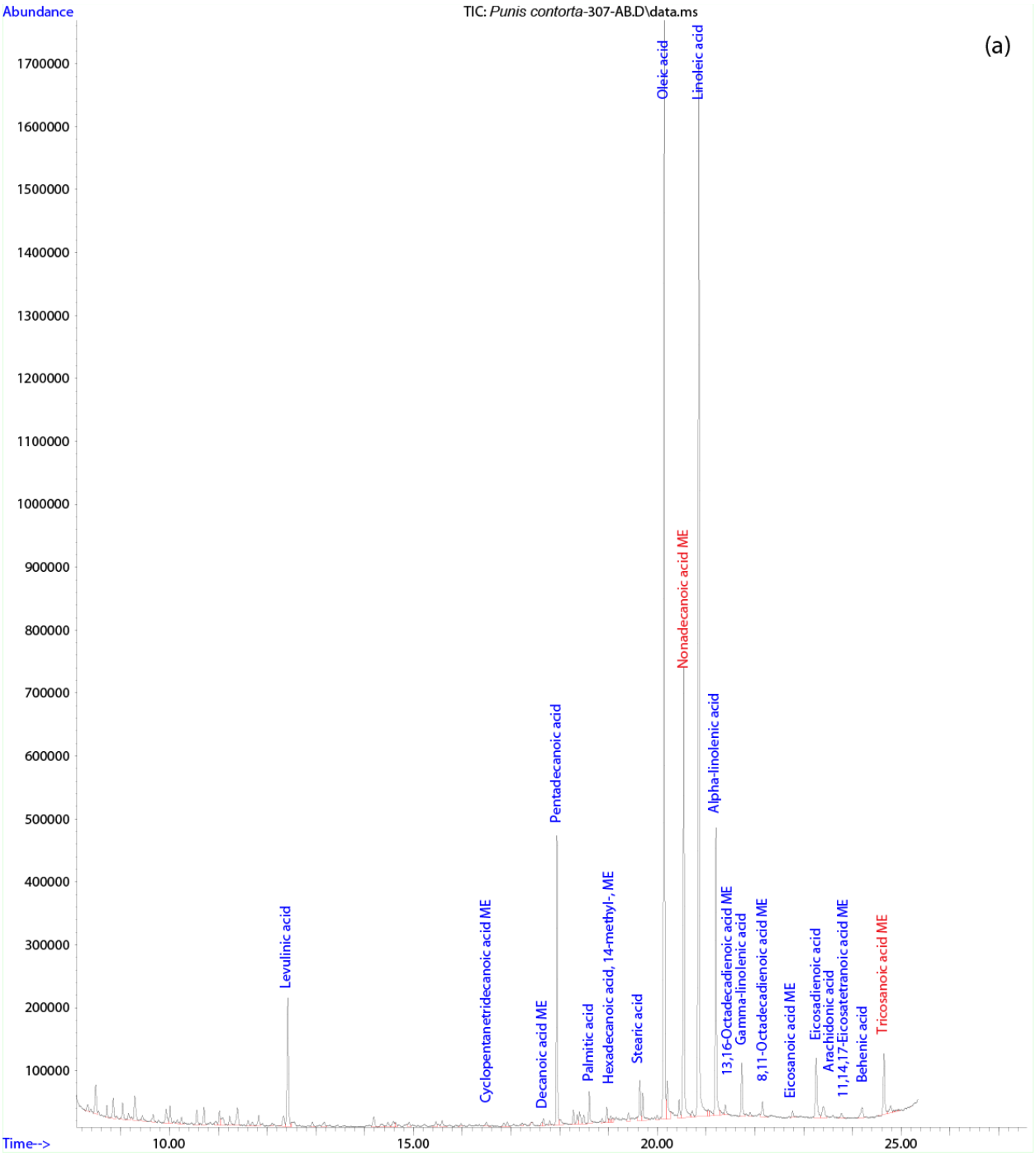
I used in the current study an array of analytical techniques in order to draw up fatty acid profiles in different tree species. We yet encountered opportunity for growth through which we could critically approach these methods. First, I noticed a coloration come in the extraction for each species ranging from green to yellow to purple. We know that the matrix of plant phloem is quite complex, yet the fact that certain colors appeared in some species but not in others which is intriguing. Also the method does not segregate free fatty acids from triacylglycerols. Perhaps in future experiments it could be to tear these apart for different functional studies. I also noticed some interference from the resin acids especially during the use of solid phase extraction (SPE) for the purification of fatty acids and optimized the technique to include a wash step that eliminates resin acids early in the process.

