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A BIOCHEMICAL, MORPHOLOGICAL, AND PHYSIOLOGICAL ANALYSIS OF DOUGLAS-FIR AND WESTERN LARCH: TWO HOSTS OF THE DOUGLAS-FIR BEETLE

presented by

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A BIOCHEMICAL, MORPHOLOGICAL, AND PHYSIOLOGICAL ANALYSIS OF DOUGLAS-FIR AND WESTERN LARCH: TWO HOSTS OF THE DOUGLAS-FIR BEETLE

By

Arden Nathan Frink Reed

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ABSTRACT

A BIOCHEMICAL, MORPHOLOGICAL, AND PHYSIOLOGICAL ANALYSIS OF DOUGLAS-FIR AND WESTERN LARCH: TWO HOSTS OF THE DOUGLAS-FIR BEETLE

By

Arden Nathan Frink Reed

Douglas-fir (*Pseudotsuga menziesii* (Beissn.) Franco) and western larch (*Larix occidentalis* Nutt.) growing in an uneven-aged, second growth stand in northern Idaho were studied for resistance mechanisms associated with Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopk.) attack. Attacks in young Douglas-fir or live western larch are rare. Successful brood production in live standing western larch has not been observed. Factors thought to be associated with resistance were chosen for study in: (1) Douglas-fir of two age and crown classes, (2) previously attacked and unattacked, mature Douglas-fir, and (3) mature, codominant western larch.

Unsuccessful brood production in western larch compared with Douglas-fir is proposed to be associated with greater phloem 3-carene content in larch. This is supported by the significant negative correlation between xylem oleoresin 3-carene content and attack rate in western larch. Significantly reduced phloem thickness, higher phloem moisture content and larger vertical sapwood resin ducts in larch were also hypothesized as factors associated with reduced beetle activity. Lack of oleoresin exudation

pressure in live standing larch indicates that oleoresin pressure is not associated with its resistance.

Analysis of phloem revealed no difference in lipid content between species, but significantly higher levels of total available carbohydrates, starch, reducing sugars, and crude protein were found in larch. Few qualitative or quantitative differences were found in xylem monoterpenes, resin acids, and core volatiles within Douglas-fir, while several quantitative species differences were found.

The greatest quantity of volatiles detected in steam distillation and gas chromatographic analysis of foliage, bark, phloem, and sapwood of Douglas-fir were monoterpenes, while higher molecular weight unknowns comprised the majority in larch. No qualitative but some quantitative differences in tissue volatile composition in relation to age and crown class in Douglas-fir and several quantitative differences between species were found. Foliage of both species had the greatest concentration of volatiles followed by bark, sapwood, and phloem in Douglas-fir and sapwood, phloem, and bark in larch. Except for 3-carene, most monoterpenes detected in oleoresin, core sections, and tissues had significantly greater concentrations in Douglas-fir than in western larch.

ACKNOWLEDGEMENTS

I wish to thank Dr. James W. Hanover (committee chairman) for guidance and the opportunity to pursue a doctoral degree and conduct this research; Drs. D. I. Dickmann, D. A. Reicosky, and G. A. Simmons for serving on my guidance committee; the U. S. Forest Service and Mr. M. M. Furniss of the Intermountain Forest and Range Experiment Station, Moscow, Idaho, for funding and expertise in selecting the study site and technical assistance in the field portion of the study. Finally, I would like to thank my wife, Susan, for her constant support which sustained me throughout the entire project.

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CHAPTER 1

RESISTANCE OF TREES TO BARK BEETLE ATTACK

Intoduction

The interaction of plants and insects is an extremely broad and complex subject that no single work has successfully researched and completely described. An analogous conclusion could also be made about the subject of resistance of plants to insects. The reasons for these conclusions are quite obvious and reside within the sheer numbers of individual species of plants and insects.

With reference to insect resistance and trees, several articles have been written specifically about trees or on principles common to both woody and non-woody species (Snelling, 1941; Painter, 1958; Vite and Rudinsky, 1962; Beck, 1965; Stark, 1965; Emden, 1973; Hanover, 1975, 1981; Hedin, 1983; Raffa and Berryman, 1983a). This review will primarily emphasize the interaction of trees and insects of the largest order, Coleoptera (roughly 40% of all known insects), and its family Scolytidae, which as a group are considered to be the most destructive of all forest pests. Examples of other forest pests which share common principles of host interactions will also be cited.

The Douglas-fir Bark Beetle

The family Scolytidae is composed of subcortical feeding bark beetles and wood boring ambrosia beetles. Rudinsky (1962) and Stark (1982) have reviewed the ecology of

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of the Scolytids and bark beetles, respectively. The majority of bark beetles favor trees which are in a less than optimum physiological state of health (Kozlowski, 1969). In most species the bark beetles are considered to be secondary pests. However, in some species and during epidemic outbreaks, bark beetles can be considered primary pests as demonstrated by their ability to attack and kill healthy standing trees.

In the family Scolytidae the genus Dendroctonus, which literally means "tree killers", contains the most destructive species (Stark, 1982). The Douglas-fir beetle (Dendroctonus pseudotsugae Hopkins) is the most destructive pest of Douglas-fir (Pseudotsuga menziesii) trees throughout most of the specie's natural range in western United States, British Columbia, and even into Mexico (Kinzer, et al., 1971).

Freshly felled, windfallen, fire scorched, and defoliated trees are preferred by Douglas-fir beetle for attacks but healthy trees may also be susceptible to attack (Atkins and McMullen 1958, 1960; Furniss, 1962, 1965; Furniss, 1941; McCowan and Rudinsky, 1958). The attack and infestation of the Douglas-fir beetle can have a very severe effect on individual trees. However, a much broader and more devastating impact may be made on an entire forest system. Estimations of timber damage inflicted by the Douglas-fir beetle in four major outbreaks in Oregon and Washington alone between 1950-1969 and in one outbreak du

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during 1978 were put at 7.4 billion and 10.8 million board feet killed, respectively. Other outbreaks in California during 1966 accounted for 809 million board feet killed and another 109 million board feet were killed in Idaho during the period 1970-1973 (Cornelius, 1955; Ciesla et al. 1971; Furniss and Orr, 1978; Orr and Brown, 1980).

Species of the genus Dendroctonus vary as to feeding habits. Some bark beetles are classified as strictly monophagous (feeding on a single species in a genus). Others are also classified as monophagous but feed on several species in a genus, while still others are classified as oligophagous (Stark, 1982). From various experiments Callaham (1966) noted that host specificity in pine bark beetles is lost if the host pines were not living.

The Douglas-fir beetle is capable of successfully attacking both live, standing, healthy Douglas-fir and physiolocially weakened Douglas-fir trees in addition to the preferred felled material. Live standing western larch (Larix occidentalis Nutt.) are also attacked on occasion in association with outbreaks of the Douglas-fir beetle. However, successful brood production in live standing western larch has not been observed (Furniss and Orr; 1978, Furniss and Caroline, 1978). Brood production in felled western larch has been documented by Ross (1967) and can occur at an equal rate with that in Douglas-fir (Furniss, 1976).

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The causes of susceptibility to attack appear complex and individual trees vary greatly in their resistance. Previous work has developed information about factors associated with host tree susceptibility and resistance with the goal of determining risk classifications for entire stands (Furniss, Livingston, and McGregor, 1981). The underlying cause(s) or mechanism(s) of individual tree resistance to the Douglas-fir beetle are unknown. In particular, the reasons for immunity of immature live Douglas-fir and live western larch of all ages are not known but are important for future forest protection. Likewise, knowledge about resistance of some mature Douglas-fir would find immediate application in Douglas-fir management.

The Douglas-fir beetle has one generation per year. Adults generally emerge and fly to a new tree between April and June while developing larvae emerge in the summer. A second attack by those adults which emerged in late spring may occur later in the summer. Both adults and larvae overwinter in trees.

The first sign of attack by the Douglas-fir beetle is the appearance of reddish orange frass on the bark of the tree. A further sign may be resin exuding from entrance holes near the upper limit of infestation in the tree. A later sign indicating beetle infestation is the discoloration of foliage from normal green to a reddish brown color.

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The characteristic boring pattern of the Douglas-fir beetle is produced as the female beetle bores through the bark and tunnels upward in the phloem, causing a light engraving in the outer sapwood. The galleries which are packed with frass, run parallel to the wood grain for lengths of 20-25 centimeters. The beetle produces 50-130 egg galleries per square meter in wind thrown trees. As the eggs mature, larvae develop and mine outward, perpendicular to the original egg galleries.

It is quite evident from the amount of damage attributed to the Douglas-fir beetle that it would be advantageous to find a means of controlling and managing the beetles. Economics rules out the feasibility of control through spraying insecticides. Trap trees have proven to be inefficient and cannot be used in most cases due to inaccessibility.

Breeding for Insect Resistance--Indirect Selection

All genetic traits including insect resistance are related to physiological processes which interact with the environment and result in phenotypic expression. Breeding insect resistant trees through the use of classical breeding methodology has the inherent problem of long generation time. Trees generally acquire some amount of insect resistance during the juvenile sapling stage, but once they reach physiological maturity their ability to withstand insect attack decreases. In the case of Douglas-fir tree/beetle relationship, the time factor is further

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confounded by the fact that trees are not attacked until long after they have reached sexual maturity. This fact by itself makes traditional screening and selection for resistance difficult.

Indirect selection, the process of improving desired trait X by selecting for genetically correlated trait Y, may be a solution to the problem of longevity and the delayed expression of trait X. Indirect selection may be beneficial if successful results can be reached in a shorter time by selecting for a correlated trait instead of the primary trait of interest.

Indirect selection theory and practice are not new. Nelson and Birkeland (1929) hypothesized from work in wheat (*Triticum aestivum*) that rust resistance and hardness could be improved by selecting varieties on the basis of the globulin fraction in grain. von Weissenberg (1970) noted that genetic correlations between biochemical processes and phenotypic traits may be present to such an extent that indirect selection methods may be a better alternative than direct selection.

Bridgen and Hanover (1979) have offered an explanation for why so little work has been done on insect resistance in trees: 1) forest genetics is relatively new, 2) long generation time is discouraging, 3) natural regeneration appears to be adequate, 4) knowledge of host/pest physiology is lacking.

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The genetic gain which can be expected from one cycle of indirect selection for a quantitative trait as defined by von Weissenberg (1976) is:

$$G_X = i_y \cdot h_y \cdot \sigma_{aX} \cdot r_a$$

where:

G_X = gain in desired trait X by indirect selection for marker trait Y

i_y = selection intensity achieved when selecting for marker trait Y

h_y = square root of heritability of marker trait Y

σ_{aX} = square root of additive genetic variance of desired trait

r_a = additive genetic correlation between desired trait X and marker trait Y

The relative efficiency of indirect selection compared with direct selection is dependent upon the genetic correlation between the two traits, the ratio of the two selection intensities, and the heritabilities of both traits.

Gerhold and Stroh (1963) and Bridgen and Hanover (1979) have discussed the advantages and situations in which indirect selection of trait Y would be favored over direct selection of trait X. Indirect selection is favored if:

1. A high genetic correlation exists between the two traits, X and Y
2. There is less cost in selecting for Y vs. X

3. Less time needed per generation when selecting for Y
4. Higher precision in recognizing trait Y (this would be an example of insect resistance selection in absence of insect infestation)
5. Higher correlation between parents and progeny for trait Y
6. Higher heritability associated with trait Y
7. Ability to apply higher selection intensity to trait Y
8. Large error associated with measuring trait X
9. If trait Y is a component of trait X
10. If trait Y can be measured earlier in the life cycle

However, the indirect selection method is not free from problems as marker traits are often difficult to identify. Also, genetic correlations and heritability estimates are often misleading and genetic correlations decrease with each generation of selection unless the correlation is due to pleiotropy.

Genetics and Selection Uses of Terpenes

Terpenoid compounds have been shown to be a useful group of marker traits in resin-producing conifer trees. Hanover (1966a) studied gene control of monoterpene levels in western white pine (*Pinus monticola*) and found inheritance to range from single gene to multigene and it also appeared to include some heterotic or epistatic effects. Monoterpene levels were also strongly associated wit

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with clonal genotypes. Estimations of broad sense heritabilities indicated moderate to strong genetic control. Hanover (1966b) further hypothesized that the concentration of 3-carene is controlled by a single gene with two alleles designated as C/c with C having complete dominance. further work by Hiltenen *et al.*, (1975) found the 3-carene content of foliage of clones and F_1 hybrids of Scotch pine (*Pinus sylvestris* L.) to be controlled by a single gene and additional modifiers.

Squillace (1971), investigating inheritance of monoterpene composition in cortical oleoresin of slash pine (*Pinus elliottii* Engelm.), found α-pinene, β-pinene, myrcene, and β-phellandrene to show strong broad sense heritability and moderately strong narrow sense heritability. Investigations of individual tree variation, found both myrcene and β-pinene to exhibit bimodality. Chi-square analysis revealed that β-pinene and myrcene were controlled by two alleles at a single locus with high concentrations being dominant over low amounts. Test crosses revealed no linkage of genes controlling either β-pinene or myrcene.

Rockwood (1973), studying variation in branch cortex monoterpenes of loblolly pine (*Pinus taeda* L.), found the same pattern in myrcene as did Squillace (1971). In addition, Rockwood found limonene to be controlled by two alleles at a single locus; however, low limonene (l) was dominant and high limonene (L), recessive.

Terpenes have several characteristics which facilitate their use in indirect selection. Monoterpenes are gene regulated and influenced little by nongenetic factors (Squillace and Fischer, 1966; Hanover, 1971). In a study of the relationship between nongenetic environmental factors and monoterpene composition, clones of western white pine planted in three separate locations showed negligible differences in monoterpenes associated with site (Hanover, 1966b). From work of Squillace (1971) in slash pine, Hanover (1966a) in western white pine, and Rockwood (1973) in loblolly pine, age effects on monoterpene composition were also found to be minimal. Heritability estimates of terpenes are fairly high (broad sense = .90, narrow sense = .50) (Squillace, 1976a). Monoterpenes have been identified as attractants and repellents (Jacobson, 1966; Harborne, 1977). Lastly, many terpenes can be sampled from seedlings at a young age and can be analyzed in each tree at the same time.

Host Selection--Terpenes and Pheromones

Numerous articles on host selection and chemical interactions between the insect and the host have been written (see reviews of Thorsteinson, 1960; Beck and Reese, 1976; Feeny, 1976; Kennedy, 1966; Detheir, 1982; Miller and Strickler, 1984). From these works it has been shown that insects select hosts through several processes including olfactory, visual, gustatory, tactile, and auditory senses. In searching for marker traits to use in an indirect

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selection method, the sensory process of olfaction would be a logical choice. Of the five sensory processes, olfaction may indeed be the farthest reaching and thus perhaps the most influential in terms of pioneer beetles.

Terpenoids have been found to be involved in the attraction of bark beetles to host trees (Renwick and Vite, 1970; Rudinsky, 1966c). In Dendroctonus species the pioneering beetles are females. The primary attraction and search for new host material is guided by host materials (Johnson and Pettinger, 1961; McMullen and Atkins, 1962; Rudinsky, 1963, 1966a, 1976; Jantz and Rudinsky, 1965; Vite and Pitman, 1969; Benett and Borden, 1971). The potency of host volatiles is exemplified by reports of attacks occurring in trees within only a few minutes after being felled (Rudinsky, 1966; Johnson and Belluschi, 1969). Once the pioneering beetle has selected suitable host material, a combination of host volatiles and insect pheromones are released which brings on mass aggregation of both sexes of beetles (McMullen and Atkins, 1962).

A complete review of pheromones is beyond the scope of this discussion but the importance of pheromones in the ecology of the beetle should not be overlooked (see reviews of Birch, 1978; Borden, 1974, 1982; Wood, 1982) as beetle attraction is much stronger than initial host attraction (Rudinsky, 1966a).

Several studies mentioned herein have identified specific monoterpenes and pheromones responsible for

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attraction and repulsion of the Douglas-fir beetle. Heikenen and Hruitford (1965) found α-pinene to be a strong attractant and β-pinene to be a repellent. Rudinsky, (1966c) from bioassays, found the terpenes α-pinene, limonene, and camphene each to be much more attractive than β-pinene, geraniol, and α-terpineol. Pitman and Vite (1970) found the Douglas-fir beetle produced an attractant pheromone, frontalin (1,5 dimethyl-6-8-dioxabicyclo (3.2.1) octane). "Douglure" a mixture of frontalin, α-pinene, and camphene was shown to successfully attract Douglas-fir beetles at a significantly higher rate to live trees (Knopf and Pitman, 1972). Further work of Rudinsky _et al_. (1972) found 3,2-MCH (3-methyl-2-cyclohexen-1-one) to be an all inclusive inhibitor to both sexes while the pheromones transverbenol (trans-4,6,6-trimethylbicyclo (3.1.1) hept-3-en-2-ol) and frontalin and the monoterpene camphene gave the highest attraction response. Dickens _et al_. (1983) in electroantennagram studies of Douglas-fir beetles found them most sensitive to antiaggregative pheromones 3,3-MCH-one and 3,3-MCH-ol. Aside from antiaggregative pheromones, male Douglas-fir beetles were most sensitive to the pheromones transverbenol and verbenone and the monoterpene camphene while female beetles were most sensitive to the pheromone frontalin and the monoterpenes limonene and camphene.

For several years the independent attraction produced by host volatiles and insect pheromones was quite apparent to researchers. However, it was not until the late 1960's tha

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that the synergistic effect of host volatiles in combination with pheromones was realized. Bedard _et_ _al_. (1969), working with the western pine beetle (_Dendroctonus_ _brevicomis_), found that the response to female production of the bicyclic ketal pheromone, exo-brevicomin, was enhanced by myrcene, which by itself is not attractive. Additional work in western pine beetle by Pitman (1969) found the attraction of frontalin to be enhanced by 3-carene. The combination of frontalin and 3-carene attracted female beetles while brevicomin plus 3-carene attracted male beetles. Renwick and Vite (1969) found the attraction of frontalin produced by the southern pine beetle (_Dendroctonus_ _frontalis_ Zimmermann) was boosted by the monoterpene α-pinene.

Pitman and Vite (1969) and Pitman (1971) found oleoresin of ponderosa pine (_Pinus_ _ponderosa_) and transverbenol to attract mountain pine beetle (_Dendroctonus_ _ponderosae_) and the mixture of transverbenol and α-pinene, "Pondelure", to attract mountain pine beetle to western white pine. Billings, Gara, and Hruitford (1976) found synthetic transverbenol by itself inactive in attracting mountain pine beetles but when applied with host ponderosa pine terpenes, myrcene and terpinolene, the mixture was attractive. Further investigations of mountain pine beetle and lodgepole pine found myrcene to have the most synergistic effect with transverbenol, but trees baited with trans-verbenol, exo-brevicomin, and 3-carene received the highest attack rates (Conn _et_ _al_. 1983; Borden _et_ _al_. 1983).

The first clues of insect pheromone production being dependent on host monoterpenes was found in the exposure of western pine beetle to α-pinene with the result of the linear production of the alcohol pheromone transverbenol (Hughes, 1973). Renwick, Hughes, and Krull (1976) were the first to demonstrate selected transformation of a host plant terpene (-) α-pinene to a geometrical isomer, cis-verbenol, a pheromone of <u>Ips</u> <u>paraconfusus</u>. Recently Byers (1983) found that both sexes of the mountain pine beetle convert the (+) and (-) enatiomers of α-pinene to the corresponding (+) and (-) enatiomers of transverbenol at equal rates. The (-) transverbenol form inhibits females and not males. Thus (-) transverbenol has been hypothesized as being a regulator of intraspecific competition and responsible for maintaining attack densities.

In addition to the synergistic effect of monoterpenes and pheromones, studies by Rudinsky <u>et</u> <u>al</u>. (1976) and Rudinsky and Ryker, (1976) found that sonic signals produced by stimulation of the opposite sex in Douglas-fir bark beetle resulted in the active release of limonene and attractive pheromones from the beetle.

From several works by Hughes (1975a,b, 1974, 1975), Renwick, Hughes, and Krull (1976), Renwick, Hughes, and Pitman (1976), Renwick, Hughes and Ty (1973), Vite, Bekke, and Renwick (1972), the biosynthetic pathway between several host oleoresin components and insect pheromones were discovered. Rudinsky (1976) proposed that in the Douglas-f

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fir beetle the pathway to MCH leads from the terpene terpinolene, while transverbenol and verbenone were derived from the oxidation of α-pinene, and pinocarvone from the oxidation of β-pinene.