In current thesis, I used phloem substrate that was extracted (to remove lipids), dried in the oven at 70°C for 24 hrs (to remove polar and non-polar compounds) and sterilized. However, I suspect that the substrate still contained some defensive chemicals, such as resin acids and phenolics, which likely affected the results, particularly larval survival. Therefore, later studies should only use phloem substrate that is free of all chemical compounds. Alternatively, researchers should rear beetles in bolts with different fatty acid compositions and determine whether this has any implications for larval survival and their fatty acid contents. Linking fatty acid content of beetles to pheromone production by adult beetles should also be investigated because some pheromone components of beetles, such as *exo*-brevicomin are produced *de novo* through utilization of long-chain fatty acids (Blomquist *et al*. 2010).

CHAPTER 4

APPENDIX

GC/MS chromatogram analyses of phloem tissue from different tree species (a) *Pinus contorta*, (b) *P. banksiana*, (c) *P. flexilis*, (d) *P. albicalulis*, (e) *P. sylvestris*, (f) *P. resinosa*, (g) *P. glauca*, and (h) *P. tremuloides* (see details in Supplementary Table 2.1.1). The compounds were identified using NIST (National Institute of Standards and Technology) 2.0 v 1997. Stars (red color) denote that compounds are not fatty acid MEs. Note: nonadecanoic acid ME (M_{C23}) and tricosanoic ME (M_{C23}) are recovery and internal standards, respectively. Eleven (levulinic, pentadecanoic, palmitic, stearic, behenic, oleic, linoleic, alpha-linolenic, gamma-linolenic, eicosadienoic, and arachidonic acids) compounds were quantified.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100000
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20.00
25.00
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Linoleic acid
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Pentadecanoic acid
Levulinic acid
Cyclopentanetridecanoic acid ME
Decanoic acid ME
Palmitic acid
Hexadecanoic acid, 14-methyl-, ME
Stearic acid
13,16-Octadecadienoic acid ME
Gamma-linolenic acid
8,11-Octadecadienoic acid ME
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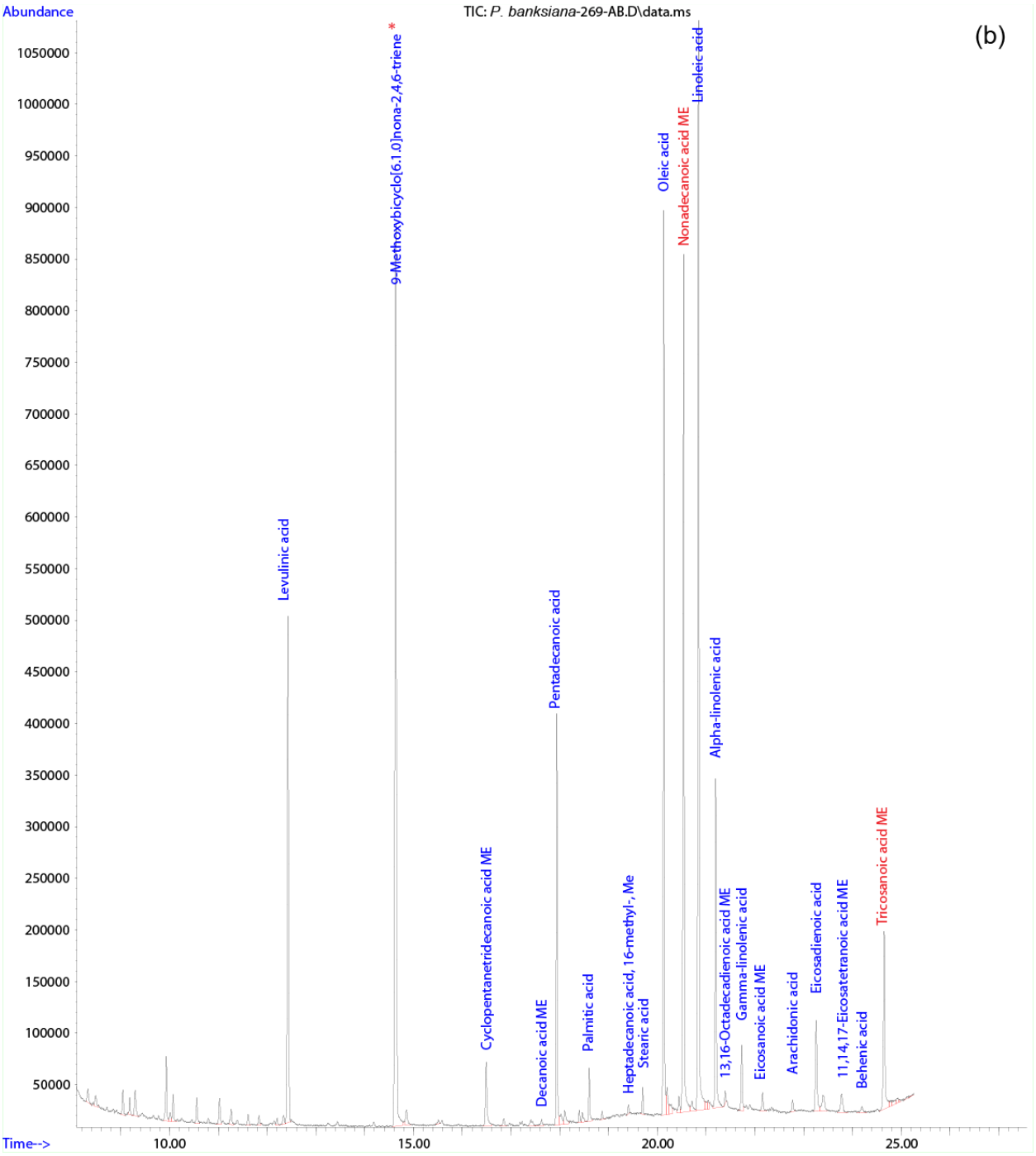
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Decanoic acid ME
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Palmitic acid