Terpenes and Resistance to Attack

The first recordings of resistance of a conifer tree exerting resistance through its oleoresin system to attack by a bark beetle was made by Hopkins (1902) from observations of the mountain pine beetle and ponderosa pine. Later Person (1931) hypothesized that the western pine beetle could select individual ponderosa pines on the basis of volatile cues. Perttunen (1957) in investigations of two bark beetle species found that *Hylurgops palliatus* Gyll. was strongly repelled by high concentrations of α-pinene but only slightly repelled by low concentrations. In contrast, *Hylorgops ater* Payk. was slightly repelled by high concentrations of α-pinene and actually slightly attracted by low α-pinene concentrations.

Much work has been completed by Smith (1961, 1963, 1965, 1966, 1969, 1972, 1975, 1982) on the relationships of bark beetles and oleoresin content of host and nonhost species. Both the western pine beetle and the Jeffrey pine beetle (*Dendroctonus jeffreyi*) could tolerate the saturated resin vapor of their hosts, ponderosa pine and Jeffrey pine, respectively. However, neither beetle could tolerate the resin of the nonhost or resin of a hybrid of the two hosts

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(Smith, 1961, 1966). In a similar study Smith (1963) found mountain pine beetle able to tolerate resin of a hybrid of a nonhost, Jeffrey pine by a host, ponderosa pine. Smith (1965) published a monoterpene vapor toxicity rating for the western pine beetle; he listed the following terpenes in the order of decreasing negative effects: limonene, 3-carene, myrcene, followed by β-pinene which was equivalent to α-pinene.

In work on the engraver bark beetles, Werner (1972) found *Ips grandicollis* responded to the quality and quantity of terpenes present in phloem tissue: male beetles were stimulated by geraniol, limonene, methyl chavicol, and myrcene while females were attracted by camphene. Elkington and Wood (1980) found that the engraver beetle, *Ips paraconfusus* selected its host ponderosa pine over a non host white fir (*Abies concolor*) only after boring through the bark and into the phloem tissue. Elkington and Wood thus hypothesized that the chemostimulants, monoterpenes, present in the phloem tissue were critical to host selection.

Grand fir (*Abies grandis* (Dougl.) Lindl.) resin has been found to have a toxic effect on fir engraver (*Scolytus ventralis* LeConte) larvae (Berryman and Ashraf, 1970). Wright, Berryman, and Gurusiddaiah (1979) found that grand fir which contained the least amounts of monoterpenes were the trees most successfully attacked by the fir engravers.

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Several studies on various weevil species have also found attraction related to monoterpene composition. Selander _et al_. (1973) found _Hylobious abiehs_ L. to be slightly attracted by (+) limonene, α-terpinene, and β-pinene while linalool, borneol, and α-terpineol were repellents. The white pine weevil (_Pissodes strobi_ Peck) is primarily a pest of Sitka spruce (_Picea sitchensis_ Carr.), Engelmann spruce (_Picea engelmannii_ Parry), and eastern white pine (_Pinus strobus_ L.). Wilkinson (1980) found that eastern white pine with low cortical concentrations of limonene and high concentrations of α-pinene were the least susceptible to attack. In studying the relationship between white pine weevil and Sitka spruce, Alfuro _et al_. (1980), found that the monoterpenes α-pinene, β-pinene, and myrcene acted synergistically with nonvolatiles of bark and foliage while piperitone was a deterrent. Limonene and camphor were also stimulants at low concentrations but were actually inhibitory at higher threshold concentrations. Further studies on Sitka spruce and white pine weevil by Harris _et al_. (1983) found cortical monoterpenes of resistant trees to be significantly lower in β-phellandrene and higher in β-pinene and 3-carene.

Four southern pine species, loblolly (_Pinus taeda_ L.), shortleaf (_P. echinata_ Mill.), longleaf (_P. palustris_ Mill.), and slash (_P. elliottii_ Engelm.) can all be attacked and killed by the southern pine beetle. In bioassays of monoterpene toxicity Coyne and Loff (1976) found limonene and 3-carene (a rare monoterpene of southern pines) to be most toxic. Gollob (1980) found loblolly pines resistant to the southern pine beetle had high myrcene oleoresin content. In contrast to the above studies, no direct effect of chemical composition on resistance to the southern pine beetle was found in any of the four southern pines (Hodges *et al*. 1979).

Limonene has also been proposed as a major factor in resistance to the western pine beetle (*Dendroctonus brevicomis* LeConte). Sturgeon (1979) found the western pine beetle to prefer ponderosa pine trees low in limonene and high in α-pinene.

Additional studies of further monoterpene involvement in resistance to attack have focused on fungi infection associated with beetle attacks. Wound reaction resin of grand fir trees contains higher concentrations of limonene, myrcene, and 3-carene which are more toxic to the attacking fir engraver beetles and associated fungi than is the preformed cortical blister resin (Bordasch and Berryman, 1977; Russell and Berryman, 1976; Raffa and Berryman, 1982a).

Raffa and Berryman (1982b, 1983b) found similar results in lodgepole pine which were infected with fungi carried by the mountain pine beetle. Artificial innoculation resulted in similar wound resin composition but greater quantities of resin were found in resistant trees than were found in susceptible trees.

The Research Project

This dissertation will present results of a comparative study of two hosts of the Douglas-fir beetle, Douglas-fir and western larch. An initial study by Hanover and Furniss (1966) found quantitative but no qualitative differences in monoterpene composition between unattacked Douglas-fir and Douglas-fir which had resisted attack of the Douglas-fir beetle.

The research of this dissertation was designed to identify traits associated with resistance to attack both within age and vigor classes of Douglas-fir and between host species. In the project several physiological, morphological, anatomical, and biochemical traits were observed. A major emphasis was placed upon the volatile monoterpene composition of several tissues within each host species. Traits which may be found to be resistance related could be further tested for inclusion in Douglas-fir tree improvement strategies utilizing the theory and method of indirect selection.

CHAPTER 2

ANALYSIS OF MORPHOLOGICAL, PHYSICAL, AND GROWTH TRAITS OF DOUGLAS-FIR AND WESTERN LARCH, TWO HOSTS OF THE DOUGLAS-FIR BEETLE

Abstract

Morphological, physical, and growth traits that may be related to Douglas-fir beetle activity were studied in (1) Douglas-fir composed of two age and crown classes, (2) two groups of previously attacked and unattacked mature Douglas-fir, and (3) a group of mature, codominant western larch. Within age and crown classes of Douglas-fir a significantly greater vertical resin duct density in mature trees was found. No other differences associated with crown or age classes were found in relation to oleoresin exudation pressure, vertical resin duct density, wounding response, and ratios of crown length to total tree height. Phloem thickness was significantly greater in both mature and codominant Douglas-fir. Within Douglas-fir codominant trees had significantly greater crown lengths, vigor indices, and vertical resin duct sizes than suppressed/intermediate trees. Previously attacked trees had significantly lower phloem and sapwood tissue moisture content and wounding response scores than did previously unattacked trees. Mature codominant Douglas-fir had significantly greater attack rates, oleoresin exudation pressure, phloem thickness, and wounding responses than mature codominant western larch. Douglas-fir and western larch were similar

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in vigor indices and in vertical resin duct density while western larch had significantly larger vertical resin ducts and higher phloem moisture content. Correlation analysis of the attack rate in felled trees with measured traits revealed significant positive relationships between attack rate and phloem thickness, size of vertical resin ducts, age, diameter breast height, and crown length in Douglas-fir and previous five years' growth increment in western larch. Within Douglas-fir of both age and crown classes, significant negative correlations existed between attack rate and the ratio of current sapwood area to basal area and between attack rate and bark moisture content. The lack of oleoresin exudation pressure in live standing western larch led to the conclusion that oleoresin pressure is not associated with its exhibited resistance. Phloem thickness and phloem moisture content are hypothesized as factors controlling Douglas-fir beetle activity.

Introduction

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., attacks Douglas-fir, *Pseudotsuga menziesii* (Beissen) Franco, throughout the specie's natural range in western United States, Mexico, and Canada. The Douglas-fir beetle also attacks western larch, *Larix occidentalis* Nutt., in northwestern United States and southwestern Canada. Successful brood production at rates comparable to that in Douglas-fir occurs only in western larch which have been felled (Furniss, 1976). Within Douglas-fir, beetles prefer older, mature, dominant trees (Furniss, Livingston, and McGregor, 1981).

Extensive research has been done on host tree physiology and its relation to insect attack (see review of Hanover, 1975). The internal physiological condition of a candidate host tree is a critical factor which is perceived by an attacking insect and transcribed into a potential of food source and/or brood production area. Kozlowski (1969) hypothesized that as trees mature, physiological activity (*i.e.* cambial activity, resin flow, sapwood moisture content) decreases or varies such that they become more vulnerable to attack.

Several physiological and morphological/physical characteristics of host tree species have been studied in relation to insect attack. Reid and Watson (1966) and Berryman (1976) investigated physical factors of size, distribution, and number of resin ducts present in lodgepole pine (*Pinus contorta* Dougl.) in relation to mountain pine beetle (*Dendroctonus ponderosae* Hopk.) attack. Number and dimensions of resin ducts associated with weevil, *Hylobius warreni* Wood, attacks in lodgepole pine have been reported by Cerezke (1972 and 1973). Stroh and Gerhold (1965) found that white pine weevil, *Pissodes strobi* (Peck), avoided resin ducts in white pine, *Pinus strobus*. The diameter of a tree as a factor of insect host selection has been studied for some bark beetles. Fargo (1979) found a positive correlation between attack rate and host diameter within the southern pine beetle (*Dendroctonus frontalis* Zimmerman)/ loblolly pine (*Pinus taeda* Laws) complex while Berryman (1976) found host diameter to be nonsignificant within the mountain pine beetle/lodgepole pine complex (see also Cole and Amman, 1969; Amman, 1973, 1978; and Cole and Cahill, 1976). Phloem thickness in lodgepole pine has been studied as an influence in mountain pine beetle attack by Amman (1969 and 1972), Amman and Pace (1976), Berryman (1976) and Cole, Guymon, and Jensen, (1981). Phloem thickness has been investigated in ponderosa pine (*Pinus ponderosa*) by Cobb *et al*. (1968) and by Amman (1972) and Berryman (1976) in lodgepole pine in relation to mountain pine beetle attacks. Moisture content of various tissues has also been associated with insect attack success. Inouye (1954) investigated sapwood moisture content of various conifers in Japan and Cobb *et al*. (1968) looked at phloem moisture of ponderosa pine in relation to bark beetle attack.

Other areas which have been investigated in terms of insect preference and attack include tree vigor (see Person, 1931; Rudinsky, 1966a; Ferrell, 1971; Mahoney, 1978; Hicks *et al*. 1978; Mitchell, Waring, and Pitman, 1983), oleoresin exudation pressure and oleoresin flow (Vite, 1961; Vite and Wood, 1961; Vite and Rudinsky, 1962; Wood, 1962), and oleoresin crystallization rate (Santamour, 1965; van Buijtenen and Santamour, 1972).

The purposes of this study were: (1) to determine preferential attack patterns of the Douglas-fir bark beetle on Douglas-fir and western larch and (2) to determine preferential attack patterns of the beetle for different age and crown classes of Douglas-fir. Factors measured included tissue moisture content, phloem thickness, oleoresin exudation pressure, oleoresin crystallization rate, wounding response, tree vigor indices, and number, sizes, and densities of vertical resin ducts in the outer sapwood.

Materials and Methods

In May, 1981, field measurements of 48 Douglas-fir and 16 western larch from the St. Joe National Forest were made. The uneven aged second growth stand was located 12.8 km southwest of Elk River, Clearwater County, Idaho at an elevation of 800 meters. The stand was comprised predominantly of Douglas-fir. Other species present in addition to Douglas-fir and western larch were ponderosa pine (*Pinus ponderosa* Laws), western white pine (*P. monticola* Dougl.), grand fir (*Abies grandis* (Dougl.) Lindl.), and western red cedar (*Thuja plicata* Donn.).

Sampled trees were selected on the basis of age and crown classification. Douglas-fir trees in this study were of the four age and crown categories: 1. mature/codominant 2. young/codominant 3. mature/suppressed or intermediate and 4. young/suppressed or intermediate. The western larch sampled in the study area were of the mature/codominant clas

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Each of the standing trees was measured for diameter at breast height (D.B.H.), wounding response, oleoresin exudation pressure and oleoresin crystallization rate. Wounding response was measured by cutting into the outer sapwood with an ax to a depth of 1.0-2.0 cm and exposing 50-60 cm^2 of xylem tissue. The qualitative measure of wounding response was made by observing the amount of oleoresin produced by each tree at the wound site after 24 and 48 hours. Wounding was scored from 0 (none or only a trace of oleoresin present) to 3 (a few milliliters of oleoresin present).

Oleoresin exudation pressure was measured with micromanometers using a modified method of Hodges and Lorio (1968) and Bridgen and Hanover (1982). Four 20.0 microliter capillary tubes, sealed in one end, were placed in holes (1.6 mm in diameter and 13 mm deep, 5 mm into the outer sapwood) drilled in bark crevices 1.8 meters from the ground, and they were secured with silicone rubber. Pressure readings were taken in the morning of the fourth day following insertion.

Oleoresin crystallization rate was measured by placing five samples of oleoresin from each tree on a microscope slide. Observations of the spotted oleoresin samples were made at 12 hour intervals with the aid of a dissecting microscope. Between observations the oleoresin slides were kept in a desiccator at ambient temperature.

For a period of two weeks beginning May 11, 1981, pairs of both age and crown classes of Douglas-fir and mature, codominant western larch were randomly selected on a daily basis for felling and further sampling from those presently under observation for oleoresin pressure and wounding response. Upon felling, age, total tree height, and live crown length of each tree were measured. Four additional micromanometers were inserted into each felled tree as described above. Two samples of bark, inner phloem, and outer sapwood were removed from each tree at 3.0-3.5 m up the bole and 25 percent of the distance into the live crown in an acropetal direction. These samples were placed in sealed polyethylene bags and put in portable ice coolers until stored at -17^{o}C in the laboratory. In the laboratory triplicate 1-2 g subsamples of outer sapwood, inner phloem, and bark were weighed to the nearest mg placed in an oven at 105^{o}C and dried to constant weight. Moisture content of the samples was calculated as the amount of water present in fresh weight condition expressed as a percent of the total oven dry weight.

Cross sections from the bole of each tree were cut at a height of 0.5 m. The following measurements from the cross sections were taken along four perpendicular radii: overall diameter, sapwood diameter, sapwood thickness, phloem thickness, bark thickness, and the width of the previous five years' growth increment. These measurements were made with the aid of a magnifying lens and calipers calibrated to the nearest 0.05 mm.

Yearly growth increments and density and dimensions of vertical resin ducts in each of the previous three years of three subsamples of outer sapwood from both upper and lower bole positions were measured. The samples of outer sapwood were placed in a 100°C water bath for 5-10 minutes. A sliding microtome was then used to cut 15 micron sections of the softened tissue. The xylem sections were then stained with a 0.05% solution of safarin-O and placed on microscope slides for observations. A dissecting microscope equipped with a calibrated scale was used to measure individual yearly growth increments of the previous three years to the nearest 0.01 mm. The number of vertical resin ducts over a measured linear distance of 1.5-2.0 cm of xylem tissue was counted for each individual year. A microscope also equipped with a calibrated scale was used to measure the longest and shortest diameter axes. Measurements were taken to the nearest 2.5 microns at a magnification of 400X of three randomly chosen resin ducts from each of the previous three years' growth increments.

Field observations of all felled trees were made in June, three weeks after initial samples were taken. The total number of frass piles visible when walking up one side and down the other of each tree (not including underside) were recorded. From these data a measure of the relative attack rate based on the number of attacks and the surface area of each tree were calculated.

Three comparisons involving species, attack history, crown class, and age class were made within the sampled trees in relation to wounding response, oleoresin exudation pressure, crystallization rate, cross sectional measurements, vertical resin duct density and dimensions, yearly incremental growth and tissue moisture content. The first comparison involved four groups, each comprised of ten Douglas-fir of the following categories: 1. mature/codominant, 2. young/codominant, 3. mature/suppressed or intermediate, and 4. young/suppressed or intermediate. The trees in this experimental comparison were felled and sampled as described above. The experimental design used for analyzing data of resin duct density and dimensions, yearly incremental growth and tissue moisture content was a 2x2x2 factorial split-split plot with the main plots arranged in a completely randomized design. The oleoresin exudation pressure and cross sectional data were analyzed as a 2x2 factorial split-plot with the main plots arranged in a completely randomized design. Wounding response was analyzed using the nonparametric one-way analysis of variance of Kruskal and Wallis (Steel and Torrie, 1960) for a completely randomized design.

The second comparison was made between two groups of four Douglas-fir trees each. One group of four mature, codominant trees was classified "resistant", since they had survived attacks from a localized Douglas-fir beetle outbreak in 1971. These trees were identified by resin pockets present in their sapwood produced by unsuccessful beetle attacks. The second group of four mature, codominant trees did not show any sign of ever being attacked. This latter group of four trees was felled and sampled as described above. The four trees which were unsuccessfully attacked were sampled for vertical resin duct density and dimensions, previous years' growth increments, and tissue moisture content at the lower position only and were not felled. These two groups were analyzed using a completely randomized design.

The third comparison was a species comparison made between 14 mature, codominant Douglas-fir (ten from the first comparison and four previously unattacked from the second comparison above) and 16 mature, codominant western larch. These two groups were felled and sampled as described above. Tissue moisture content, yearly growth increments, vertical resin duct densities and sizes were analyzed as a 2x2 split plot design with the main plots arranged in a completely randomized design. Cross sectional measurements and oleoresin exudation pressure were analyzed as completely randomized designs. The Kruskal-Wallis nonparametric one-way analysis of variance was again used in analyzing data of wounding response.

Results

Within Douglas-fir comparisons of age and crown classes

Table 2.1 presents the results of field and cross section measurements within age and crown classifications of

Table 2.1. Comparison of growth and physiological characteristics of Douglas-fir age and crown class groups.

Character	-Age Class- Young n=20	Mature n=20	Sig.	-Crown Class- Codom. n=20	Supp./Int. n=20	Sig.
Age (yrs)	52.1	85.0	**	70.6	66.5	*
D.B.H. (cm)	27.5	37.5	**	41.9	23.1	**
Height (m)	23.1	27.6	**	29.3	21.4	**
Crown length (m)	10.0	10.7	ns	12.6	8.1	**
Crown length/total height	0.43	0.39	ns	0.43	0.38	ns
Previous 5 year growth area (cm^2)	72.9	111.3	ns	147.4	36.8	*
Sapwood area (cm^2)	258.5	357.3	**	466.1	149.8	**
Basal area (cm^2)	697.2	1239.3	**	1478.7	457.7	**
5 year growth area/sapwood area	0.25	0.27	ns	0.31	0.22	*
Sapwood area/basal area	0.37	0.29	**	0.33	0.33	ns
Phloem thickness (mm)	2.62	3.68	**	3.67	2.63	**
Oleoresin pressure (atms.)	1.47	1.21	ns	1.20	1.48	ns
Surface area (m^2)	12.1	19.0	**	21.5	9.6	**
Attack rate (No./m^2)	4.08	7.08	**	6.50	4.65	**

*, ** significant at the 0.05 and 0.01 levels, respectively; ns nonsignificant

Douglas-fir. A statistically significant difference in ages of the two crown classes was found. Diameters and heights of mature and codominant classes were greater than younger and suppressed/intermediate classes, respectively. Crown length did not vary significantly between age classes but was significantly greater in the codominant crown class. Nonsignificant differences between ratios of crown length to total tree height were found within both age and crown classifications.

Codominant trees produced 4.0 times (significant at the 0.01 level) the amount of growth over the last five years as did the suppressed/intermediate trees. No difference in previous five years' growth was found between age classes.

Basal area, which is defined in this study as the total inside bark cross sectional area of each tree at 0.5 meters height, and sapwood area at the same height showed trends similar to 5-year stem growth increment. Sapwood and basal areas were greater in mature trees by factors of 1.4 and 1.8, respectively, than in younger trees and greater in codominant trees by factors of 3.1 and 3.2, respectively than in suppressed/intermediate trees.

Age classes within Douglas-fir did not differ significantly in vigor ratings (amount of stemwood area production over the last five years to the total area of sapwood). However the ratio of sapwood to total basal area was significantly greater in younger trees than in mature trees. Codominant trees had a significantly higher vigor rating than did suppressed/intermediate trees. No difference in the ratio of sapwood to total basal area was found between crown classes.

Phloem thickness, surface area, and attack rate were significantly greater in both mature and codominant class trees. In addition, a highly significant position effect was also demonstrated in the phloem thickness character. Lower bole sampling positions averaged 3.95 mm and upper sampling positions averaged 2.35 mm in thickness.

No significant difference in oleoresin exudation pressure associated with either age or crown class within standing Douglas-fir was found. Only one tree, a mature suppressed/intermediate tree, exhibited oleoresin pressure in the micromanometers inserted after felling. The pressure in the felled tree was 90% of the average value exhibited in the standing tree.

Nonparametric tests of qualitative wounding responses associated with both age and crown classes indicated no significance at the 0.05 level (Table 2.2).

Tissue moisture content analysis (Table 2.3) showed that phloem had the highest and bark the lowest water content. The higher percentage of water in bark tissues of young trees compared with older trees was the only significant variation within the three tissues in relation to age or crown classification. Bark, phloem, and sapwood exhibited significant variation due to sampling position.

Table 2.2. Kruskal-Wallis nonparametric one-way analysis of variance of wounding response within Douglas-fir age and crown classes.

Class	n	Mean Rank	Chi-square value	Significance
		---Age Classification---		
Young	20	19.8	0.14	ns
Mature	20	21.2	--	--
		---Crown Classification---		
Codominant	20	23.7	3.04	ns
Supp./Int.	20	17.3	--	--

ns = nonsignificant

Variation was also shown in annual growth rates, and sizes and densities of vertical resin ducts within Douglas-fir (Table 2.4). Young, codominant trees and the upper sampling position consistently had significantly greater stemwood production in all three years measured. The density of vertical resin ducts was more uniform across both age and crown classes and sampling position. Within age class, crown class, and sampling position a nonsignificant trend of a higher density of vertical ducts in mature, suppressed/intermediate trees and in the lower sampling position was found in all years except sampling position for 1979. The average area of individual vertical resin ducts did not vary with age class but larger ducts were found in codominant trees. Larger ducts were present in the lower sampling position in 1978 and 1980 and in the overall mean of the three years sampled. Highly significant interactions

Table 2.3. Tissue moisture contents of two age, two crown classifications, and two sampling positions within Douglas-fir.

Tissue	-Age Class- Young n=40	Mature n=40	Sig.	-Crown Class- Codom. n=40	Supp./Int. n=40	Sig.	-Sample Position- Upper n=40	Lower n=40	Sig.
				---Mean % Moisture ---					
Phloem	116	105	ns	106	115	ns	105	116	*
Bark	58	43	**	49	53	ns	76	26	**
Sapwood	102	106	ns	104	105	ns	118	91	**

ns nonsignificant
* significant at 0.05 level
** significant at 0.01 level

Table 2.4. *Mean yearly growth* increments and vertical resin duct sizes and densities within Douglas fir age and crown classifications and sampling positions.

Table 2.4. Mean yearly growth increments and vertical resin duct sizes and densities within Douglas-fir age and crown classifications and sampling positions.

Year	-Age Class- Young n=40	-Age Class- Mature n=40	Sig.	-Crown Class- Codom. n=40	-Crown Class- Supp./Int. n=40	Sig.	-Sample Position- Upper n=40	-Sample Position- Lower n=40	Sig.
---Average Yearly Growth Increments (mm)---									
1978	1.54	0.93	**	1.64	0.83	**	1.50	0.98	**
1979	1.71	1.21	*	1.95	0.98	**	1.82	1.11	**
1980	1.81	1.19	*	1.98	1.02	**	1.87	1.13	**
mean a/	1.69	1.11	*	1.86	0.94	**	1.73	1.07	**
---Average Number of Ducts per mm^2---									
1978	0.48	0.99	ns	0.67	0.79	ns	0.46	1.00	ns
1979	0.48	0.75	ns	0.46	0.77	ns	0.62	0.61	ns
1980	0.27	0.42	ns	0.30	0.39	ns	0.33	0.36	ns
mean a/	0.41	0.72	*	0.48	0.65	ns	0.47	0.66	ns
---Average Area of Ducts ($microns^2$)---									
1978	2578	2939	ns	3245	2272	**	2395	3122	**
1979	2610	2951	ns	3322	2229	**	2583	2974	ns
1980	2283	2379	ns	2641	2021	*	2114	2547	*
mean a/	2466	2843	ns	3148	2161	**	2383	2926	**

a/ mean of 3 years
ns nonsignificant
* significant at 0.05 level
** significant at 0.01 level

between age class and sampling position in average yearly growth increment were found in all three years and the mean of the three years sampled. No interactions between age, crown class and/or sampling position were found in any of the three years.

Comparison of previously attacked and unattacked Douglas-fir

Results of the comparison of previously attacked and unattacked mature, codominant Douglas-fir are presented in Tables 2.5 and 2.6. Unattacked trees had significantly higher moisture levels in phloem and sapwood tissues (Table 2.5). In 1979 unattacked trees had vertical resin ducts with significantly greater densities and areas (Table 2.6). Wounding response as analyzed by Kruskal-Wallis nonparametric one-way analysis of variance revealed unattacked Douglas-fir had significantly higher wounding response scores than did previously attacked Douglas-fir (Table 2.7).

Species comparison of mature codominant Douglas-fir and western larch

Mean values of field and cross sectional measurements of the species comparison of mature, codominant Douglas-fir and western larch are presented in Table 2.8. Mature codominant western larch and Douglas-fir were similar in total height, crown length, ratio of crown length to total height, and surface area. The significant difference between species in D.B.H. was reflected in significant

Table 2.5. Comparison of previously attacked and unattacked mature, codominant Douglas-fir tissue moisture content.

Tissue	Unattacked	Attacked	Significance
	---Mean % moisture---		
Phloem	109	72	**
Bark	23	25	ns
Sapwood	116	58	**

ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

Table 2.6. Mean yearly growth increments and vertical resin duct sizes and densities of previously attacked and unattacked mature, codominant Douglas-fir.

Year	Unattacked	Attacked	Significance
---Average Yearly Growth Increments (mm)---			
1978	1.10	3.95	ns
1979	1.26	2.00	ns
1980	1.22	1.97	ns
mean[a/]	1.19	2.64	ns
---Average Number of Ducts per mm^2---			
1978	1.08	0.08	ns
1979	0.42	0.06	*
1980	0.20	0.05	ns
mean[a/]	0.57	0.06	ns
---Average Area of Ducts ($microns^2$)---			
1978	2838	4221	ns
1979	3312	603	**
1980	2604	2459	ns
mean[a/]	2987	3623	ns

[a/] mean of 3 years
ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

Table 2.7. Kruskal-Wallis nonparametric one-way analysis of variance of wounding response scores in previously attacked and unattacked Douglas-fir.

Class	n	Mean Rank	Chi-square value	Significance
Unattacked	4	6.5	5.33	*
Attacked	4	2.5	--	

* significant at the 0.05 level

differences in sapwood, basal, and the previous five years' growth areas. There was no significant difference in the quantitative measure of tree vigor or in the ratio of sapwood to basal area between the two species.

There was a highly significant difference between species in oleoresin exudation pressure. No western larch either standing or felled, produced any measurable oleoresin exudation pressure. One mature, codominant Douglas-fir exhibited oleoresin exudation pressure in one of the four micromanometers which were inserted after felling equivalent to 46% of the average pressure in the standing state.

Douglas-fir mean phloem thickness was 2.5 times greater than western larch. No significant difference was found in average phloem thickness associated with sampling position. Also, in relation to phloem thickness, no significant interaction was found between species and sampling position.

The Douglas-fir beetle showed a strong preference for felled Douglas-fir over felled western larch. Douglas-fir was attacked 14 times more than western larch.

Table 2.8. Species comparison of mature, codominant Douglas-fir and western larch mean field and cross sectional characteristics.

Character	Douglas-fir n=14	Western larch n=16	Significance
Age (years)	90.6	108.3	**
D.B.H. (cm)	53.0	41.9	**
Height (m)	32.9	34.6	ns
Crown length (m)	14.2	14.1	ns
Ratio: crown length/ total height	0.43	0.41	ns
Previous 5 year growth area (cm^2)	179.9	57.2	**
Sapwood area (cm^2)	609.1	262.0	**
Basal area (cm^2)	2352.1	1214.9	**
Ratio: 5 year growth area/ sapwood area	0.30	0.22	ns
Ratio: Sapwood area/ basal area	0.27	0.23	ns
Oleoresin exudation pressure (atmospheres)	1.82	0.00	**
Phloem thickness (mm)	4.53	1.83	**
Surface area (m^2)	29.5	25.9	ns
Attack rate (No./m^2)	5.19	0.37	**

ns nonsignificant
* significant at 0.05 level
** significant at 0.01 level

The Kruskal-Wallis nonparametric test for differences in wounding response resulted in a significantly higher rating for Douglas-fir (Table 2.9).

Significant differences were found between species in all three tissue moisture contents (Table 2.10). The interaction between species and sampling position was significant in phloem at the 0.01 level and in bark at the 0.05 level. Phloem of the bottom sampling position of western larch was 69.7%, 85.7%, and 56.3% more moist than upper western larch, upper Douglas-fir, and lower Douglas-fir phloem samples, respectively. Bark from the upper sampling position of Douglas-fir was 32.4%, 32.8%, and 23.3% more moist than lower Douglas-fir, lower western larch, and upper western larch bark samples, respectively.

Douglas-fir produced stemwood at a significantly faster rate than was western larch (Table 2.11) with greater increment growth rate at the upper bole position. Density of the vertical resin ducts also presented in Table 2.11, showed no difference associated with either species or sampling position. Consistently larger vertical resin ducts

Table 2.9. Kruskal-Wallis nonparametric one-way analysis of variance of wounding response in mature, codominant Douglas-fir and western larch.

Species	n	Mean Rank	Chi-square value	Significance
Douglas-fir	14	22.6	17.28	**
Western larch	16	9.3	--	--

** significant at the 0.01 level

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Table 2.10. Species and sample position comparison of mature, codominant Douglas-fir and western larch mean tissue moisture content.

Tissue	-Species- Douglas-fir n=28	western larch n=32	Sig.	-Sample Position- Upper n=30	Lower n=30	Sig.
			---Mean % Moisture ---			
Phloem	100	135	**	93	144	**
Bark	38	26	**	42	22	**
Sapwood	114	97	*	119	92	**

* significant at the 0.05 level
** significant at the 0.01 level

Table 2.11. Mean yearly growth increments and vertical resin duct sizes and densities of mature, codominant Douglas-fir and western larch.

Year	-Species- Douglas-fir n=28	Western larch n=32	Sig.	-Sample Position- Upper n=30	Lower n=30	Sig.
	---Average Yearly Growth Increments (mm)---					
1978	1.20	0.83	*	1.16	0.85	**
1979	1.64	0.58	**	1.29	0.86	**
1980	1.60	0.71	**	1.34	0.92	**
mean[a/]	1.48	0.71	**	1.26	0.88	**
	---Average Number of Ducts per mm^2---					
1978	0.89	0.36	ns	0.33	0.88	ns
1979	0.56	0.74	ns	0.55	0.75	ns
1980	0.33	0.21	ns	0.33	0.20	ns
mean[a/]	0.59	0.44	ns	0.41	0.61	ns
	---Average Area of Ducts ($microns^2$)---					
1978	3302	5060	**	3813	4674	*
1979	3438	4742	**	3571	4708	**
1980	2581	4802	**	3538	3994	ns
mean[a/]	3243	4717	**	3560	4502	**

[a/] mean of 3 years
ns nonsignificant
* significant at 0.05 level
** significant at 0.01 level

were found in western larch in all three years sampled. Significantly larger ducts were also measured in lower sample positions of both species in two of the three years sampled. Analysis of variance revealed no species x sampling position interaction for mean yearly growth increments, mean number of vertical ducts, and mean area of vertical ducts in any of the three years sampled.

Oleoresin samples from only two of the 48 Douglas-fir and one of the 16 western larch crystallized during the first three weeks of observation. Three of the others crystallized over the next month. Thus, no statistics were computed on the crystallization data.

Correlation analysis of attack rate with the other physical characteristics measured is presented in Table 2.12. Spearman's nonparametric method (Steel and Torrie, 1960) was used in correlation analysis of qualitative wounding response with attack rate. The only significant correlations found in western larch characteristics were positive relationships between previous 5-years' area growth and the ratio of 5-year growth area to sapwood area with attack rate. Within Douglas-fir the strongest positive relationships were between age, D.B.H., and height with attack rate. Significant negative correlations were found between attack rate and the ratio of sapwood area to basal area and between attack rate and the bark moisture content of both the upper and lower bole positions.

Table 2.12. Correlation analysis of attack rate of Douglas-fir beetle in Douglas-fir and western larch with measured tree characteristics.

Character	Douglas-fir n=44	Western Larch n=16
Age	.69***	-.12
D.B.H.	.51***	.12
Height	.53***	.01
Crown length	.33*	.21
Crown length/height	.00	.26
Previous 5 year growth area	.20	.66**
Sapwood area	.36*	.29
Basal area	.44**	.19
5 yr growth area/sapwood area	-.04	.57*
Sapwood area/basal area	-.54***	-.12
Phloem thickness top	.26	.30
Phloem thickness bottom	.31*	.41
Mean phloem thickness	.31*	.42
Oleoresin exudation pressure	.04	---
Phloem moisture top	-.17	.28
Phloem moisture bottom	-.11	.32
Bark moisture top	-.36*	.05
Bark moisture bottom	-.40**	.23
Sapwood moisture top	.14	.21
Sapwood moisture bottom	.03	.04
Mean growth increment top	-.16	.18
Mean growth increment bottom	-.06	.46
Mean area of ducts top	.38**	-.08
Mean area of ducts bottom	.39**	.29
Mean duct density-top	.14	-.20
Mean duct density-bottom	.28	-.22
Wounding response@	.17	.02

* significant at the 0.05 level
** significant at the 0.01 level
*** significant at the 0.001 level
--- correlation uncomputable
@ Spearman correlation coefficient

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Discussion

Observations similar to those of Furniss, Livingston, and McGregor (1981) of higher attack rates in older, larger, and more dominant trees in unmanaged stands were also made in the felled Douglas-fir trees of this study. In attempting to determine which factors the attacking Douglas-fir beetle may be keying on, one must compare the attack rates and the associated characters which are significantly different between age and crown classifications. Environmental conditions, such as bark temperature from exposure to sunlight which also effects the distribution of attacks (Atkins and McMullen, 1960), were uniform throughout the field study site.

The statistically significant four-year mean age differential between the codominant and the suppressed/ intermediate crown classes does not necessarily signal a biologically significant difference which a Douglas-fir beetle could detect. I feel the 33-year mean differential between the two age categories is a biologically significant effect which could be associated with factors discernable to a pioneering bark beetle.

Of these first five descriptive characters, D.B.H. is the only factor which bark beetles can truly assess in its search for a suitable host tree (Cole and Amman, 1969). Thus, the associations of higher attack rates for the older age class and for the codominant crown class could be biologically significant. The Douglas-fir beetle probably dif

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differentiates between the age and crown classifications or it may respond directly to the greater diameters of mature and codominant trees.

Measurements of tissue areas, growth rates, and the ratios derived from them are also not sensed directly by the Douglas-fir beetle. However, these quantitative values of tree vigor are directly related to the physiological state (vigor) of the host tree and in turn may effect behavioral responses of the beetle. Factors which effect the overall physiological state of the tree and are sensed directly by the bark beetle include, phloem thickness, oleoresin exudation pressure, tissue moisture content, and size and density of resin ducts.

Rationale for studying the size and density of vertical resin ducts of western larch and Douglas-fir lies in the physical behavior of an attacking beetle. In colonizing a tree the Douglas-fir beetle bores through the outer bark and into the phloem where it proceeds to create brood galleries. During gallery construction the bark beetle also leaves the exact outline of the phloem galleries etched in the outer portion of sapwood which remains visible for a period of several years following attack.

The implications of average density and size of vertical resin ducts in relation to the actual qualitative and quantitative amounts of odoriferous oleoresin contained within the ducts are discussed in Chapters 3 and 4. The larger size vertical ducts in western larch imply that larch contains greater quantities of xylem oleoresin.

Oleoresin crystallization rate in any of the comparisons within classes of Douglas-fir or in the comparison between species did not appear to be a factor in colonization success. The lack of crystallization in over 90% of all trees up to the time of attack observations indicates the fluidity of resin did not deter attack.

Comparison of age and crown classes within Douglas-fir

From the within Douglas-fir comparison of those factors which can be directly sensed by an attacking bark beetle, only the phloem thickness factor shows a significant difference associated with attack rate and both age and crown classifications. Both Amman (1972) and Berryman (1976) working with lodgepole pine/mountain pine beetle interaction found that phloem thickness was the single most important variable affecting brood production. The significant positive correlation between phloem thickness of both lower position and mean of lower and upper sampling positions with attack rate in Douglas-fir supports the hypothesis that thick phloem is a factor which Douglas-fir beetles are keying on in their colonization behavior.

The quantitative measure of tree vigor (previous five years' growth area/sapwood area) as defined by Waring, Thies, and Muscato (1980) revealed a significant difference with respect to crown class. Lack of a significant difference in tree vigor within age classes is a result of both codominant and suppressed/intermediate crown classes being equally represented within each age class. The absolute value of these vigor ratios for codominant and suppressed/intermediate crown classes were not numerically as large as those for coastal Douglas-fir published earlier by Waring, Thies, and Muscato (1980). This finding is not surprising in that the vigor index is dynamic and sensitive to different environmental conditions associated with each individual stand. The lower ratios would tend to indicate the trees of this study were from a lower quality site. In examining the ratios of vigor indices from individual crown classes it was found that the ratio of the vigor index of codominant trees to the vigor index of suppressed/ intermediate trees equalled 1.39. From data of Waring, Thies, and Muscato (1980), the ratio of the vigor index of codominant trees to suppressed trees equalled 1.43. Thus the trees in this study, which were labeled as a mix of suppressed/intermediate types, might actually tend to be more suppressed.

The association of higher attack rates occurring in trees with higher vigor indices found here are in direct contrast to findings of Waring and Pitman (1980) in study of lodgepole pine and mountain pine beetle. This finding indicates different mechanisms controlling colonization patterns within the Dendroctonus genus.

High phloem moisture content has been found to adversely effect bark beetle development (Berryman, 1974; Webb and Franklin, 1978). The adverse effect of high tissue moisture content on attack rate is implicated by the trend of high average bark and phloem moisture values associated with young and suppressed/intermediate classes. Support for this hypothesis is found in the significant negative correlations between attack rate and bark moisture content of both the upper and lower bole positions. The significantly greater phloem moisture present in lower bole sampling positions may be associated with the observed preference of Douglas-fir beetle attack at positions of mid bole and above (Furniss, 1962).

The number of vertical ducts per unit area varied extensively (as much as a two fold difference in mature class trees) from year to year, and were similar to results reported by Reid and Watson (1966) for vertical resin ducts of lodgepole pine. Two statistically supported attack preference theories arise from the mean density and size measurements taken from individual year samples. First, the attack preference for older age class trees is associated with the greater mean density of vertical ducts in mature trees rather than the mean area of individual ducts which is roughly equal between each age class. The second theory is that attack preference for codominant trees is associated with the greater mean area of vertical ducts in codominant trees rather than the mean density which is also roughly equal between each crown class. The assumption which ties these two theories together is that large ducts or high frequency of ducts leads to a greater production of attractive oleoresin.

Comparison of previously attacked and unattacked trees

In comparing unattacked trees with those which were attacked 10 years previous, significant differences in phloem and sapwood tissue moisture content during the study sampling year probably do not reflect values present 10 years earlier. The most recent years' growth increments and vertical resin duct measurements offer no clue to an inherent resistance mechanism present in the previously attacked trees. Observation of the 10-year-old sapwood in those trees which had been attacked revealed resinous cavities and the occurrence of numerous traumatic resin ducts. The older sapwood tissue of these trees exhibited what Raffa and Berryman (1982) describe as the "secondary" or "hypersensitive response" to bark beetle attack. The hypersensitive response results in the autolysis of parenchyma cells, formation of traumatic resin ducts, an increase in concentration of monoterpenes and phenolics and the secretion of secondary resin. In total this reaction renders the phloem tissue unsuitable for brood production.