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Alpha-linolenic acid
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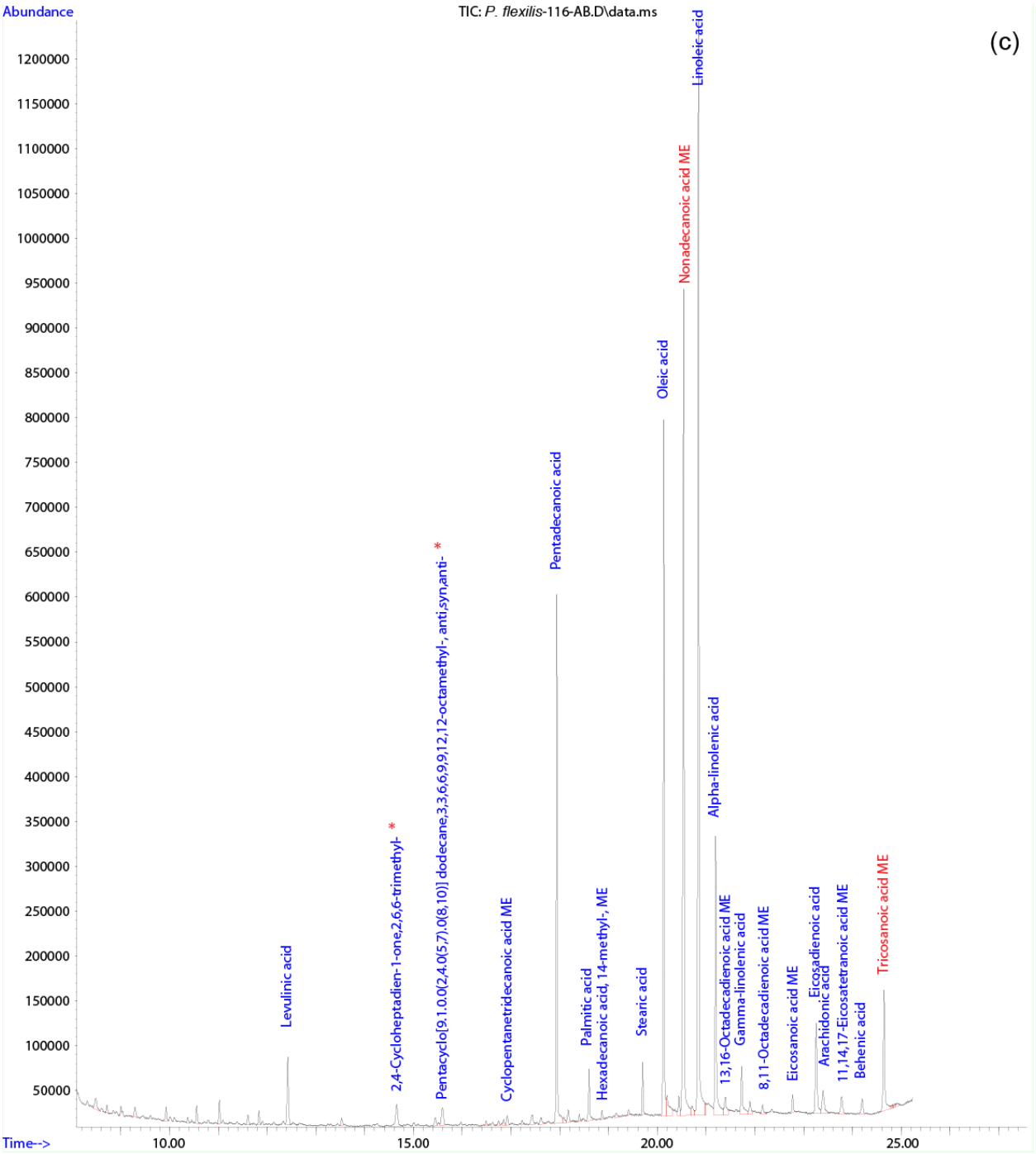
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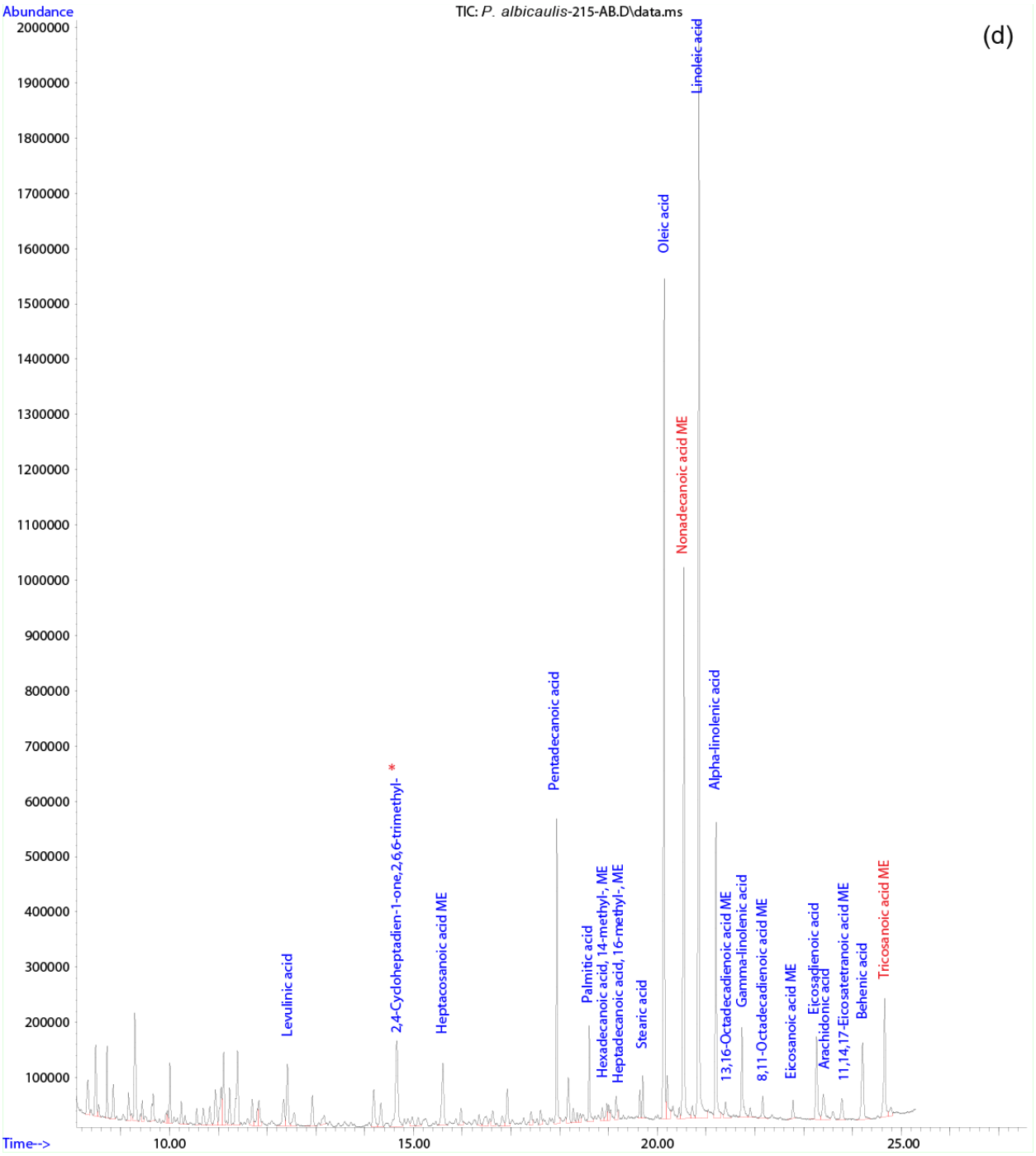
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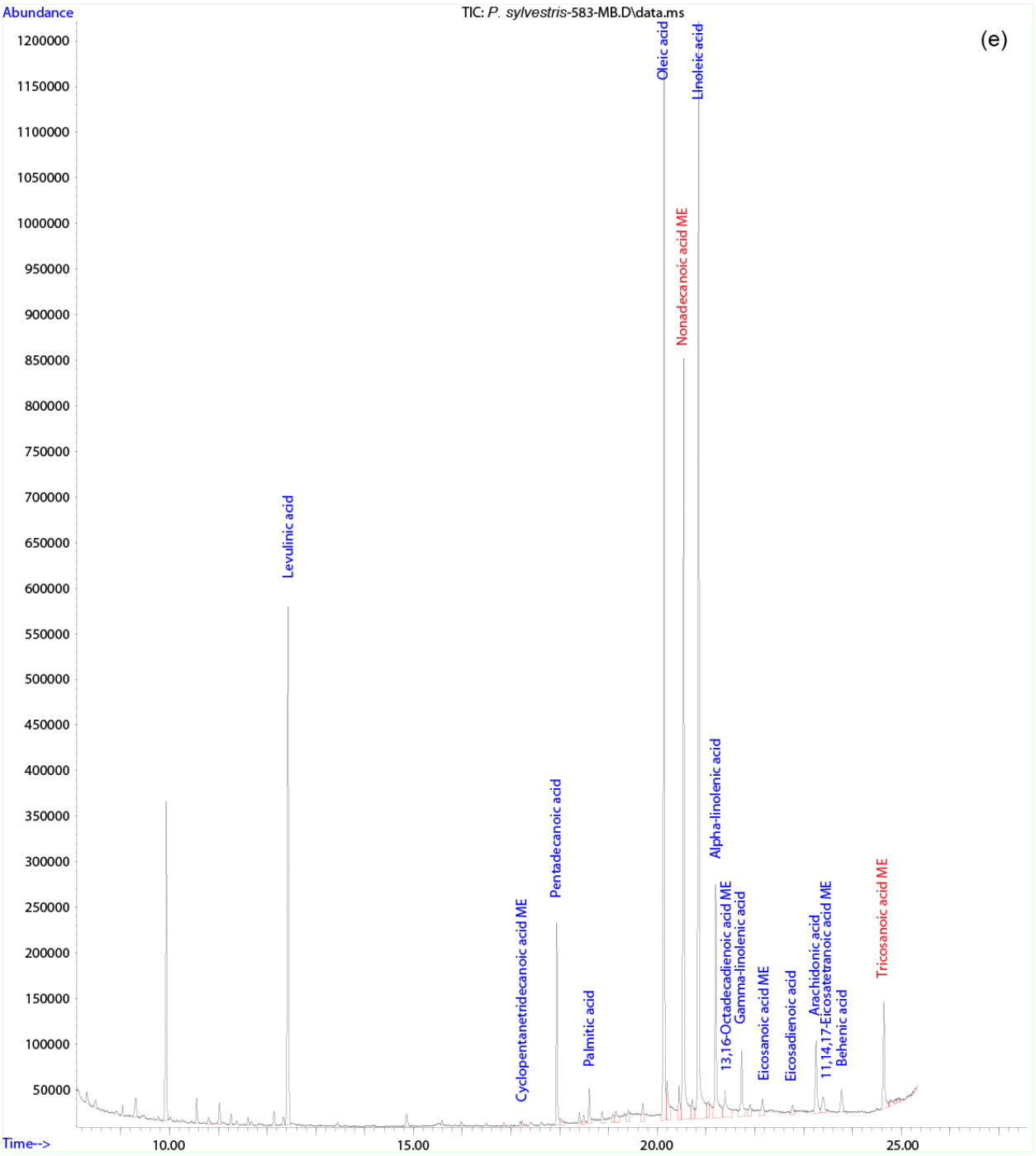
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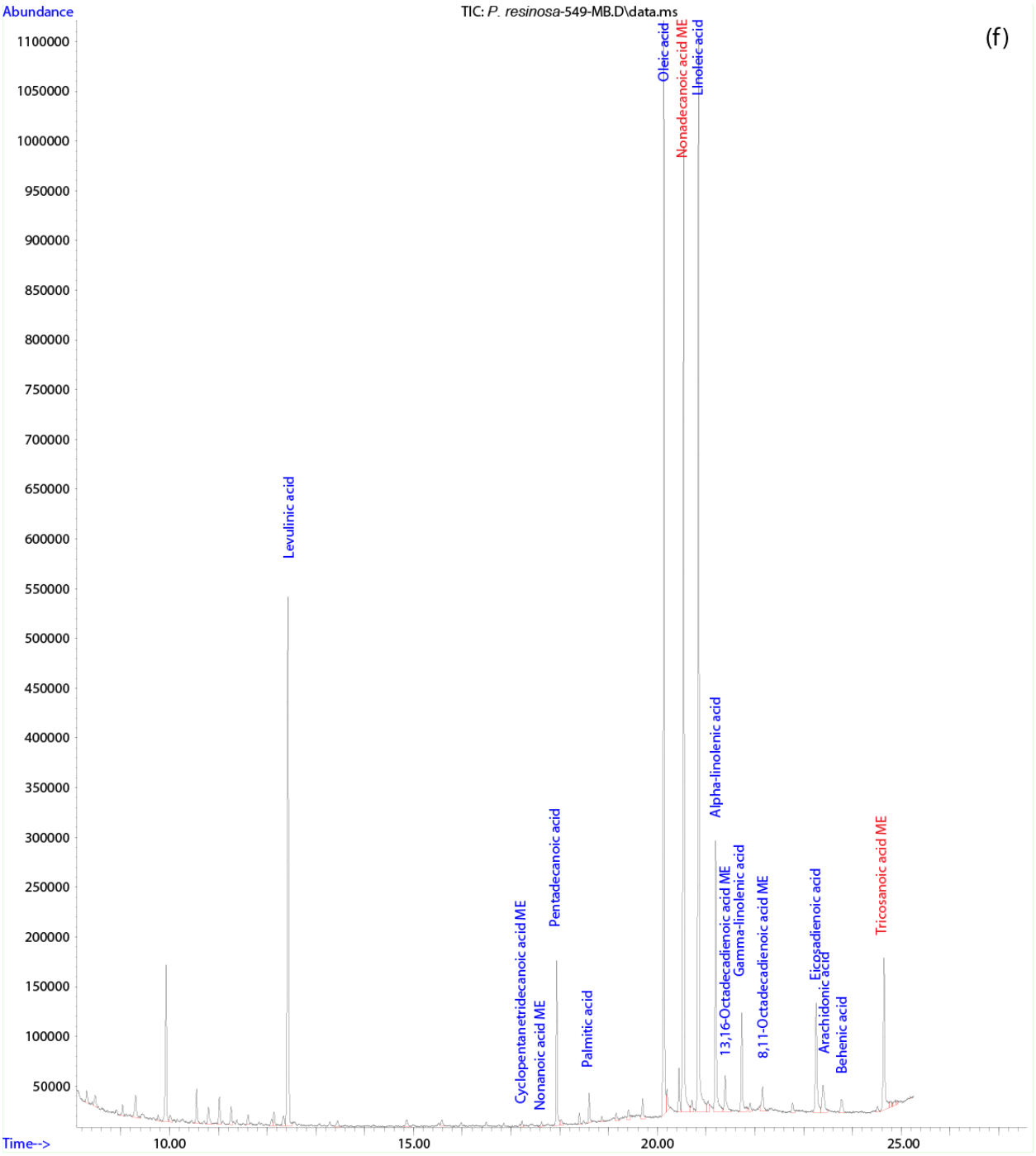