As in moisture content, wounding response values exhibited during the study may not reflect responses 10 years ago. Trees with greater wounding response may be defensing themselves against further attack by lowering the optimal ratio of insect pheromone to host volatile as hypothesized by Raffa and Berryman (1983a) from work with the mountain pine beetle/lodgepole pine complex.

<u>Species comparison of mature codominant Douglas-fir and western larch</u>

The significantly higher oleoresin exudation pressure exhibited by Douglas-fir coincides with the significant qualitative difference in wounding response demonstrated by the two species. The values of oleoresin pressure were lower than the maximum values (5-7 atmospheres) found in Douglas-fir (Vite and Rudinsky, 1962), lower than the values of trees classified as vigorous (5-10 atmospheres), and about equal to those classified as susceptible (0-2 atmospheres) as reported by Rudinsky (1966a).

The very low resin flow found in western larch reflected observations of Vite and Rudinsky (1960) of four species (*Pinus contorta*, *P. silvestris*, *Larix occidentalis*, and *L. decidua*) which were unable to produce measurable oleoresin pressure. Rudinsky (1966a) hypothesized that trees with high oleoresin exudation pressure are able to resist successful colonization by repelling the beetles with high concentrations of vapors or physically suffocating them. The latter alternative of repulsion by concentrated vapors may be a reason for the observed attack differential between species. The low attack rate in western larch is in contrast to findings in ponderosa pine by Vite (1961) in relation to the attack rate of the western pine beetle, *Dendroctonus brevicomis*. Ponderosa pine with oleoresin exudation pressures less than 1.0 atmosphere contained more western pine beetle larvae than did trees with greater than 1.5 atmospheres of pressure.

Oleoresin exudation pressure has also been postulated to be associated with tree water status (Rudinsky, 1966a). Trees which lack water stress should have a tendency to exhibit a greater oleoresin exudation pressure than trees under water stress. Lower levels of sapwood moisture content in western larch combined with its significantly larger duct areas may be associated with its near lack of oleoresin pressure.

Findings of Berryman (1974) and Webb and Franklin (1978) that high phloem moisture adversely effects bark beetle development may also explain the failure of successful brood production within western larch which has relatively high phloem moisture content.

The absolute number of attempts to initiate brood production on any of the individual felled trees could not be measured; many beetles may have tested the felled tree (either larch or Douglas-fir) and opted for continued flight in search for a more suitable host. The large frass piles which were counted in this study were those of *bona fide* attempts at brood production. It is possible that abandoned initial attempts on a western larch with very thin phloem may have gone unnoticed for lack of significant frass/saw dust build up. The significantly lower attack rate found for western larch compared with Douglas-fir could be related to its narrow phloem band, as was found in lodgepole pine by

Amman (1972) and Berryman (1976).

Further studies should focus on the aspects of phloem thickness, and phloem and bark moisture content. In addition qualitative and quantitative investigation of the outer sapwood oleoresin monoterpene and resin acid content of both Douglas-fir and western larch should be undertaken. Such an investigation would provide information on vertical resin duct sizes and components of sapwood oleoresin of each species in relation to Douglas-fir beetle activity.

CHAPTER 3

COMPARATIVE BIOCHEMISTRY OF TWO HOSTS OF THE DOUGLAS-FIR BARK BEETLE: XYLEM AND EMITTED VOLATILES

Abstract

Forty-eight Douglas-fir of two age, two crown, and two bark beetle resistance classes and 16 mature, codominant western larch from an uneven aged stand in northern Idaho were analyzed by gas chromatography for xylem oleoresin monoterpene and resin acid composition and for volatiles released from core sections. Few qualitative or quantitative differences were found in oleoresin, resin acids, and core samples of the two age, two crown, and two resistance classes of Douglas-fir. The majority of the resin acids in each species were identified as either abietic or pimaric types. The absence of the resin acid pair, pimarate/communate, in Douglas-fir was one of the few qualitative species differences found. Several quantitative differences were found between species. Alpha-pinene was the predominant component of oleoresin and core volatiles in all trees. With the exception of 3-carene, most monoterpenes comprised a greater percent of the total oleoresin and core volatiles in Douglas-fir than in western larch. In both species concentrations of α-pinene and camphene were greater in the vapor spectrums emitted from core samples than in the xylem oleoresin. Live western larch apparently avoid beetle attack by producing the

repellent 3-carene in greater quantity than Douglas-fir. Support for this hypothesis is based on the significant negative correlation found between relative attack rate and concentration of 3-carene in xylem oleoresin of western larch.

Introduction

The Douglas-fir beetle (Dendroctonus pseudotsugae Hopkins) is the most destructive beetle of North American Douglas-fir forests. Within recent years the Douglas-fir beetle has accounted for annual losses averaging more than ten million board feet in eastern Washington and Oregon alone (Orr and Brown, 1980).

Douglas-fir (Pseudotsuga menziesii (Beissn.) Franco) is attacked by the Douglas-fir beetle throughout its natural range in Western United States, Canada, and Mexico. Only two tree species are known to produce Douglas-fir beetle broods in nature: Douglas-fir and western larch (Larix occidentalis (Nutt.)). Freshly felled material is the preferred attack site for the Douglas-fir beetle (Lejeune, McMullen, and Atkins, 1961) yet the beetle is also capable of colonizing and killing a living tree. Live standing western larch trees are sometimes attacked in association with outbreaks in Douglas-fir trees. However, successful brood production in live standing western larch has not been observed (Furniss and Orr, 1978). Brood production has been documented in felled western larch (Ross, 1967) and can occur at an equal rate with brood production in Douglas-fir (Furniss, 1976).

Much work in relation to host attraction and chemical communication within bark beetles has been accomplished (see reviews of Renwick and Vite, 1970; Wood, 1972, 1982; Borden, 1974, 1977; Birch, 1978). The synergistic effect produced by pheromones in combination with host monoterpenes, primarily α-pinene, has been studied by Jantz and Rudinsky (1970), Vite (1970), Knopf and Pitman (1972), and Rudinsky (1976). Heikkenen and Hrutfiord (1965) found α-pinene attracts Douglas-fir beetles while β-pinene repels them. Rudinsky (1966) found that in addition to α-pinene, camphene and limonene were also attractants but β-pinene and myrcene were not. Frontalin, the Douglas-fir beetle produced pheromone, in combination with camphene, produced the greatest attraction of several synergistic frontalin/monoterpene combinations (Pitman and Vite, 1970). Results of work of Furniss and Schmitz (1971) differed from those of Pitman and Vite (1970) in that frontalin in combination with α-pinene was found to produce the greatest attraction. Furniss, Livingston, and McGregor (1981) hypothesized that trees with maximum α-pinene and minimum 3-carene concentrations would attract beetles while trees with minimum α-pinene and maximum 3-carene would repel beetles. In testing the response of Douglas-fir beetles to various monoterpenes using the method of electroantennagram analysis, male beetles were found to be most sensitive to camphene and female beetles, the pioneering host finders, were found to be most sensitive to camphene and limonene (Dickens *et al*. 1983).

Insect resistance in relation to oleoresin content in conifers has been reviewed by Hanover (1975). From numerous studies on the mountain pine beetle (*Dendroctonus ponderosae*), western pine beetle (*D. brevicomis*), and the Jeffrey pine beetle (*D. jeffreyi*), Smith (1963, 1965, 1966, 1969, 1972, 1975, and 1982) has found that: 1) bark beetles in general can tolerate resin vapors from their specific hosts but not from nonhosts, 2) individual vapors of conifer trees differ in their effect on beetles, and 3) the overall success of attack depends on the composition of xylem oleoresin. In specific, Smith (1965, 1966) and Sturgeon (1979) both found that high concentrations of limonene in ponderosa pine (*Pinus ponderosa* Laws.) are associated with resistance to attack by the western pine beetle. Harris *et al*. (1983) found that cortical resin of Sitka spruce (*Picea sitchensis* (Bong.) Car.) resistant to the white pine weevil (*Pissodes strobi* Peck) had significantly lower concentrations of β-phellandrene and higher concentrations of β-pinene and 3-carene than did susceptible trees.

In natural unmanaged stands successful attack rates of the Douglas-fir beetle are greater in larger, more dominant trees (Furniss *et al*. 1981). The underlying cause(s) or mechanism(s) of resistance to the Douglas-fir beetle are virtually unknown. In particular the reasons for observed differential resistance of mature Douglas-fir and the immunity of immature live Douglas-fir and live western larch of all ages are not known but have immense potential significance to future forest protection. A comprehensive analysis of the oleoresin systems of hosts of the Douglas-fir beetle might provide an indication of a mechanism of resistance to attack. An initial study of monoterpene concentrations within Douglas-fir in relation to geographic location and resistance to attack was made by Hanover and Furniss (1966).

The purpose of this study was to investigate: 1) the volatile monoterpene composition of xylem oleoresin, 2) the non-volatile resin acid content of xylem oleoresin which along with the monoterpene fraction comprise the majority of the oleoresin within conifer trees, and 3) the volatile vapor spectrum of individual tree core sections from Douglas-fir and western larch trees. Within this study, variation in oleoresin composition and emitted vapors of core sections are reported in relation to age and crown classification within Douglas-fir, previous patterns of attack of the Douglas-fir beetle within Douglas-fir, and species differences between Douglas-fir and western larch.

Materials and Methods

In May, 1981, 48 Douglas-fir and 16 western larch trees of an uneven aged second growth stand in the St. Joe National Forest were selected for study. The stand was located 12.8 km southwest of Elk River, Clearwater County, Idaho at an elevation of 800 meters. Other species common to the study area were ponderosa pine (*Pinus ponderosa* Laws), western white pine (*P. monticola* Dougl.), grand fir (*Abies grandis* (Dougl.) Lindl.), and western red cedar (*Thuja plicata* Donn.).

This study was comprised of three comparative tests of xylem oleoresin monoterpenes and resin acids and volatile monoterpenes produced from core sections. For the first comparison ten Douglas-fir were selected for each of the following age and crown categories: mature/codominant, young/codominant, mature/suppressed or intermediate, and young/suppressed or intermediate. The second test compared two groups of mature, codominant Douglas-fir. The first group of four Douglas-fir had been attacked by a localized outbreak of Douglas-fir beetles ten years prior to this study and survived. The second group of four Douglas-fir were of the same relative age but had not sustained attack ten years prior to this study. The third test was a species comparison between 14 mature, codominant Douglas-fir (ten from the first comparison above and four of the unattacked trees from the second comparison) and 16 mature, codominant western larch.

Core Sampling and Vapor Analysis

Core samples for vapor analysis were taken from each tree at breast height (1.8 m) with an increment borer (5.1 mm diameter). Upon extraction, the core samples were immediately placed in test tubes (16.4 ml volume), sealed with silicone rubber stoppers, and placed in a portable ice cooler until they were returned to the laboratory and placed in a freezer at -17°C.

Core sections of each tree contained tissues of outer bark, inner phloem, sapwood, and heartwood. In the analysis of volatiles, test tubes containing the core sections were removed from storage (-17°C) and allowed to warm to ambient room temperature for one hour before being submerged in a 35°C water bath. After 0.5 hours the core sections were removed from the water bath and a 3.0 ml air sample was drawn from each tube with a 5.0 ml gas tight syringe which was preheated to 45°C in an oven to prevent condensation of the vapor sample. The vapor sample was immediately injected into a Hewlett Packard 5700A gas chromatograph equipped with a flame ionization detector and dual 244 cm by 6 mm packed columns of 10% β, β' oxidipropionitrile on 60/80 mesh Chromasorb W-AW. Operating temperatures were column 68°C, injector 120°C, and detector temperature 150°C. Helium, at a flow rate of 45 ml/min was used as the carrier gas. Monoterpenes in the vapor phase were identified by relative retention times of pure samples analyzed by external standard techniques. Individual peak areas were measured by a Hewlett-Packard 3390A integrator. The amount of each monoterpene present in the vapor samples was adjusted for individual detector response as determined from the external standard procedure. Monoterpenes of the vapor spectrum from each core section were quantified as to the relative proportion present on a ppm basis. Following each analysis, total length and average diameter of the core sections were taken with calibrated calipers to calculate individual core surface areas. To standardize vapor measurements across all core sections, a ratio of relative ppm of individual terpenes produced to total core surface area was calculated.

Oleoresin Sampling

Oleoresin samples for monoterpene and resin acid analysis were obtained by drilling a hole (6.35 mm diameter) at an angle approximately 45^{o} from vertical to a depth of 1.0 cm into the outer sapwood of each tree. A sealed culture tube was placed in each hole and secured with silicone rubber. Three measured volumes of oleoresin from each tree were collected from the culture tubes with calibrated 20.0 microliter capillary tubes. The capillary tubes were then placed in sealed culture tubes and refrigerated at 2^{o}C until analyzed by gas-liquid chromatography. Trees which produced insufficient oleoresin for collection in the sealed culture tubes were cut with an ax to a depth of 1.0-1.5 cm into the outer sapwood, exposing an area of 50-60 cm^2. Oleoresin samples were then collected from these wound sites as described above.

Oleoresin Analysis of Monoterpenes

Oleoresin samples stored at 2^{o}C in capillary tubes were diluted with measured volumes of pentane and a known amount of heptane was added as an internal standard. The culture tubes were then centrifuged to insure all oleoresin from the capillary tube had dissolved in the pentane/heptane solvent. A Hewlett Packard 5700A gas chromatograph equipped with a flame ionization detector and 244 cm by 6 mm dual packed stainless steel columns of 7.5% SE-30 on 60/80 mesh Chromasorb W-AW was used to analyze 3.0 microliter injections of the diluted oleoresin samples. The initial column temperature was held at 70°C for eight minutes followed by a programmed increase in temperature at a rate of 8°C/min to a final temperature of 220°C. Injection and detector temperatures were each 250°C. Helium was used as the carrier gas at a flow rate of 80 ml/min. A second gas chromatograph (F & M model 700) equipped with dual flame ionization detectors and the same stainless steel packed columns of 10% β,β' oxidipropionitrile and operating conditions as described above was used to separate the pairs of compounds β-pinene/sabinene and limonene/β-phellandrene, which were not separated on the SE-30 column.

Pure samples of known amounts of individual monoterpenes were chromatographed individually under the identical operating conditions to determine relative retention times and individual detector response values relative to the internal standard heptane. Unidentified compounds from oleoresin samples were assigned a response factor of 1.0 in relation to the response of heptane.

Resin Acid Analysis

Trees which produced sufficient quantities of xylem oleoresin were sampled by collecting 20.0 microliters of oleoresin in calibrated capillary tubes. These samples were placed in sealed culture tubes and stored at 2^{o}C as described previously. All but one Douglas-fir and only seven western larch produced sufficient 20.0 microliter samples of oleoresin for resin acid analysis.

Methyl esters of individual resin acids were analyzed using the gas chromatography method of Bridgen, Hanover, and Wilkinson (1979). Individual resin acids in the oleoresin samples were identified by comparison with retention times of pure standards run under identical isothermal conditions on a Versamide-900 column. Quantification of resin acids was obtained by determining individual detector response values relative to the internal standard, methyl arachidate. Unidentified compounds were assigned a response factor of 1.0 in relation to the internal standard.

After sampling trees for oleoresin and core samples, each tree was cut down to facilitate further studies and sampling. Three weeks after the last tree had been sampled and felled, field observations of relative attack rates were determined by walking up one side and down the other to count the number of attacks incurred (not counting the underside) by each tree. Trees which had sustained attack ten years previously and survived were not felled and scored for attacks but were left standing for further analysis.

Statistical Procedures

Monoterpene and resin acid content of xylem oleoresin were reported on the basis of percent composition of total oleoresin. These percentages were transformed by the arcsine of square root transformation and then analyzed by procedures of analysis of variance. The experimental design used for analyzing data of monoterpene and resin acid oleoresin composition and core vapors within Douglas-fir trees of two age and two crown classifications was a 2x2 factorial split-plot with the main plots (crown classes) arranged in a completely randomized design. The second comparison of previously attacked and unattacked mature, codominant Douglas-fir was made only on data of oleoresin monoterpene and resin acid content using a completely randomized design. The comparison of species was analyzed as a completely randomized design.

Results

Analysis of Xylem Oleoresin Monoterpenes

Nineteen monoterpenes and 27 other unknown compounds were found within two age and two crown classes of Douglas-fir. With the exceptions of significantly greater amounts of myrcene and terpinolene in the codominant class trees and α-terpineol in the older class trees, monoterpenes showed no qualitative and little quantitative variation across age and crown classifications. The mean percent oleoresin values for each terpene and several unknown compounds in age and crown classes are listed in Table 3.1. Four unknown compounds (Nos. 42, 44, 47, and 55) did show significantly greater concentrations in younger age class trees. Compounds which have greater retention times than bornyl acetate are probably higher molecular weight nonterpenoid aromatics, sesquiterpene hydrocarbons, and sesquiterpene alcohols. For purposes of analysis and discussion these unknowns were labelled as "group II unknowns". Total monoterpenes, which included all compounds eluting from α-pinene to bornyl acetate inclusive, and the overall total of monoterpenes plus group II unknowns showed no significant quantitative difference in relation to age or crown classification. The total of group II unknowns also showed no significant quantitative difference in relation to crown classification but did between age classes. Younger age trees had two times the total concentration of group II unknowns than older age trees. Age by crown class interactions occurred for camphene and unknown 21. The mean percentages of individual monoterpenes from xylem oleoresin based upon the total monoterpene percent oleoresin values are presented in Table A.1.

The comparison of mean percent monoterpenes and unknowns of xylem oleoresin from four previously attacked and four previously unattacked codominant Douglas-fir is presented in Table 3.2. Seventeen monoterpenes and 17 additional unknowns were detected. No significant quantitative differences in any of the individual or total

Table 3.1. Mean percent monoterpenes and unknowns of xylem oleoresin of two age and two crown classes of Douglas-fir.

	-Age Class-			-Crown Class-		
Compound	Young	Mature	Sig.	Codom.	Supp./ Int.	Sig.
			---Percent Oleoresin---			
α-pinene	24.12	22.02	ns	24.08	22.06	ns
Camphene	0.54	0.39	ns	0.49	0.43	ns
β-pinene	2.83	2.79	ns	2.92	2.70	ns
Sabinene	0.26	0.30	ns	0.28	0.29	ns
Myrcene	0.36	0.29	ns	0.38	0.28	*
3-Carene	0.30	0.49	ns	0.57	0.23	ns
Limonene	1.06	1.43	ns	1.39	1.11	ns
β-phellandrene	1.37	1.25	ns	1.37	1.25	ns
γ-terpinene	0.02	trace	ns	0.01	0.01	ns
Terpinolene	0.14	0.22	ns	0.24	0.12	**
Camphor	0.01	trace	ns	0.01	trace	ns
Borneol	trace	0.06	ns	trace	0.06	ns
Terpinen-4-ol	0.01	0.07	ns	0.01	0.06	ns
α-terpineol	trace	0.04	**	0.01	0.03	ns
Unknown 10	0.03	0.02	ns	0.04	0.01	ns
Unknown 12	0.01	0.04	ns	0.01	0.04	ns
Citronellol	trace	0.02	ns	0.01	0.01	ns
Unknown 13	trace	0.01	ns	trace	trace	ns
Bornyl acetate	0.16	0.14	ns	0.18	0.13	ns
Unknown 15	0.02	0.04	ns	0.03	0.04	ns
Unknown 18	0.17	0.11	ns	0.17	0.11	ns
Unknown 19	0.11	0.07	ns	0.11	0.06	ns
Unknown 21	0.44	0.36	ns	0.44	0.36	ns
Unknown 24	0.01	0.01	ns	0.01	trace	ns
Unknown 26	0.12	0.01	ns	0.12	0.01	ns
Unknown 28	trace	0.01	ns	trace	0.01	ns
Unknown 30	0.10	0.01	ns	0.09	0.01	ns
Unknown 32	0.09	trace	ns	0.08	trace	ns
Unknown 36	0.0	trace	ns	trace	trace	ns
Unknown 37	0.09	trace	ns	0.09	trace	ns
Unknown 38	0.06	trace	ns	0.05	0.01	ns
Unknown 39	0.14	trace	ns	0.14	trace	ns
Unknown 40	0.05	trace	ns	0.05	trace	ns
Unknown 42	0.80	0.28	*	0.58	0.51	ns
Unknown 44	0.91	0.42	*	0.74	0.59	ns
Unknown 45	0.42	0.14	ns	0.33	0.23	ns
Unknown 47	0.21	0.03	*	0.14	0.09	ns
Unknown 48	3.10	1.59	ns	2.97	1.71	ns
Unknown 50	0.0	0.04	ns	0.02	0.02	ns
Unknown 52	2.66	1.78	ns	2.58	1.85	ns
Unknown 55	2.55	1.50	*	2.29	1.76	ns
Unknown 58	1.52	0.66	ns	1.18	0.99	ns
Unknown 60	1.20	0.12	ns	0.67	0.65	ns

Table 3.1 continued.

Compound	Young	Mature	Sig.	Codom.	Supp./ Int.	Sig.
Total monoterpenes	31.22	29.58	ns	32.00	28.82	ns
Total group II unknowns	14.76	7.19	**	12.89	9.06	ns
Total	45.98	36.77	ns	44.89	37.88	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

compound categories were detected. Mean xylem oleoresin monoterpene composition as percent of total monoterpene oleoresin fraction in unattacked and attacked Douglas-fir is presented in Table A.2.

The species comparison of xylem oleoresin revealed a total of 19 monoterpenes present in at least trace amounts in mature, codominant Douglas-fir and 18 monoterpenes present in at least trace amounts in mature, codominant western larch. The mean oleoresin composition of monoterpenes and unknown compounds are listed in Table 3.3. The presence of sabinene in Douglas-fir and its absence in western larch was the only qualitative monoterpene difference found between the species. Additionally, some group II unknowns were detected in western larch only.

Several quantitative differences between species were found in both the monoterpenes and the group II unknowns. Six monoterpenes, α-pinene, β-pinene, sabinene, myrcene, limonene, and β-phellandrene occurred in greater concentrations in Douglas-fir than in western larch, whereas

Table 3.2. Mean percent monoterpenes and unknowns of xylem oleoresin of previously attacked and unattacked mature, codominant Douglas-fir.

Compound	Unattacked	Attacked	Significance
	---Percent	Oleoresin---	
α-pinene	28.19	23.51	ns
Camphene	0.55	0.38	ns
β-pinene	3.77	3.73	ns
Sabinene	0.30	0.23	ns
Myrcene	0.91	0.70	ns
3-Carene	0.27	0.33	ns
Limonene	2.32	1.54	ns
β-phellandrene	1.85	1.86	ns
γ-terpinene	0.04	0.05	ns
Terpinolene	0.15	0.12	ns
Camphor	trace	trace	ns
Borneol	trace	trace	ns
Terpinen-4-ol	trace	trace	ns
α-terpineol	trace	trace	ns
Unknown 10	0.04	0.02	ns
Citronellol	0.01	0.01	ns
Bornyl acetate	0.13	0.08	ns
Unknown 18	0.10	0.16	ns
Unknown 19	0.08	0.06	ns
Unknown 21	0.41	0.61	ns
Unknown 24	0.01	0.02	ns
Unknown 26	0.05	0.0	ns
Unknown 30	0.04	0.0	ns
Unknown 37	0.0	0.03	ns
Unknown 42	1.49	1.41	ns
Unknown 44	1.47	1.02	ns
Unknown 45	0.26	0.47	ns
Unknown 47	0.43	0.13	ns
Unknown 48	2.47	1.33	ns
Unknown 50	0.10	0.32	ns
Unknown 52	6.09	2.61	ns
Unknown 55	5.76	2.37	ns
Unknown 58	2.55	1.22	ns
Unknown 60	2.57	0.73	ns
Total monoterpenes	38.53	32.55	ns
Total group II unknowns	23.87	12.08	ns
Total	62.40	44.63	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Table 3.3. Mean percent monoterpenes and unknowns of xylem oleoresin of mature codominant western larch and Douglas-fir.

Compound	Douglas-fir	Western Larch	Significance
	---Percent Oleoresin---		
α-pinene	22.70	11.70	**
Camphene	0.39	0.29	ns
β-pinene	3.21	1.11	**
Sabinene	0.29	0.0	**
Myrcene	0.49	0.12	**
3-Carene	0.56	4.99	**
Limonene	1.63	0.77	*
β-phellandrene	1.61	0.13	**
γ-terpinene	0.01	0.08	*
Terpinolene	0.26	0.30	ns
Camphor	trace	0.01	ns
Borneol	trace	0.02	ns
Terpinen-4-ol	0.01	0.05	**
α-terpineol	0.01	0.04	ns
Unknown 10	0.03	0.05	ns
Unknown 12	0.01	0.08	ns
Citronellol	0.02	0.01	ns
Unknown 13	0.01	trace	ns
Bornyl acetate	0.10	0.02	ns
Unknown 15	0.01	trace	ns
Unknown 18	0.10	0.02	**
Unknown 19	0.07	0.01	**
Unknown 21	0.31	0.03	**
Unknown 22	0.0	0.01	**
Unknown 24	trace	0.05	**
Unknown 26	0.02	0.09	**
Unknown 28	trace	0.09	**
Unknown 29	0.0	0.07	*
Unknown 30	0.02	0.05	ns
Unknown 32	trace	0.06	**
Unknown 34	0.0	0.05	**
Unknown 35	0.0	0.03	*
Unknown 36	trace	0.02	ns
Unknown 37	trace	0.11	**
Unknown 38	trace	0.07	*
Unknown 39	trace	0.07	**
Unknown 40	trace	0.02	**
Unknown 42	0.69	6.72	**
Unknown 44	0.72	0.70	ns
Unknown 45	0.18	3.48	**
Unknown 47	0.14	0.09	ns
Unknown 48	2.07	0.22	**
Unknown 49	0.0	0.18	*
Unknown 50	0.06	0.54	**
Unknown 52	3.13	2.37	ns
Unknown 53	0.0	0.34	*

Table 3.3 continued.

Compound	Douglas-fir	Western larch	Significance
Unknown 54	0.0	0.29	**
Unknown 55	2.69	2.81	ns
Unknown 58	1.12	2.20	**
Unknown 60	0.82	0.59	ns
Unknown 61	0.0	0.18	*
Total monoterpenes	31.34	19.76	**
Total Group II unknowns	12.11	21.54	**
Total	43.45	41.30	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

western larch had significantly more 3-carene, γ-terpinene, and terpinen-4-ol than Douglas-fir. Both the total percent monoterpenes and total of group II unknowns differed significantly between species. The monoterpene composition of Douglas-fir oleoresin (31.34%) was more than 1.5 times greater than that of western larch (19.76%), whereas the total concentration of the 32 group II unknowns was significantly higher in western larch. Of the 32 unknowns detected, 19 were more concentrated in western larch and four were more with concentrated in Douglas-fir. Analysis of variance indicated no significant difference in the combined total of monoterpenes and group II unknowns. The xylem oleoresin composition as percent of total monoterpenes of mature, codominant Douglas-fir and western larch are listed in Table A.3. The most striking difference between the two species is the very high proportion of 3-carene in western larch.

Xylem Resin Acid Analysis

Thirty-six compounds were detected in xylem oleoresin resin acid analysis of the two age and two crown classes within Douglas-fir (Table 3.4). Of the 36 compounds, eight were identified resin acids. Two pairs of acids, levopimarate/palustrate and dehydroabietate/strobate were not separated on the Versamide-900 column and were thus reported on a combined percentage basis.

Only one qualitative difference (unknown 119) was found between age classes and no qualitative differences were found in crown classes within Douglas-fir. No quantitative differences were found between young and mature trees in any of the 36 compounds or the total of all resin acids. Two quantitative differences occurred between codominant and suppressed/intermediate crown class trees. Significantly higher concentrations of sandaracopimarate and dehydroabietate/strobate occurred in suppressed/intermediate crown class trees compared with codominant class trees.

Identified resin acids were consistent both within and between age and crown classes and formed the majority of the resin acid fraction, with 84.25%, 86.48%, 85.13%, and 85.87% present in young and mature age classes and codominant and suppressed/intermediate crown classes, respectively. Xylem oleoresin resin acid composition in age and crown classes of Douglas-fir is presented in Table A.4.

In the comparison of previously attacked and unattacked mature, codominant Douglas-fir trees, 34 compounds were

Table 3.4. Mean percent resin acids of xylem oleoresin of two age and two crown classes of Douglas-fir.

	-Age Class-			-Crown Class-		
Resin Acid	Young	Mature	Sig.	Codom.	Supp./ Int.	Sig.
	---Percent Oleoresin---					
Unknown 102	0.07	0.09	ns	0.11	0.05	ns
Unknown 103	0.21	0.29	ns	0.24	0.26	ns
Unknown 104	0.15	0.20	ns	0.15	0.19	ns
Unknown 105	0.05	0.12	ns	0.05	0.12	ns
Unknown 106	0.63	0.44	ns	0.48	0.59	ns
Unknown 108	0.02	0.01	ns	trace	0.03	ns
Unknown 109	0.09	0.06	ns	0.01	0.14	ns
Unknown 110	0.03	0.04	ns	0.02	0.05	ns
Unknown 112	0.03	0.03	ns	0.02	0.04	ns
Unknown 114	0.17	0.18	ns	0.12	0.22	ns
Unknown 115	0.03	0.03	ns	0.04	0.02	ns
Unknown 117	0.07	0.11	ns	0.09	0.09	ns
Unknown 118	0.17	0.19	ns	0.17	0.19	ns
Unknown 119	0.0	0.02	ns	0.01	0.01	ns
Unknown 120	0.15	0.12	ns	0.07	0.21	ns
Unknown 121	0.08	0.04	ns	0.04	0.08	ns
Unknown 122	0.14	0.11	ns	0.13	0.12	ns
Unknown 123	0.25	0.21	ns	0.20	0.24	ns
Unknown 124	0.02	0.02	ns	0.03	0.01	ns
Unknown 126	0.06	0.04	ns	0.07	0.02	ns
Unknown 127	0.04	0.01	ns	trace	0.05	ns
Unknown 128	0.64	0.51	ns	0.67	0.48	ns
Unknown 129	0.06	0.08	ns	0.05	0.09	ns
Unknown 130	0.30	0.19	ns	0.21	0.27	ns
Sandaracopimarate	0.66	0.74	ns	0.60	0.80	*
Levopimarate + Palustrate	9.11	9.78	ns	8.84	10.05	ns
Isopimarate	6.31	7.84	ns	6.54	7.61	ns
Unknown 131	0.46	0.54	ns	0.47	0.52	ns
Dehydroabietate + Strobate	1.87	1.94	ns	1.30	2.52	*
Unknown 132	0.23	0.23	ns	0.24	0.23	ns
Abietate	3.30	3.47	ns	3.19	3.58	ns
Unknown 133	0.34	0.34	ns	0.35	0.33	ns
Neoabietate	4.14	4.68	ns	4.13	4.69	ns
Unknown 134	0.25	0.19	ns	0.26	0.17	ns
Total Acids	30.14	32.90	ns	28.90	34.07	ns

* significant at the 0.05 level

** significant at the 0.01 level

ns nonsignificant

detected. The mean concentrations of resin acids found in xylem oleoresin are listed in Table 3.5. Eight of the 34 compounds were identified. No quantitative differences were detected in any of the resin acids or in the total of all resin acids. As previously reported for Douglas-fir, the majority of the resin acid fraction, 77.22% in the attacked trees and 84.40% in the unattacked trees, was composed of the eight identified resin acids. The mean xylem oleoresin resin acid composition as percent total resin acid of attacked and unattacked trees is presented in Table A.5.

Forty compounds, 10 identified and 30 unknown, were detected in the xylem oleoresin resin acid analysis in the comparison of mature, codominant Douglas-fir and western larch. The means of these resin percentages are listed in Table 3.6. Western larch lacked unknowns 124 and 129 while Douglas-fir lacked the pair of identified resin acids pimarate/communate which were not separated from each other on the Versamide-900 column. Of the 18 significant quantitative differences between the species, 12 were more concentrated in western larch than in Douglas-fir. The mean total of resin acids was not significantly different between the two species but was slightly higher in western larch.

The majority (84.06%) of the resin acid fraction in Douglas-fir was found in the identified resin acids and was considerably more than the relative portion (57.68%) of identified resin acids in western larch. The mean resin acid composition as percent of total resin acid for both

Table 3.5. Mean percent resin acids of xylem oleoresin of previously attacked and unattacked mature, codominant Douglas-fir.

Resin Acid	Attacked	Unattacked	Significance
	---Percent	Oleoresin---	
Unknown 102	0.06	0.30	ns
Unknown 103	0.21	0.09	ns
Unknown 104	0.11	0.13	ns
Unknown 105	0.29	0.51	ns
Unknown 106	0.22	0.37	ns
Unknown 108	0.19	0.03	ns
Unknown 109	0.11	0.15	ns
Unknown 113	0.0	0.02	ns
Unknown 114	0.15	0.14	ns
Unknown 115	0.09	0.10	ns
Unknown 117	0.17	0.20	ns
Unknown 118	0.31	0.40	ns
Unknown 120	0.21	0.12	ns
Unknown 121	0.08	0.07	ns
Unknown 122	0.12	0.12	ns
Unknown 123	0.19	0.26	ns
Unknown 124	0.0	0.03	ns
Unknown 126	0.07	0.0	ns
Unknown 127	0.06	0.08	ns
Unknown 128	0.31	0.46	ns
Unknown 129	0.33	0.0	ns
Unknown 130	0.19	0.08	ns
Sandaracopimarate	0.46	0.73	ns
Levopimarate + Palustrate	5.56	9.38	ns
Isopimarate	4.98	7.99	ns
Unknown 131	0.60	0.61	ns
Dehydroabietate + Strobate	0.98	1.73	ns
Unknown 132	0.14	0.25	ns
Abietate	1.96	3.43	ns
Unknown 133	0.37	0.40	ns
Neoabietate	2.93	4.76	ns
Unknown 134	0.40	0.26	ns
Total resin acids	21.85	33.20	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Table 3.6. Mean percent resin acids of xylem oleoresin of mature, codominant Douglas-fir and western larch.

Resin Acid	Douglas-fir	Western Larch	Significance
	---Percent	Oleoresin---	
Unknown 102	0.10	0.13	ns
Unknown 103	0.26	0.20	ns
Unknown 104	0.14	0.11	ns
Unknown 105	0.14	0.57	**
Unknown 106	0.42	0.03	**
Unknown 108	0.05	0.16	ns
Unknown 109	0.05	0.11	ns
Unknown 110	0.03	0.25	**
Unknown 112	0.02	0.10	*
Unknown 113	trace	0.17	**
Unknown 114	0.13	0.32	ns
Unknown 115	0.05	0.49	**
Unknown 116	0.0	0.18	ns
Unknown 117	0.12	0.99	**
Unknown 118	0.22	2.06	**
Unknown 119	0.02	0.24	**
Unknown 120	0.10	0.16	ns
Unknown 121	0.05	0.31	**
Unknown 122	0.13	0.14	ns
Unknown 123	0.22	0.13	*
Unknown 124	0.01	0.0	ns
Unknown 126	0.06	0.08	ns
Unknown 127	0.02	0.15	**
Unknown 128	0.56	4.72	**
Unknown 129	0.17	0.0	*
Unknown 130	0.20	0.27	ns
Pimarate + Communate	0.0	0.65	**
Sandaracopimarate	0.62	0.53	ns
Levopimarate + Palustrate	8.60	4.40	*
Isopimarate	6.95	6.82	ns
Unknown 131	0.55	0.46	ns
Dehydroabietate + Strobate	1.36	0.67	**
Unknown 132	0.22	0.22	ns
Abietate	3.02	2.86	ns
Unknown 133	0.35	0.54	ns
Neoabietate	4.10	2.74	*
Unknown 134	0.28	0.41	ns
Total resin acids	29.32	32.37	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Douglas-fir and western larch is presented in Table A.6.

Core Volatile Analysis

Ten monoterpenes and three unknown compounds were detected in the analysis of air samples of heated core sections of two age and two crown classes within Douglas-fir. The mean ratios of concentrations of volatiles detected to total surface area of individual core sections are listed in Table 3.7. Unknown 5 which was present only in samples of younger, codominant class trees, while a significantly greater ratio of relative ppm of β-pinene to core section surface area within the codominant crown class was detected.

Table 3.7. Mean ratios of monoterpene volatiles (ppm) to surface area of core sections within two age and two crown classes of Douglas-fir.

	-Age Class-			-Crown Class-		
Monoterpene	Young	Mature	Sig.	Codom.	Supp./ Int.	Sig.
	---Ratio of ppm to Surface Area---					
Unknown 2	1.68	1.21	ns	1.46	1.44	ns
Unknown 4	1.58	0.74	ns	0.92	1.41	ns
α-pinene	108.05	138.55	ns	145.85	100.74	ns
Unknown 5	0.28	0.0	ns	0.27	0.0	ns
Camphene	8.99	4.57	ns	4.33	9.66	ns
β-pinene	6.14	8.24	ns	9.54	4.90	**
3-Carene	0.43	1.10	ns	0.78	0.72	ns
Sabinene	0.15	0.07	ns	0.06	0.03	ns
Myrcene	1.18	3.08	ns	3.30	0.95	ns
Limonene	2.51	3.73	ns	3.47	2.76	ns
β-phellandrene	1.05	2.06	ns	2.43	0.69	ns
γ-terpinene	0.01	0.05	ns	0.05	0.01	ns
Terpinolene	0.02	0.10	ns	0.09	0.03	ns
Total volatiles	129.76	162.30	ns	172.55	123.33	ns

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Alpha-pinene was the predominant volatile released from all core sections and accounted for approximately 83-84% of the total vapor spectrum. The individual mean percent monoterpene volatiles released from each core section within two age and two crown classes of Douglas-fir are presented in Table A.7.

From the comparison of volatiles released from core sections of mature codominant Douglas-fir and western larch, 10 monoterpenes and two unknowns were detected. The mean ratios of concentrations of volatiles detected from core sections to total core surface area are presented in Table 3.8. Three qualitative differences were found. Sabinene, β-phellandrene, and γ-terpinene were not volatilized from western larch core sections. In addition to the significant difference in total volatiles given off, Douglas-fir showed significantly greater ratios for α-pinene, β-pinene, myrcene, limonene, β-phellandrene, and total volatiles. Western larch had significantly greater ratios for unknown 4 and 3-carene.

The mean percent of α-pinene was roughly 20% greater in Douglas-fir than in western larch while the mean percents of unknown 4 and 3-carene in western larch were 60 and 39 times greater, respectively, than those of Douglas-fir cores. The concentrations of monoterpene volatiles based on the total ppm of volatiles released by each core section are listed in Table A.8.

Table 3.8. Mean ratios of monoterpene volatiles (ppm) to surface area of core sections of mature, codominant Douglas-fir and western larch.

Monoterpene	Douglas-fir	Western Larch	Significance
	---Ratio of ppm to Surface Area---		
Unknown 2	1.22	0.85	ns
Unknown 4	0.35	5.48	**
α-pinene	179.18	34.25	**
Camphene	5.54	2.13	ns
β-pinene	13.56	1.72	**
3-Carene	0.78	7.57	**
Sabinene	0.15	0.0	ns
Myrcene	4.67	0.25	*
Limonene	5.14	1.21	*
β-phellandrene	3.36	0.0	**
γ-terpinene	0.06	0.0	ns
Terpinolene	0.13	0.04	ns
Total Volatiles	214.14	51.73	**

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Beetle Attack

Attacks in freshly felled Douglas-fir were in some cases observed within 48 hours after felling. No attacks in freshly felled western larch were observed during the sampling period. However, observations made three weeks following the final sampling date revealed that all western larch had been attacked but at a relatively low level. One Douglas-fir of the young, suppressed/intermediate class was not attacked. Attack rates within age and crown classes of Douglas-fir were both significantly different at the 0.01 level. Young, mature, codominant, and suppressed trees received attacks at an average rate of 4.08, 7.08, 6.50, and 4.65 attacks/m^2, respectively. Mature, codominant Douglas-fir had a mean attack rate of 5.19 attacks/m^2, which was significantly greater ($p \leq 0.01$) than the 0.37 attacks/m^2 found in western larch.

Correlation Analysis

Correlation analysis of the observed relative attack rates with xylem oleoresin monoterpenes, resin acids, and volatiles of core sections gave the following results. From the analysis of xylem oleoresin, monoterpenes and group II unknowns within Douglas-fir, only the monoterpene limonene was positively correlated at a significant level with attack rate ($r=.30$, $p \leq .047$). Within western larch, three monoterpenes showed significant relationships. Beta-pinene and bornyl acetate were positively correlated with attack rate ($r=.55$, $p \leq .027$ and $r=.65$, $p \leq .006$, respectively) while 3-carene was negatively correlated ($r=-.52$, $p \leq .038$).