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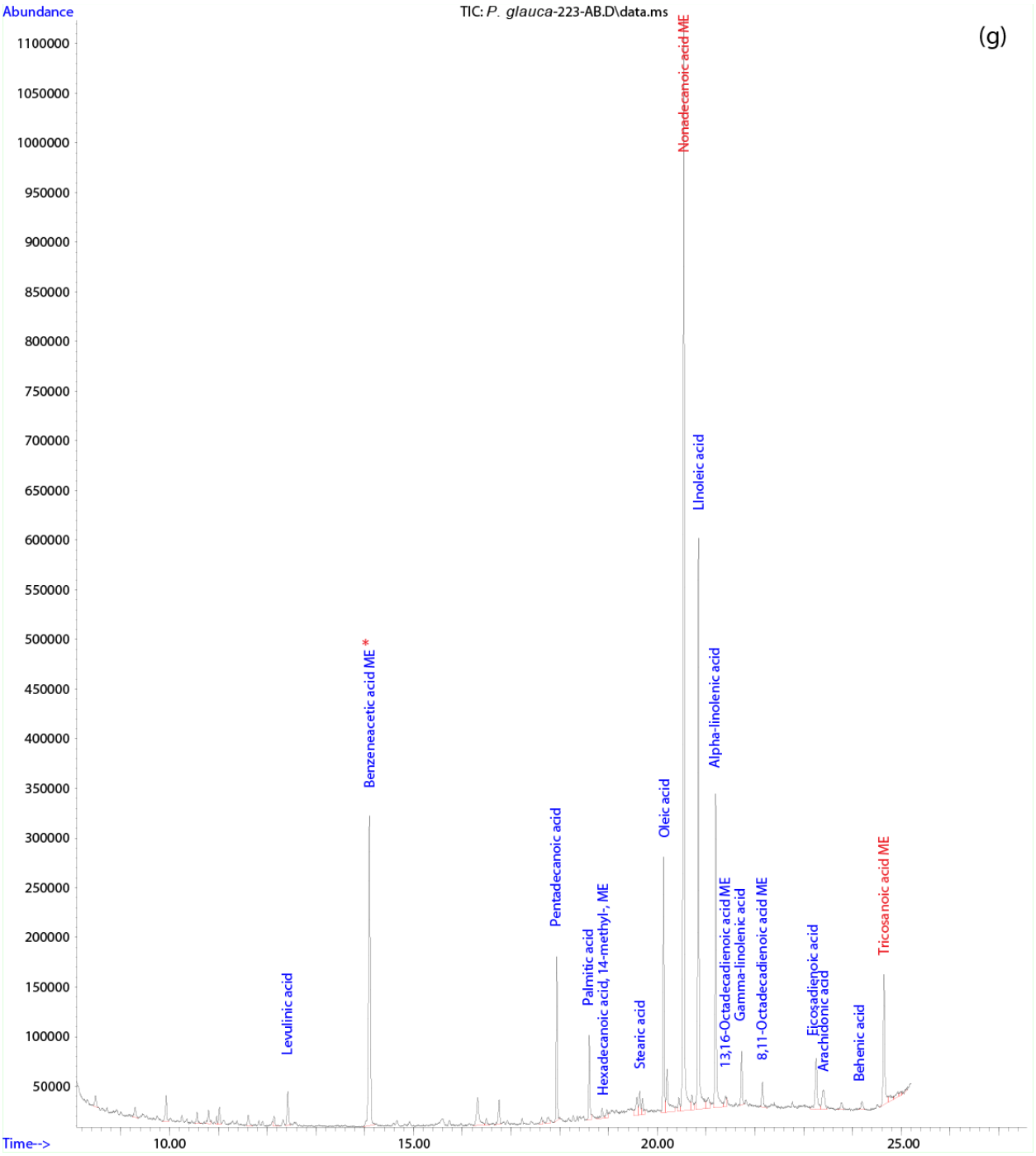
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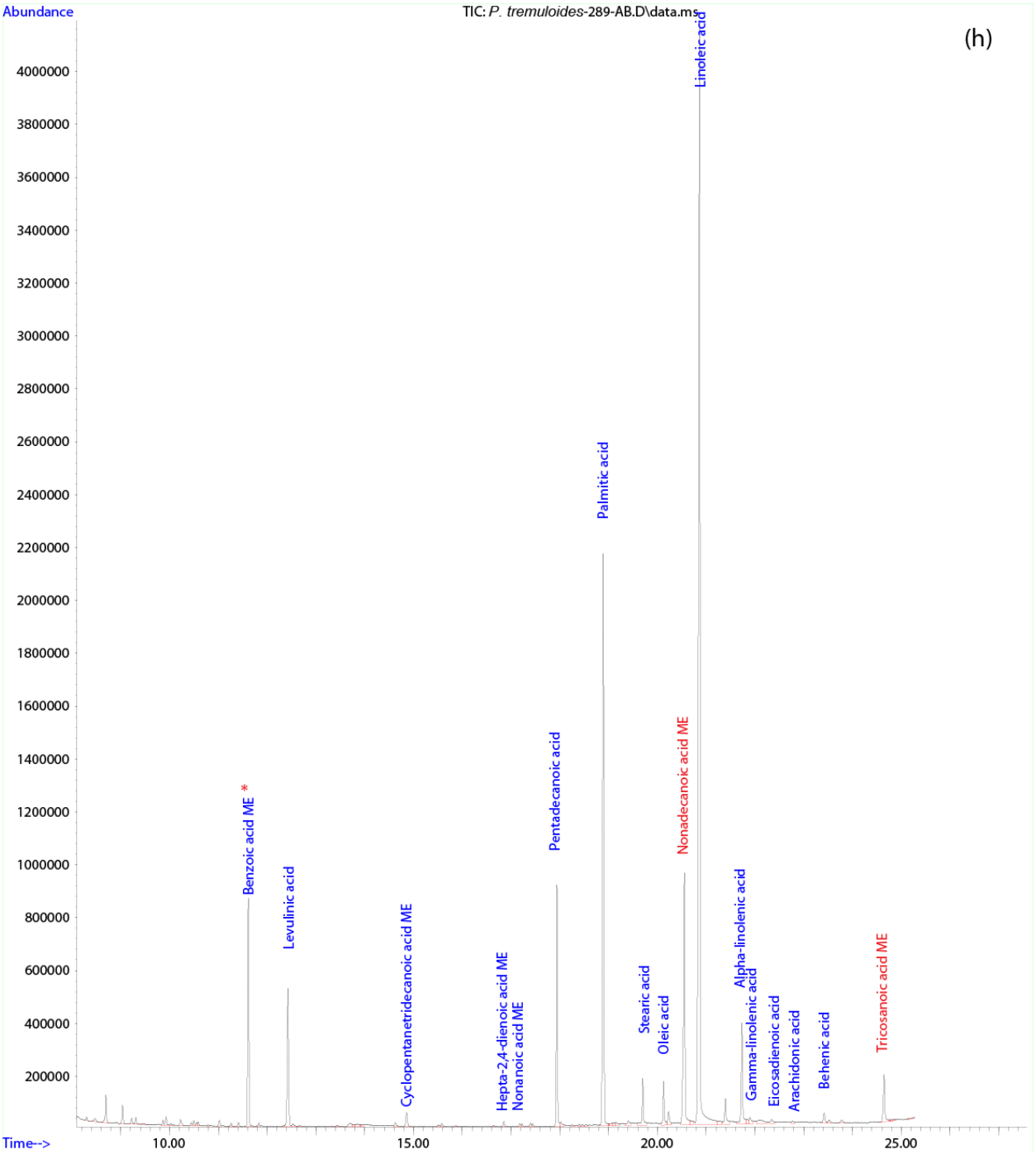
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25.00
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Cyclopentanetridecanoic acid ME
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Arachidonic acid
Behenic acid
Tricosanoic acid ME

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