For Douglas-fir, two resin acids, unknown 122 and the combination of levopimarate/palustrate, were related to attack rate: $r=-.38$, $p \leq .015$ and $r=-.35$, $p \leq .023$, respectively. No significant relationship between resin acids and relative attack rates was found in western larch.

Correlation analysis of the ratios of core volatiles expressed on the basis of ppm evolved to total core section surface area, with the relative attack rate observed in Douglas-fir revealed a weak negative relationship with unknown 2 ($r=-.30$ $p \leq .050$) and a weak positive relationship with β-pinene ($r=.33$ $p \leq .042$). In western larch a positive correlation between unknown 4 and relative attack rate was found ($r=.64$ $p \leq .001$).

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Discussion

The lack of any current epidemic outbreaks of the Douglas-fir beetle population did not allow the comparative investigation of heavily attacked live trees versus nonattacked trees. The sampling procedures and designs did, however, permit the evaluation of factors which have been previously hypothesized as being related to beetle selection mechanisms. Sampling time (mid to late May) of xylem oleoresin and core sections and climatic conditions were within the seasonal conditions and period of the previously documented beetle spring flight (McCowan and Rudinsky, 1958; Atkins and McMullen, 1960; McMullen and Atkins, 1962). Thus, the method of sampling was representative of those physiological tree conditions normally encountered by an attacking Douglas-fir beetle. Observations of beetles attacking Douglas-fir trees within 48 hours after felling while not attacking the surrounding live trees underscores the preference for freshly felled material. The qualitative and quantitative differences in volatiles detected in core samples represent those emitted from freshly felled trees.

The results revealed a significant difference in the monoterpene concentration of α-terpineol with age classification within Douglas-fir which contrasts with the earlier report by Hanover and Furniss (1966). In that study significant negative correlations were found between tree age and the concentrations of the monoterpenes 3-carene and limonene. A possible explanation of this difference is related to the range of tree ages within the two studies. The range in tree age in the initial study was more than 100 years (81-184), while the range in this study was 66 years (42-108). The difference in ages of trees classified as being young (42-64 yrs.) and those classified as being mature (74-103 yrs.) may not have been great enough to detect an age class difference.

The monoterpene profile of Douglas-fir xylem oleoresin was very similar to the values found in the Hanover and Furniss (1966) study. The additional compounds identified in this study were not major ones in terms of relative contributions to total oleoresin percent. Therefore, the monoterpene fraction was confirmed as being consistent at 32-33%.

This author is aware of only two previous publications, Drew and Pylant (1966) and Stairs (1968), reporting on the concentrations of monoterpenes in western larch. Both studies were based upon sample sizes of only one and five tree(s), respectively, and the results were reported on the basis of percent monoterpenes rather than on a percent oleoresin basis. The results of this study and the two previous ones agree that on a relative percent monoterpene basis, α-pinene, β-pinene, and 3-carene comprise 90 to 94% of the total monoterpenes present. In the previous studies, percentages of α-pinene and 3-carene varied considerably (Stairs, 1968; Drew and Pylant, 1966). The percentages found here (α-pinene, 59.2% and 3-carene, 25.3%) were intermediate to the values reported previously. This information suggests that some variability may exist for western larch monoterpenes. However, the lack of adequate sample sizes in the earlier studies may be distorting the true picture.

The dominance of α-pinene as the major monoterpene component was also demonstrated in the vapor analysis. In both western larch and Douglas-fir and for both age and crown classes of Douglas-fir, the concentrations of α-pinene and camphene were both greater in the vapor extracts than in the xylem oleoresin. Myrcene also exhibited this same trend in the species comparison and within the mature age and codominant crown classes of Douglas-fir. All other monoterpene percentages decreased in the core vapor profile. These contrasting patterns are most likely associated with the relatively lower boiling points of α-pinene and camphene. This same pattern of higher percentages of α-pinene and camphene in the vapor phase has been shown in gas chromatographic analysis of cortical oleoresin and volatiles released from foliage samples of various pine and spruce species (Hanover, 1972) and in oleoresin of grand fir, (*Abies grandis*), (Bordash and Berryman, 1977).

The results of the Douglas-fir resin acid analysis were comparable to earlier investigations of "pocket resin" by Erdtman *et al*. (1968). In both studies the majority of the resin acids were of the pimaric and abietic types. This study identified an additional resin acid, sandaraco-pimarate, in Douglas-fir xylem oleoresin. Observations here showing resin acids accounting for approximately 30% of the xylem oleoresin varies from the 54% reported earlier by Erdtman et al. (1968). Explanation of this difference could lie in the fundamental difference of sampling xylem oleoresin versus sampling "pocket resin". On a total percent resin acid basis, there was general agreement between the two studies on relative contributions of individual resin acids.

Analysis of resin acids of western larch resulted in the identification of ten resin acids which had previously been identified in studies of western larch heartwood extractives by Mills (1973). No quantitative comparisons can be made between this study and that of Mills because in the earlier study results were reported on a total resin acid percent basis. Comparisons of individual resin acid percents on a total percent resin acid basis revealed only minor differences between the studies.

The hypothesis that α-pinene attracts and β-pinene repels the Douglas-fir bark beetle first proposed by Heikkenen and Hrutfiord (1965) was tested by correlation of individual concentrations of α-pinene, β-pinene and the ratio of the two monoterpenes with relative attack rates observed in both species. The positive significant correlations between attack rate and β-pinene in Douglas-fir core volatiles and between attack rate and concentration of β-pinene in western larch xylem oleoresin are directly opposed to the hypothesis offered by Heikkenen and Hrutfiord

(1965) and would suggest classifying β-pinene as an attractant rather than a repellent. No significant correlations were found in either species between relative attack rate and the ratios of concentrations of α-pinene to 3-carene in xylem oleoresin or volatiles emitted from core sections to support the theory of attraction/repulsion offered by Furniss _et al_. (1981).

Based upon the results of this study the following hypothesis is offered to explain the apparent resistance to beetle attack exhibited by western larch: high 3-carene in xylem oleoresin is a repellent to the Douglas-fir beetle. Support for this hypothesis is based on the significant negative correlation between observed relative attack rate and the concentration of 3-carene in the xylem oleoresin of western larch. The lack of the corresponding negative correlation in Douglas-fir could be a result of the consistently low concentrations of 3-carene in this species. Data from the comparison of previously attacked with unattacked trees in this study offered no support for either 3-carene involvement or for any other aspect of monoterpene attraction/repulsion in Douglas-fir.

Data of "attacked/resistant" and "unattacked" Douglas-fir from the initial study of Hanover and Furniss (1966) supported the repulsive features of the 3-carene proposed here. Douglas-fir from two geographic locations which were unattacked had higher concentrations of 3-carene than those attacked. These observations illustrate the problem of

classification and terminology which is common to studies of insect/tree relations. Trees which are classified as being "attacked/resistant" may not actually be true "resistant" trees. The "attacked/resistant" trees may have survived because the attack was not overwhelming. In addition, the unattacked tree group may very well be confounding the entire situation by containing some trees which are truly resistant or not susceptible to attack.

Additional evidence in support of the hypothesis of high 3-carene concentrations inhibiting Douglas-fir beetle attack is found in the results of Furniss and Schmitz (1971). Their study focused on the degree of attraction attributable to the insect pheromone frontalin and several individual host monoterpenes. They concluded that the aggregating mechanism of the Douglas-fir beetle rested in part on the combination of the beetle's pheromone frontalin and the host monoterpene, α-pinene. Closer analysis of their data indicates that 3-carene alone or in combination with frontalin attracted the fewest number of beetles relative to any other individual monoterpene or pheromone/monoterpene combination.

An analogy to the hypothesis stated herein exists in the case of susceptibility of the favored hosts, shortleaf (*Pinus echinata*) and loblolly (*P. taeda*) pines, to attack by the southern pine beetle (*Dendroctonus frontalis* Zimmermann). Coyne and Lott (1976) found that while limonene was toxic to the southern pine beetle low concentrations of 3-carene, an uncommon monoterpene of southern pines, was even more toxic.

Theoretically the Douglas-fir beetle avoids trees with high concentrations of 3-carene. This would explain the lack of observations of "immediate" attacks on freshly felled western larch trees. I might further hypothesize that concentrations of 3-carene in felled western larch decrease to tolerable levels over time although no experimental data are available to support or refute the hypothesis.

To test the proposed hypotheses, analysis of monoterpene concentrations of xylem oleoresin of both hosts should be made in areas of epidemic outbreaks of the Douglas-fir beetle. In such studies emphasis should be placed on measuring concentrations of 3-carene with respect to attack rate over time. Bioassays of the relative toxicity of 3-carene to the Douglas-fir beetle should also be studied. In electroantennagram studies of the southern pine beetle and the western pine beetle, α-pinene and 3-carene have been found to occupy some of the same antennal receptor sites with the attractant pheromone, frontalin (Payne and Dickens, 1976; Dickens and Payne, 1977). Frontalin is also an attractant pheromone produced by the Douglas-fir beetle (Pitman and Vite, 1970). Electroantennagram studies of the Douglas-fir beetle in relation to frontalin and 3-carene should be done. These studies should test whether the repellent effect of 3-carene is magnified by competitive exclusion with the pheromone attractant, frontalin, in antennal receptor sites. Additional analysis of individual outer bark, inner phloem, and sapwood tissues of the two hosts of the Douglas-fir beetle for qualitative and quantitative volatile monoterpene composition should be undertaken to further elucidate mechanisms of resistance.

CHAPTER 4

COMPARATIVE ANALYSIS OF VOLATILES FROM TWO HOSTS OF THE DOUGLAS-FIR BEETLE

Abstract

Foliage, bark, phloem, and sapwood tissues of 44 Douglas-fir and 16 western larch from an uneven aged stand in northern Idaho were analyzed for volatile composition by steam distillation and gas-liquid chromatography. No qualitative differences in tissue composition in relation to age, crown, or sample position were found in Douglas-fir. Few quantitative differences between Douglas-fir crown classes were found. Some quantitative differences were found in Douglas-fir foliage, bark, phloem, and sapwood monoterpenes between age classes. In 34 of the 50 compounds detected in Douglas-fir bark tissue concentrations were greater in the upper bole. Western larch foliage lacked santene and tricyclene and of the 14 quantitative differences in monoterpenes it contained only two, sabinene and 3-carene, in greater concentrations than Douglas-fir. In the species comparison of bark tissue no qualitative differences in the 22 monoterpenes detected were found, but each of the 15 quantitative differences were associated with greater concentrations in Douglas-fir. Douglas-fir phloem had greater concentrations of santene, sabinene, and β-phellandrene while western larch had greater concentrations of α-pinene, camphene, 3-carene, limonene, and terpinolene.

Six of the seven quantitative species differences in sapwood tissue monoterpenes were associated with greater concentrations of α-pinene, β-pinene, sabinene, myrcene, β-phellandrene, and bornyl acetate in Douglas-fir. Monoterpenes comprised the majority of volatiles detected in the four tissues of Douglas-fir. Higher molecular weight unidentified compounds comprised the majority of the volatile composition of western larch. Foliage of both species had the greatest concentration of volatiles followed by bark, sapwood, and phloem in Douglas-fir and sapwood, phloem, and bark in western larch. Evidence was found in support of the hypothesis that 3-carene is a repellent of the Douglas-fir bark beetle because it occurs in much larger concentrations in western larch phloem, sapwood, and foliage compared with Douglas-fir.

Introduction

The Douglas-fir bark beetle (*Dendroctonus pseudotsugae* Hopkins) is the most destructive pest of Douglas-fir forests throughout their natural range in western United States and Canada. The Douglas-fir beetle has two hosts, Douglas-fir (*Pseudotsuga menziesii* (Beissn.) Franco) and western larch (*Larix occidentalis* (Nutt.)). Freshly felled material is preferred for attack but live trees may also be attacked and killed. Live standing western larch are occasionally attacked in association with outbreaks of Douglas-fir beetles. However, successful brood production in live standing western larch has not been observed (Furniss and

Orr, 1978). Brood production in felled western larch has been documented by Ross (1967) and can occur at an intensity equal to that in Douglas-fir (Furniss, 1976).

Numerous studies of bark beetle/tree relationships have focused on the cortical and xylem oleoresin content in relation to attack success (Person, 1931; Smith 1961, 1963, 1965, 1966, 1969, 1972; Hanover and Furniss, 1966; Rudinsky, 1966; Coyne and Lott, 1976; Bordasch and Berryman, 1977; Sturgeon, 1979; Gollob, 1980). Results of these studies have suggested that the presence of various monoterpenes may be associated with natural resistance to attack.

Many investigations of the effects of volatile monoterpenes on bark beetles consider only the xylem oleoresin monoterpene composition and report results in terms of individual percent of total monoterpenes. However it is also important to differentiate between tissues and quantify those components within each tissue which may be associated with resistance.

Experiments exposing certain bark beetles and weevils to bark extracts have revealed that insects can and do respond differentially depending on the species source and extract composition (Anderson and Fisher, 1960; Gilbert and Norris, 1968). Alfaro _et al_. (1980) found that volatile monoterpenes of phloem, foliage, and bark of Sitka spruce (_Picea sitchensis_ (Bong.) Carr.) can attract or stimulate white pine weevil feeding. The monoterpenes α-pinene, β-pinene, and myrcene combined with nonvolatile bark constituents stimulate feeding while specific concentrations of piperitone, camphor, and limonene were feeding deterrents. In a study of phloem tissue of lodgepole pine in relation to mountain pine beetle attacks, Cole et al. (1981) found monoterpene concentrations of phloem tissue to be below toxic levels.

To further elucidate possible reasons for the observed immunity of live standing western larch to bark beetle attack and the beetle's preference for mature, codominant Douglas-fir, the following study was undertaken. From the previous study of xylem oleoresin monoterpenes and resin acids of Douglas-fir and western larch (Chapter 3) a hypothesis linking limited bark beetle attack success and high levels of 3-carene was proposed. The present study attempts to substantiate or refute this hypothesis or to provide evidence for alternative resistance mechanisms. Concentrations of volatiles were measured and compared in four tissues, foliage, outer bark, inner phloem, and sapwood of two age and crown classes of Douglas-fir and mature, codominant western larch trees.

Materials and Methods

Foliage, bark, phloem, and sapwood were analyzed for volatile composition using a modification (Figure 4.1) of Hefendehl's (1962) circulatory distillation apparatus as diagrammed in von Rudloff (1969). Hefendehl's technique has been successfully tested for use in steam distillation of

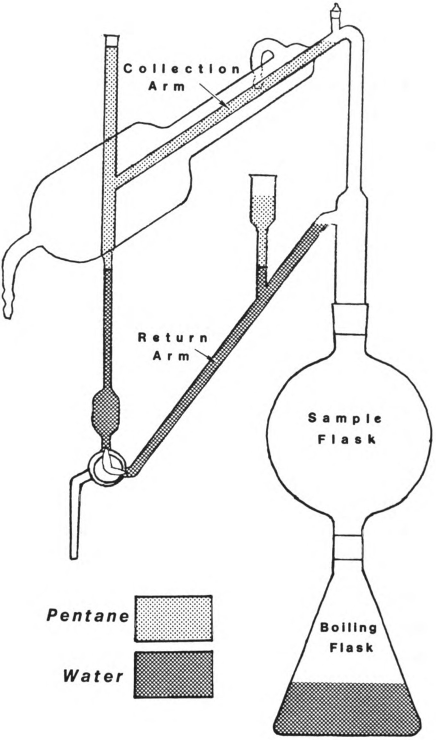

Figure 4.1. Circulatory steam distillation apparatus for distillation of foliage, bark, phloem, and sapwood tissues as modification of Hefendehl (1962).

plant materials over the range of 1 gram to 1 kilogram (von Rudloff, 1967).

Preliminary methodology

To test the reproducibility of the circulatory distillation apparatus, 10 random foliage samples of the previous years' growth were obtained from the lower crown (0.5 to 2.5 m) of a 20-year-old Douglas-fir tree growing at Michigan State University. Individual 10 g needle samples were obtained after immersing the branches in liquid nitrogen. From each foliage sample moisture percentages were calculated after oven drying preweighed samples.

Each 10 g needle sample was placed in the sample flask and distilled water and pentane were added to the return and collection side arms (Figure 4.1). Samples were distilled for six hours and then the distillate was collected from the side arm. The distillation apparatus was rinsed three times with pentane and the three rinses were then added to the original distillate. The pentane and volatile components were separated from water using a separatory funnel. The final volume of pentane/distillate and separatory funnel rinses (approximately 75 ml) was concentrated to five ml under a nitrogen stream in a constant temperature (30°C) water bath.

The volatile foliage monoterpene composition was analyzed using gas liquid chromatography and a modified method of Moore (1980). Analyses were performed on a Hewlett-Packard model 5700A gas chromatograph equipped with dual flame ionization detectors and columns 240 cm in length and six mm in diameter packed with 7.5% SE-30 on 60/80 mesh chromasorb W-AW. Injection and detector temperatures were 250°C and 300°C, respectively. Initial oven temperature was held at 70°C for eight minutes followed by a programmed increase of 8°C/min to a final temperature of 220°C. Helium at a flow rate of 80 ml/min was used as the carrier gas. An internal standard of 5.0 microliters of heptane was added to each concentrated 5.0 ml pentane/distillate sample. A Hewlett-Packard 3390A electronic integrator was used to record individual peak areas and retention times for each 3.0 microliter injection. A second gas chromatograph, F & M model 700, equipped with dual flame ionization detectors and stainless steel columns 240 cm in length and six mm in diameter packed with 10% β,β' oxidipropionitrile on 60/80 mesh chromasorb W-AW was used to separate the pairs of compounds β-pinene/sabinene and limonene/β-phellandrene, which are not separated on the packed SE-30 column. Injector and detector temperatures for the second gas chromatograph were 120°C and 150°C, respectively. Isothermal column temperature was 68°C with helium carrier gas at a flow rate of 45 ml/min.

Monoterpene distillate components were identified by comparison of retention times of pure monoterpene standards chromatographed individually under identical operating conditions and by individual peak enhancement techniques. For quantification purposes, detector response values relative to the internal standard, heptane, were calculated for each individual monoterpene. Unidentified compounds were assigned a response factor of 1.0 in relation to the response of heptane.

To determine the recovery efficiency of the circulatory distillation apparatus, a series of two determinations each of 15.0, 10.0, and 2.0 microliters of pure α-pinene standard were added to individual sample flasks, distilled for six hours, and concentrated using the procedure described above. Heptane was added as an internal standard and 3.0 microliters of the pentane/α-pinene concentrates were injected into the gas chromatograph. The mean recovery percentages for the initial 15.0, 10.0, and 2.0 microliter α-pinene samples were 42.8%, 47.4%, and 46.1%, respectively. The overall mean recovery percentage was 45.4%.

An additional test was run on the recovery efficiency associated with the nitrogen stream/water bath concentration process. In this test a series of individual 15.0, 10.0, 5.0, and 2.0 microliter samples of pure α-pinene standard were added to 75.0 ml volumes of pentane and concentrated to 5.0 ml under a nitrogen stream in a water bath (30°C). Heptane (5.0 microliters) was added to the concentrated pentane solution and 3.0 microliter samples were injected into the gas chromatograph. The mean recovery percentages for 15.0, 10.0, 5.0, and 2.0 microliter samples of α-pinene standards were 61.2%, 72.9%, 69.1%, and 78.7%, respectively. The overall mean recovery percentage for the concentration process was 73.2%. Thus just over half of the total loss (27.8%) was in the distillation process itself while just under half of the total loss (26.8%) was attributable to the nitrogen stream concentration process.

Field methods

In May, 1981, 44 Douglas-fir and 16 western larch trees in an uneven-aged second growth stand in the St. Joe National Forest were selected for comparative analysis of tissue volatile composition. The stand was located 12.8 km southeast of Elk River, Clearwater County, Idaho at an elevation of 800 m.

This study was composed of two comparative tests in relation to the volatile composition of foliage, outer bark, inner phloem, and outer sapwood tissues of Douglas-fir and western larch. In the first test 10 Douglas-fir were selected from each of the following age and crown categories: mature/codominant, young/codominant, mature/suppressed or intermediate, and young/suppressed or intermediate. The second test was a comparison between 14 mature, codominant Douglas-fir and 16 mature, codominant western larch.

For a period of two weeks beginning May 11, 1981, randomly chosen pairs of both age and crown classes of Douglas-fir and mature, codominant western larch were selected for felling and sampling on a daily basis. Upon felling, duplicate samples 12 cm wide and 15-20 cm in length

(length varied with diameter of main stem at sampling position) of outer bark, inner phloem, and outer sapwood were removed from each tree at positions of 3.0-3.5 m up the bole and 25% of the distance into the live crown in an acropetal direction. Four random samples of the previous years foliage were taken from the lower third of the live crown. All samples were placed in polyethylene bags, sealed, and transported in portable ice coolers until stored at -17°C in the laboratory. In the laboratory triplicate 1-2 gram subsamples from each of the four tissues of every tree and both sample positions were weighed to the nearest mg placed in an oven at 105°C and dried to a constant weight for purposes of calculating moisture percentage.

Small branches of Douglas-fir and western larch were immersed in liquid nitrogen to remove the needles. Inner phloem samples were cut into 3 mm cubes with a razor blade. Outer bark and sapwood samples were cut into 5 mm cubes. Distillation samples were weighed to the nearest 0.01 g and were 10, 15, 25, and 25 grams for for foliage, phloem, bark, and sapwood, respectively. Each of the tissue samples were analyzed using the circulatory steam distillation apparatus and gas-liquid chromatography technique described above.

Statistical procedures

Volatile composition of each tissue was reported as microliters of each component per g tissue dry weight. The experimental design used in analyzing foliage volatiles within Douglas-fir of two age and two crown classes was a

2x2 factorial split-plot with the main plots (age classes) arranged in a completely randomized design. The species comparison of Douglas-fir and western larch was analyzed as a completely randomized design with two treatments. Bark, phloem, and sapwood within Douglas-fir of two age, two crown, and two sample positions were analyzed as a 2x2x2 factorial split-split plot with the main plots (age classes) arranged as a completely randomized design. The experimental design of the species comparison of three tissues was a completely randomized design.

Data from all tissues were tested for normal distribution using the Kolmogurov-Smirnov test (Hull and Nie, 1981). Tissue components which did have normal distributions were analyzed by parametric analysis of variance techniques and significance was determined by the standard F-test (Steel and Torrie, 1960). The Kruskal-Wallis non-parametric one-way analysis of variance (Steel and Torrie, 1960) was used to test for significant differences in components which exhibited skewed, non-normal distributions.

Results

Results of reproducibility tests of foliage distillations are presented in Table 4.1. Overall reproducibility among the 10 samples was good. Values of coefficients of variation ranged from a low of 4.4% for bornyl acetate to a high of 17.8% for myrcene.

Table 4.1. Results of reproducibility study of the circulatory distillation apparatus using ten, 10 g foliage samples of a single Douglas-fir.

Monoterpene	Mean	Range	Standard Deviation	Coefficient of Variation (%)
	---μl per g dry weight---			
Santene	0.176	0.160-0.195	0.013	7.3
Tricyclene	0.090	0.085-1.000	0.006	6.8
α-pinene	0.587	0.545-0.654	0.042	7.2
Camphene	1.090	0.976-1.214	0.083	7.6
β-pinene	0.427	0.385-0.475	0.031	7.3
Myrcene	0.048	0.038-0.063	0.009	17.8
Limonene	0.314	0.284-0.335	0.018	5.9
Terpinolene	0.023	0.016-0.026	0.003	13.4
Camphor	0.056	0.043-0.065	0.006	9.9
Borneol	0.050	0.039-0.058	0.006	12.4
α-terpineol	0.046	0.035-0.051	0.005	11.1
Bornyl acetate	0.756	0.703-0.814	0.033	4.4
Total monoterpenes	3.664	3.366-4.000	0.228	6.2

In each tissue analysis several monoterpene hydrocarbons were identified. Several compounds which have greater retention times than bornyl acetate were found and are probably higher molecular weight nonterpenoid aromatic compounds, sequiterpene hydrocarbons, and oxygenated sesquiterpenes. For the purposes of analysis and discussion, these unknowns were labelled as "group II unknowns".

The majority of monoterpenes and unknowns found in bark, phloem, and sapwood tissues deviated from normal distributions. In bark tissue only two monoterpenes, santene and α-terpineol demonstrated normal distributions while all others deviated strongly from normality. In phloem tissue α-pinene and the total monoterpenes were the only elements with normal distributions. In the sapwood tissue all compounds exhibited skewed distributions. Of the foliage monoterpenes, santene, tricyclene, α-pinene, camphene, β-pinene, limonene, β-phellandrene, α-terpineol, citronellol, bornyl acetate, and total monoterpenes were distributed normally.

Foliage analysis

Eighteen monoterpenes and 25 unknown compounds were detected in the foliage of two age and two crown classes of Douglas-fir. The mean composition of monoterpenes and unknowns found in the two age and two crown classes expressed as microliters per g on a dry weight basis are presented in Table 4.2. Except for myrcene, camphor, and unknown 19, no quantitative differences were observed between crown classes. Significantly greater concentrations of α-pinene and unknowns Nos. 18, 24, 26, 27, 28, 29, 31, and the total of group II unknowns were found in foliage of younger age class trees. Only camphor and unknown 19 occurred in greater concentrations in older trees.

The mean composition of individual monoterpenes as a percent of total foliage monoterpenes is presented in Table B.1. Camphene was the major monoterpene component, comprising approximately 30% of the total. Approximately 70% of the foliage monoterpenes of both age classes and both crown classes consisted of camphene, α-pinene, and bornyl acetate. Each monoterpene had a narrow percentage range

Table 4.2. Composition of volatiles present in foliage tissue from two age and two crown classes within Douglas-fir.

		-Age Class-			-Crown Class-		
Compound	Test	Young	Mature	Sig.	Codominant	Suppressed/ Intermediate	Sig.
		---µl per g dry weight---					
Santene	F	0.206	0.181	ns	0.165	0.222	ns
Tricyclene	F	0.095	0.080	ns	0.079	0.096	ns
α-pinene	F	0.618	0.496	*	0.493	0.620	ns
Camphene	F	1.209	0.942	ns	0.961	1.190	ns
β-pinene	F	0.285	0.301	ns	0.260	0.326	ns
Sabinene	KW	0.008	0.016	ns	0.004	0.020	ns
Myrcene	KW	0.054	0.046	ns	0.046	0.054	*
3-Carene	KW	0.008	0.005	ns	0.004	0.010	ns
Limonene	F	0.241	0.210	ns	0.203	0.248	ns
β-phellandrene	F	0.037	0.023	ns	0.025	0.029	ns
γ-terpinene	KW	0.040	0.028	ns	0.021	0.046	ns
Terpinolene	KW	0.045	0.037	ns	0.028	0.053	ns
Camphor	KW	0.008	0.010	*	0.008	0.010	*
Borneol	KW	0.014	0.015	ns	0.014	0.014	ns
Terpinen-4-ol	KW	0.014	0.020	ns	0.010	0.024	ns
α-terpineol	F	0.027	0.029	ns	0.028	0.028	ns
Citronellol	F	0.024	0.021	ns	0.023	0.023	ns
Bornyl acetate	F	0.880	0.696	ns	0.716	0.860	ns
Unknown 18	F	0.018	0.008	**	0.010	0.016	ns
Unknown 19	KW	0.004	0.006	*	0.006	0.004	*
Unknown 20	KW	0.020	0.009	ns	0.011	0.019	ns
Unknown 21	KW	0.030	0.023	ns	0.025	0.028	ns
Unknown 22	F	0.015	0.009	ns	0.011	0.012	ns
Unknown 24	F	0.032	0.022	**	0.024	0.030	ns
Unknown 26	KW	0.039	0.014	*	0.021	0.032	ns
Unknown 27	F	0.043	0.024	*	0.035	0.031	ns
Unknown 28	F	0.039	0.018	*	0.026	0.030	ns

Table 4.2. continued.

		-Age Class-			-Crown Class-		
Compound	Test	Young	Mature	Sig.	Codominant	Suppressed/ Intermediate	Sig.
Unknown 29	KW	0.027	0.015	*	0.016	0.026	ns
Unknown 30	KW	0.012	0.015	ns	0.015	0.013	ns
Unknown 31	F	0.036	0.016	**	0.027	0.025	ns
Unknown 32	F	0.068	0.075	ns	0.069	0.074	ns
Unknown 34	F	0.053	0.044	ns	0.045	0.052	ns
Unknown 36	KW	0.007	0.002	ns	0.005	0.004	ns
Unknown 37	F	0.024	0.023	ns	0.023	0.023	ns
Unknown 39	KW	0.005	0.004	ns	0.005	0.004	ns
Unknown 40	KW	0.001	trace	ns	trace	0.001	ns
Unknown 41	KW	0.001	0.003	ns	0.003	0.001	ns
Unknown 42	KW	0.003	0.002	ns	0.004	0.002	ns
Unknown 44	KW	0.001	0.002	ns	0.001	0.002	ns
Unknown 58	F	0.010	0.013	ns	0.012	0.010	ns
Unknown 60	KW	0.002	0.002	ns	0.004	trace	ns
Unknown 61	KW	0.001	0.003	ns	0.002	0.002	ns
Unknown 65	KW	0.008	0.013	ns	0.010	0.011	ns
Total Monoterpenes	F	3.807	3.155	ns	3.087	3.874	ns
Total Group II unknowns	F	0.491	0.363	**	0.404	0.450	ns
Total	F	4.298	3.518	ns	3.491	4.324	ns

F F-test, parametric analysis of variance
KW Kruskal-Wallis, nonparametric analysis of variance
ns nonsignificant
* significant at the .05 level
** significant at the .01 level

over the two age and two crown classes.

The volatile composition of foliage of mature, codominant Douglas-fir and western larch is presented in Table 4.3. Western larch foliage lacked santene and tricyclene and unknown 19. Douglas-fir foliage lacked unknowns Nos. 45, 50, 54, 59, and 69. Thirty-two quantitative differences in individual compounds were found between the species. Concentrations of 12 of the 14 individual monoterpenes were greater in foliage of Douglas-fir. The only two monoterpenes present in greater amounts in western larch were sabinene and 3-carene. As a result, the total of foliage monoterpenes was more than three times greater in Douglas-fir. Concentrations of 13 of the 31 group II unknowns were significantly greater in western larch, and the total of group II unknowns was also significantly greater in western larch. The large amounts of monoterpenes in foliage of Douglas-fir was the basis for the total of unknowns and monoterpenes being significantly greater in Douglas-fir foliage.

As was found in the within Douglas-fir comparisons, approximately 70% of the monoterpenes in mature, codominant, Douglas-fir foliage was accounted for by camphene, α-pinene, and bornyl acetate, with camphene predominating. In foliage of western larch, α-pinene was dominant (44.8%). In contrast to Douglas-fir, approximately 70% of the total monoterpenes in western larch consisted of α-pinene, β-pinene, and 3-carene. Mean foliage monoterpene composition

Table 4.3. Composition of volatiles present in foliage tissue of mature, codominant Douglas-fir and western larch.

Compound	Test	Douglas-fir	Western Larch	Significance
		---µl per g dry weight---		
Santene	F	0.195	0.0	**
Tricyclene	KW	0.083	0.0	**
α-pinene	F	0.526	0.504	ns
Camphene	F	1.051	0.059	**
β-pinene	F	0.304	0.149	**
Sabinene	KW	0.005	0.009	*
Myrcene	F	0.054	0.070	ns
3-Carene	KW	0.003	0.159	**
Limonene	F	0.230	0.031	**
β-phellandrene	F	0.026	0.014	*
γ-terpinene	KW	0.026	0.014	*
Terpinolene	F	0.030	0.030	ns
Camphor	KW	0.010	0.001	**
Borneol	KW	0.012	0.002	**
Terpinen-4-ol	KW	0.015	0.004	ns
α-terpineol	F	0.027	0.010	*
Citronellol	KW	0.029	0.009	*
Bornyl acetate	F	0.828	0.059	**
Unknown 18	KW	0.009	0.003	*
Unknown 19	KW	0.006	0.0	*
Unknown 20	KW	0.006	0.003	ns
Unknown 21	KW	0.025	0.030	ns
Unknown 22	KW	0.010	0.109	**
Unknown 24	KW	0.019	0.050	**
Unknown 26	KW	0.015	0.276	**
Unknown 27	KW	0.026	0.081	**
Unknown 28	KW	0.019	0.041	ns
Unknown 29	KW	0.012	0.010	ns
Unknown 30	KW	0.022	0.007	ns
Unknown 31	F	0.018	0.023	ns
Unknown 32	F	0.067	0.047	ns
Unknown 34	KW	0.037	0.073	ns
Unknown 36	KW	0.002	0.023	*
Unknown 37	F	0.019	0.008	*
Unknown 39	KW	0.004	0.001	ns
Unknown 40	KW	trace	0.007	ns
Unknown 41	KW	0.005	0.018	ns
Unknown 42	KW	0.004	0.016	ns
Unknown 44	KW	0.001	0.018	*
Unknown 45	KW	0.0	0.011	*
Unknown 50	KW	0.0	0.005	ns
Unknown 54	KW	0.0	0.013	**
Unknown 55	KW	0.001	0.016	**
Unknown 58	KW	0.014	0.089	**
Unknown 59	KW	0.0	0.028	**

Table 4.3. continued

Compound	Test	Douglas-fir	Western Larch	Significance
Unknown 60	KW	0.005	0.030	**
Unknown 61	KW	0.002	0.008	**
Unknown 65	KW	0.014	0.093	**
Unknown 69	KW	0.0	0.062	*
Total monoterpenes	F	3.454	1.124	**
Total group II unknowns	KW	0.356	1.183	**
Total	F	3.810	2.305	*

F F-test, parametric analysis of variance
KW Kruskal-Wallis, nonparametric analysis of variance
ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

as percent of total foliage monoterpenes is presented in Table B.2.

Bark analysis

Twenty-two monoterpenes and 29 unknown compounds were found in bark tissue of Douglas-fir. Mean volatile composition on a microliter per g dry weight basis is presented in Table 4.4. Of the 22 quantitative differences found between young and mature age classes, 20 compounds were found in significantly greater concentrations in younger trees. Borneol and unknown 66 were the only compounds in mature trees which were present in significantly greater quantities than in the bark of younger trees. Few quantitative differences were found between crown classes. A position effect was demonstrated in 36 of the 50 individual compounds, with 34 of these 36 differences representing significantly greater amounts in the upper

Table 4.4. Composition of volatiles present in bark tissue of two age classes, two crown classes, and two sample positions within Douglas-fir.

	-Age Class-			-Crown Class-			-Position-		
Compound	Young	Mature	Sig.	Codominant	Suppressed/ Intermediate	Sig.	Upper	Lower	Sig.
			---µl per gram dry weight---						
Santene	0.002	0.002	ns	0.002	0.002	ns	0.003	0.001	**
α-pinene	0.931	0.451	**	0.674	0.708	ns	1.148	0.234	**
Camphene	0.033	0.023	*	0.026	0.031	ns	0.040	0.017	**
β-pinene	0.073	0.050	*	0.057	0.066	ns	0.107	0.016	**
Sabinene	0.024	0.006	**	0.010	0.020	ns	0.026	0.004	*
Myrcene	0.024	0.011	**	0.019	0.016	ns	0.031	0.004	**
3-Carene	0.017	0.021	ns	0.015	0.022	ns	0.028	0.010	ns
Limonene	0.107	0.078	*	0.085	0.100	ns	0.152	0.033	**
β-phellandrene	0.035	0.018	**	0.024	0.028	ns	0.044	0.009	**
γ-terpinene	0.012	0.006	**	0.006	0.012	ns	0.013	0.005	**
Terpinolene	0.023	0.013	**	0.013	0.023	**	0.022	0.014	ns
Unknown 6	0.007	0.004	ns	0.007	0.005	**	0.010	0.001	**
Unknown 7	0.005	0.005	ns	0.005	0.005	ns	0.003	0.008	**
Camphor	0.029	0.018	*	0.026	0.022	ns	0.026	0.022	ns
Borneol	0.017	0.020	*	0.015	0.023	ns	0.018	0.020	ns
Terpinen-4-ol	0.061	0.045	**	0.035	0.071	*	0.049	0.057	ns
α-terpineol	0.044	0.032	ns	0.037	0.039	ns	0.042	0.033	*
Unknown 10	0.010	0.009	ns	0.008	0.011	ns	0.011	0.008	ns
Unknown 12	0.020	0.010	ns	0.014	0.015	ns	0.006	0.014	ns
Citronellol	0.016	0.009	ns	0.007	0.017	ns	0.016	0.008	ns
Piperitone	0.007	0.008	ns	0.008	0.006	ns	0.009	0.006	ns
Bornyl acetate	0.036	0.021	**	0.030	0.026	ns	0.041	0.015	**
Unknown 15	0.003	0.001	ns	0.002	0.002	ns	0.001	0.002	ns
Unknown 18	0.013	0.011	*	0.014	0.010	ns	0.018	0.006	**
Unknown 19	0.009	0.005	ns	0.009	0.005	ns	0.012	0.003	ns
Unknown 21	0.024	0.015	**	0.019	0.021	ns	0.026	0.013	**
Unknown 24	0.001	0.001	ns	0.001	0.001	ns	0.002	0.001	**

Table 4.4. continued

Table 4.4. continued.

Compound	-Age Class- Young	Mature	Sig.	-Crown Class- Codominant	Suppressed/ Intermediate	Sig.	-Position- Upper	Lower	Sig.
Unknown 26	0.005	0.004	*	0.004	0.005	*	0.006	0.003	*
Unknown 29	0.004	0.002	*	0.003	0.004	ns	0.004	0.002	**
Unknown 30	0.005	0.002	*	0.004	0.003	ns	0.005	0.002	**
Unknown 32	0.002	0.001	ns	0.002	0.001	ns	0.003	trace	**
Unknown 34	trace	0.001	ns	0.001	0.001	ns	0.001	0.001	ns
Unknown 36	0.003	0.002	ns	0.003	0.003	ns	0.005	0.001	**
Unknown 38	0.003	0.001	ns	0.003	0.001	ns	0.003	0.001	**
Unknown 39	0.002	0.001	ns	0.002	0.001	ns	0.002	0.001	**
Unknown 42	0.018	0.008	**	0.012	0.014	ns	0.013	0.013	ns
Unknown 44	0.018	0.013	ns	0.015	0.017	ns	0.021	0.010	**
Unknown 45	0.008	0.004	ns	0.006	0.006	ns	0.009	0.003	*
Unknown 47	0.004	0.003	ns	0.003	0.004	ns	0.004	0.003	*
Unknown 48	0.071	0.041	ns	0.060	0.052	ns	0.104	0.008	**
Unknown 52	0.028	0.019	ns	0.025	0.022	ns	0.042	0.004	**
Unknown 55	0.019	0.014	ns	0.016	0.017	ns	0.028	0.005	**
Unknown 58	0.006	0.005	ns	0.005	0.006	ns	0.010	0.002	**
Unknown 60	0.007	0.007	ns	0.008	0.006	ns	0.011	0.003	**
Unknown 62	0.003	0.004	ns	0.004	0.002	ns	0.006	0.001	**
Unknown 64	0.009	0.008	ns	0.008	0.009	ns	0.011	0.007	ns
Unknown 65	0.003	0.004	ns	0.003	0.003	ns	0.002	0.004	**
Unknown 66	0.003	0.005	*	0.004	0.004	ns	0.005	0.003	ns
Unknown 68	0.002	0.003	ns	0.003	0.002	ns	0.003	0.002	ns
Unknown 72	0.002	0.006	ns	0.005	0.002	ns	0.005	0.003	ns
Total Monoterpenes	1.534	0.860	**	1.125	1.269	ns	1.858	0.537	**
Total Group II unknowns	0.276	0.189	**	0.240	0.225	ns	0.356	0.108	**
Total	1.810	1.049	**	1.365	1.494	ns	2.214	0.645	**

ns nonsignificant

*,** significant at the .05 and .01 level, respectively

sampling position.

The predominant monoterpene in all categories was α-pinene with an average of about 55%. Mean volatile monoterpene percent based upon total monoterpene composition for age, crown, and sample position are presented in Table B.3.

The quantitative composition of volatiles present in bark tissue of mature, codominant Douglas-fir and western larch is presented in Table 4.5. Twenty-four quantitative and no qualitative differences were found between the species. All 15 of the significant quantitative differences in the 22 monoterpene compounds detected were greater concentrations in Douglas-fir. As a result, the total of all monoterpenes was also significantly different and more than three fold greater in Douglas-fir than larch.

Of the group II unknowns, nine quantitative differences were found. Four of these differences were associated with larger concentrations in western larch. No significant difference in the total of group II unknowns was found. The grand total of monoterpenes and group II unknowns was significantly greater in Douglas-fir at the 0.05 level.

Alpha-pinene was the major bark monoterpene in both species followed by limonene and β-pinene in Douglas-fir and α-terpineol, β-pinene, and 3-carene in western larch. The mean volatile monoterpene composition as a percent of the total monoterpenes of bark tissue in Douglas-fir and western larch is listed in Table B.2.

Table 4.5. Composition of volatiles present in bark tissue of mature, codominant Douglas-fir and western larch.

Compound	Douglas-fir	Western Larch	Significance
	---μl per g dry weight---		
Santene	0.002	trace	**
α-pinene	0.278	0.043	**
Camphene	0.016	0.005	**
β-pinene	0.037	0.012	**
Sabinene	0.004	trace	*
Myrcene	0.007	0.001	**
3-Carene	0.018	0.012	*
Limonene	0.052	0.008	**
β-phellandrene	0.016	0.002	**
γ-terpinene	0.004	0.001	**
Terpinolene	0.010	0.004	*
Unknown 6	0.003	0.002	ns
Unknown 7	0.005	0.002	ns
Camphor	0.018	0.009	ns
Borneol	0.014	0.009	ns
Terpinen-4-ol	0.029	0.008	**
α-terpineol	0.031	0.017	**
Unknown 10	0.009	0.005	ns
Unknown 12	0.007	0.004	ns
Citronellol	0.007	0.004	ns
Piperitone	0.006	0.005	*
Bornyl acetate	0.014	0.004	*
Unknown 15	0.002	0.001	ns
Unknown 18	0.010	0.001	*
Unknown 19	0.004	0.001	*
Unknown 21	0.011	0.001	**
Unknown 24	0.001	0.001	ns
Unknown 26	0.003	0.003	ns
Unknown 29	0.002	0.001	ns
Unknown 30	0.002	0.001	ns
Unknown 32	0.001	0.001	ns
Unknown 34	0.001	0.002	ns
Unknown 36	0.001	0.001	ns
Unknown 38	0.001	0.001	ns
Unknown 39	0.001	0.001	ns
Unknown 42	0.008	0.038	ns
Unknown 44	0.011	0.010	ns
Unknown 45	0.004	0.029	**
Unknown 47	0.002	0.012	**
Unknown 48	0.035	0.006	*
Unknown 50	0.001	0.003	ns
Unknown 52	0.010	0.012	ns
Unknown 55	0.010	0.020	ns
Unknown 58	0.004	0.005	ns
Unknown 60	0.006	0.010	ns
Unknown 62	0.005	0.014	**

Table 4.5. continued

Compound	Douglas-fir	Western Larch	Significance
Unknown 64	0.006	0.020	**
Unknown 65	0.004	0.004	ns
Unknown 66	0.005	0.002	**
Unknown 68	0.004	0.003	ns
Unknown 72	0.007	0.004	ns
Total Monoterpenes	0.584	0.156	**
Total group II unknowns	0.160	0.208	ns
Total	0.744	0.363	*

ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

<u>Phloem analysis</u>

Seventeen identified and 23 unknown compounds were detected in phloem tissue of two age classes, two crown classes, and two sample positions in Douglas-fir. The quantitative amounts of these compounds in each of the three categories are presented in Table 4.6. Only one quantitative difference was found in both age and crown classes and each were associated with monoterpenes. Unknown 6 was significantly greater in mature trees and terpinolene was significantly greater in the suppressed/intermediate crown class. Ten of the 12 position differences in phloem monoterpenes were reflected as higher concentrations in the upper rather than lower sample position. Santene and unknown 12 were the two exceptions and were found in greater quantities in the lower sample position. All 12 group II unknowns were found in greater quantities in the lower position.

Table 4.6. Composition of volatiles present in phloem tissue of two age classes, two crown classes, and two sample positions within Douglas-fir.

	-Age Class-			-Crown Class-			-Position-		
Compound	Young	Mature	Sig.	Codominant	Suppressed/ Intermediate	Sig.	Upper	Lower	Sig.
	---μl per gram dry weight---								
Santene	0.002	0.003	ns	0.002	0.003	ns	0.002	0.004	**
α-pinene	0.120	0.110	ns	0.115	0.115	ns	0.132	0.098	**
Camphene	0.004	0.004	ns	0.004	0.004	ns	0.005	0.003	**
β-pinene	0.012	0.013	ns	0.013	0.012	ns	0.015	0.010	**
Sabinene	0.001	0.001	ns	0.001	0.001	ns	0.002	trace	**
Myrcene	0.003	0.006	ns	0.005	0.004	ns	0.005	0.004	ns
3-Carene	0.002	0.005	ns	0.003	0.004	ns	0.004	0.002	ns
Limonene	0.013	0.014	ns	0.012	0.015	ns	0.014	0.013	ns
β-phellandrene	0.007	0.008	ns	0.008	0.007	ns	0.008	0.007	ns
γ-terpinene	0.002	0.002	ns	0.002	0.003	ns	0.003	0.001	**
Terpinolene	0.003	0.003	ns	0.003	0.004	*	0.004	0.002	*
Unknown 6	0.002	0.003	*	0.002	0.002	ns	0.004	0.001	**
Camphor	trace	0.001	ns	0.001	trace	ns	trace	0.001	ns
Borneol	trace	0.001	ns	0.001	0.001	ns	0.001	trace	**
Terpinen-4-ol	0.002	0.003	ns	0.003	0.002	ns	0.003	0.002	**
α-terpineol	0.003	0.003	ns	0.003	0.003	ns	0.004	0.002	**
Unknown 10	0.005	0.004	ns	0.004	0.005	ns	0.004	0.006	ns
Unknown 12	0.004	0.004	ns	0.004	0.004	ns	0.001	0.006	**
Citronellol	0.002	0.001	ns	0.002	0.001	ns	0.001	0.002	ns
Bornyl acetate	0.003	0.002	ns	0.003	0.002	ns	0.002	0.003	ns
Unknown 18	0.001	0.001	ns	0.001	0.001	ns	0.001	0.001	ns
Unknown 21	0.004	0.003	ns	0.004	0.003	ns	0.002	0.005	**
Unknown 24	0.001	0.002	ns	0.002	0.001	ns	0.001	0.002	**
Unknown 26	0.005	0.004	ns	0.004	0.005	ns	0.002	0.007	**
Unknown 30	0.006	0.004	ns	0.005	0.005	ns	0.002	0.008	**
Unknown 34	0.002	0.002	ns	0.002	0.002	ns	trace	0.004	**
Unknown 36	0.004	0.003	ns	0.004	0.003	ns	0.001	0.006	**

Table 4.6. continued.

Compound	-Age Class- Young	Mature	Sig.	-Crown Class- Codominant	Suppressed/ Intermediate	Sig.	-Position- Upper	Lower	Sig.
Unknown 42	0.003	0.003	ns	0.004	0.002	ns	0.001	0.004	ns
Unknown 44	0.004	0.004	ns	0.004	0.003	ns	0.003	0.004	ns
Unknown 45	0.006	0.002	ns	0.007	0.002	ns	0.001	0.008	ns
Unknown 47	0.001	0.001	ns	0.001	0.001	ns	0.001	0.001	ns
Unknown 48	0.016	0.014	ns	0.020	0.011	ns	0.015	0.016	ns
Unknown 52	0.012	0.015	ns	0.015	0.012	ns	0.10	0.017	**
Unknown 55	0.011	0.012	ns	0.014	0.010	ns	0.010	0.014	*
Unknown 58	0.007	0.008	ns	0.008	0.007	ns	0.004	0.010	**
Unknown 59	0.002	0.003	ns	0.004	0.001	ns	0.002	0.003	ns
Unknown 60	0.004	0.005	ns	0.004	0.005	ns	0.002	0.008	**
Unknown 62	0.003	0.005	ns	0.003	0.001	ns	0.002	0.002	ns
Unknown 64	0.012	0.016	ns	0.012	0.015	ns	0.006	0.022	**
Unknown 72	0.016	0.020	ns	0.017	0.019	ns	0.006	0.030	**
Total Monoterpenes	0.192	0.191	ns	0.190	0.193	ns	0.217	0.166	**
Total Group II unknowns	0.120	0.124	ns	0.133	0.111	ns	0.071	0.173	**
Total	0.312	0.315	ns	0.323	0.304	ns	0.288	0.339	ns

ns nonsignificant
* significant at the .05 level
** significant at the .01 level

The quantities of total monoterpenes and total group II unknowns did not vary significantly across age and crown classifications. Both groups of chemicals did, however differ significantly in relation to sample position in accordance with the previous patterns found in individual monoterpenes and group II unknowns. The overall total of monoterpenes and group II unknowns did not differ significantly in relation to age, crown, or position categories.

All phloem monoterpenes exhibited a narrow range in percent composition. Alpha-pinene was the predominant monoterpene with a mean of about 60% and a range of 5.6% across age, crown, and position categories. The mean volatile monoterpene composition as percent of the total phloem monoterpenes is presented in Table B.4.

Seventeen identified monoterpenes and 31 unknown compounds were detected in the distillate of phloem tissue of mature, codominant Douglas-fir and western larch. The mean quantitative composition of the phloem compounds is presented in Table 4.7. Western larch phloem lacked the monoterpene sabinene while Douglas-fir phloem lacked group II unknowns Nos. 20, 27, 29, 32, 35, 49, 50, and 61. Mature, codominant Douglas-fir phloem had significantly greater quantities of santene, sabinene, and β-phellandrene while mature, codominant western larch had significantly greater quantities of α-pinene, camphene, 3-carene, limonene, terpinolene, and unknown 10. The total of phloem

Table 4.7. Composition of volatiles present in phloem tissue of mature, codominant Douglas-fir and western larch.

Compound	Douglas-fir	Western Larch	Significance
	---μl per g dry weight---		
Santene	0.003	0.002	*
α-pinene	0.118	0.149	**
Camphene	0.004	0.006	**
β-pinene	0.017	0.018	ns
Sabinene	0.001	0.0	**
Myrcene	0.007	0.004	ns
3-Carene	0.004	0.060	**
Limonene	0.015	0.020	**
β-phellandrene	0.010	0.004	*
γ-terpinene	0.002	0.003	ns
Terpinolene	0.003	0.006	**
Unknown 6	0.003	0.003	ns
Camphor	0.001	trace	ns
Borneol	0.001	0.001	ns
Terpinen-4-ol	0.003	0.001	ns
α-terpineol	0.004	0.002	ns
Unknown 10	0.003	0.008	*
Unknown 12	0.003	0.004	ns
Citronellol	0.003	0.004	ns
Bornyl acetate	0.002	0.001	ns
Unknown 18	0.001	trace	*
Unknown 20	0.0	trace	ns
Unknown 21	0.003	0.001	**
Unknown 24	0.002	0.002	ns
Unknown 26	0.004	0.011	**
Unknown 27	0.0	0.002	**
Unknown 29	0.0	0.002	**
Unknown 30	0.004	0.006	ns
Unknown 32	0.0	0.001	ns
Unknown 34	0.003	0.006	**
Unknown 35	0.0	0.003	**
Unknown 36	0.003	0.004	ns
Unknown 42	0.005	0.048	**
Unknown 44	0.007	0.013	**
Unknown 45	0.003	0.009	**
Unknown 47	0.001	0.004	**
Unknown 48	0.018	0.005	**
Unknown 49	0.0	0.002	ns
Unknown 50	0.0	0.008	**
Unknown 52	0.017	0.047	**
Unknown 55	0.013	0.050	**
Unknown 58	0.008	0.030	**
Unknown 59	0.004	0.007	ns
Unknown 60	0.005	0.012	**
Unknown 61	0.0	0.006	**
Unknown 62	0.003	0.010	**

Table 4.7. continued.

Compound	Douglas-fir	Western Larch	Significance
Unknown 64	0.012	0.017	ns
Unknown 72	0.019	0.022	ns
Total Monoterpenes	0.207	0.295	**
Total group II unknowns	0.135	0.328	**
Total	0.342	0.623	**

ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

monoterpenes in western larch was 50% greater than that of Douglas-fir.

Sixteen out of the 28 group II unknowns were in significantly greater concentrations in western larch. These 16 differences accounted for the total of group II unknowns also being significantly greater in western larch.

Alpha-pinene was the major monoterpene of phloem tissue in both species while β-pinene and 3-carene are ranked second in Douglas-fir and western larch, respectively. The mean volatile monoterpene composition as a percent of the total monoterpenes of phloem tissue in Douglas-fir and western larch is presented in Table B.2.

<u>Sapwood analysis</u>

Sixteen identified monoterpenes and an additional 22 unknown compounds (Table 4.8) were detected in the distillate of sapwood tissue of two age classes, two crown classes, and two sample positions within Douglas-fir. Within the age class comparison in Douglas-fir, 14

Table 4.8. Composition of volatiles present in sapwood tissue of two age classes, two crown classes, and two sample positions within Douglas-fir.

	-Age Class-			-Crown Class-			-Position-		
Compound	Young	Mature	Sig.	Codominant	Suppressed/ Intermediate	Sig.	Upper	Lower	Sig.
	---µl per gram dry weight---								
α-pinene	0.220	0.320	*	0.261	0.279	ns	0.241	0.298	ns
Camphene	0.005	0.007	ns	0.005	0.006	ns	0.004	0.008	**
β-pinene	0.026	0.035	*	0.034	0.027	ns	0.028	0.033	ns
Sabinene	0.006	0.007	ns	0.006	0.007	ns	0.010	0.002	**
Myrcene	0.005	0.010	*	0.007	0.008	ns	0.007	0.008	ns
3-Carene	0.003	0.008	ns	0.007	0.003	ns	0.006	0.004	ns
Limonene	0.013	0.024	**	0.015	0.021	ns	0.015	0.021	ns
β-phellandrene	0.013	0.017	ns	0.017	0.012	ns	0.013	0.016	ns
γ-terpinene	0.002	0.003	ns	0.002	0.003	ns	0.004	0.001	**
Terpinolene	0.005	0.005	ns	0.005	0.005	ns	0.007	0.002	**
Camphor	trace	trace	ns	trace	trace	ns	trace	trace	ns
Borneol	trace	trace	ns	trace	trace	ns	0.001	trace	**
Terpinen-4-ol	0.001	0.001	ns	0.001	0.001	ns	0.001	trace	**
α-terpineol	0.001	0.001	ns	0.001	0.001	ns	0.002	trace	**
Unknown 10	0.001	0.002	*	0.002	0.002	ns	0.002	0.001	ns
Unknown 12	0.001	0.001	ns	0.001	0.001	ns	0.002	0.001	ns
Citronellol	0.001	0.001	ns	0.001	0.001	ns	0.001	0.002	**
Bornyl acetate	0.001	0.002	ns	0.002	0.002	ns	0.001	0.002	ns
Unknown 18	0.001	0.002	*	0.002	0.001	*	0.001	0.002	ns
Unknown 19	trace	0.001	*	0.001	0.001	ns	0.001	0.001	ns
Unknown 21	0.004	0.006	ns	0.005	0.005	ns	0.004	0.006	*
Unknown 24	0.001	0.001	ns	0.001	0.001	ns	0.001	0.001	ns
Unknown 26	0.001	0.002	ns	0.002	0.002	ns	0.001	0.002	**
Unknown 30	0.001	0.001	ns	0.001	0.001	ns	trace	0.002	**
Unknown 36	0.001	0.001	ns	0.001	0.002	ns	trace	0.002	**
Unknown 38	0.001	0.001	ns	0.001	0.001	ns	trace	0.002	**
Unknown 42	0.006	0.011	ns	0.008	0.009	ns	0.007	0.010	ns

Table 4.8. continued.

Compound	-Age Class- Young	Mature	Sig.	-Crown Class- Codominant	Suppressed/ Intermediate	Sig.	-Position- Upper	Lower	Sig.
Unknown 44	0.009	0.013	**	0.010	0.012	ns	0.010	0.012	ns
Unknown 45	0.001	0.002	ns	0.002	0.001	ns	0.002	0.001	**
Unknown 47	0.002	0.002	ns	0.002	0.001	ns	0.002	0.002	ns
Unknown 48	0.021	0.026	ns	0.027	0.019	ns	0.024	0.023	ns
Unknown 52	0.027	0.049	**	0.036	0.039	ns	0.037	0.039	ns
Unknown 55	0.018	0.031	**	0.023	0.025	ns	0.025	0.024	ns
Unknown 58	0.008	0.011	**	0.009	0.010	ns	0.010	0.009	ns
Unknown 60	0.005	0.005	ns	0.005	0.006	ns	0.007	0.004	**
Unknown 62	0.004	0.004	ns	0.004	0.005	ns	0.005	0.004	ns
Unknown 64	0.003	0.002	ns	0.003	0.003	ns	0.004	0.001	**
Unknown 72	0.005	0.005	ns	0.005	0.005	ns	0.006	0.013	**
Total Monoterpenes	0.304	0.443	**	0.368	0.379	ns	0.347	0.400	ns
Total Group II unknowns	0.117	0.176	**	0.146	0.147	ns	0.145	0.148	ns
Total	0.421	0.619	**	0.514	0.526	ns	0.491	0.549	ns

ns nonsignificant
* significant at the .05 level
** significant at the .01 level

quantitative differences were found, all reflecting greater concentrations in the mature age class. Only one significant difference, unknown 18, was found between crown classes. Seventeen quantitative differences occurred with sample position. Of the identified monoterpenes, sabinene, γ-terpinene, terpinolene, borneol, terpinen-4-ol, and α-terpineol were significantly greater in the upper position while camphene and citronellol were significantly greater in the lower position. Significant differences in total monoterpenes, total group II unknowns, and the overall total were found only in association with the age class comparison, with the mature age class predominating in each individual total.

Alpha-pinene constituted 70-75% of the volatile monoterpenes in all three categories of age, crown, and sample position. The other monoterpenes accounted for a much smaller fraction of the total but their percentages were also consistent across all categories. The mean volatile monoterpene composition as a percent of total monoterpenes in distillate of sapwood tissue is presented in Table B.5.

In the species comparison of sapwood tissue 16 identified monoterpenes and 31 unknown compounds were detected. The mean composition of sapwood tissue distillate of both species is presented in Table 4.9. From the species analysis, nine qualitative differences of group II unknowns not detected in Douglas-fir were found. Alpha-pinene,

Table 4.9. Composition of volatiles present in sapwood tissue of mature, codominant Douglas-fir and western larch.

Compound	Douglas-fir	Western Larch	Significance
	---μl per g dry weight---		
α-pinene	0.357	0.174	**
Camphene	0.006	0.005	ns
β-pinene	0.051	0.026	**
Sabinene	0.008	0.001	**
Myrcene	0.010	0.004	**
3-Carene	0.009	0.062	**
Limonene	0.026	0.018	ns
β-phellandrene	0.027	0.004	**
γ-terpinene	0.004	0.002	ns
Terpinolene	0.007	0.005	ns
Camphor	trace	trace	ns
Borneol	trace	trace	ns
Terpinen-4-ol	0.001	trace	ns
α-terpineol	0.001	trace	ns
Unknown 10	0.002	0.001	ns
Unknown 12	0.002	0.001	ns
Citronellol	0.001	trace	ns
Bornyl acetate	0.002	trace	**
Unknown 18	0.002	trace	**
Unknown 19	0.001	trace	**
Unknown 21	0.005	0.001	**
Unknown 23	0.0	0.002	**
Unknown 24	0.001	0.005	**
Unknown 26	0.002	0.008	**
Unknown 27	0.0	0.002	**
Unknown 30	0.001	0.001	ns
Unknown 34	0.0	0.001	*
Unknown 35	0.0	0.003	**
Unknown 36	0.001	trace	**
Unknown 38	0.001	trace	ns
Unknown 42	0.013	0.146	**
Unknown 44	0.014	0.020	ns
Unknown 45	0.003	0.031	**
Unknown 47	0.002	0.005	**
Unknown 48	0.039	0.016	**
Unknown 49	0.0	0.001	ns
Unknown 50	0.0	0.011	**
Unknown 52	0.050	0.110	**
Unknown 53	0.0	0.007	ns
Unknown 54	0.0	0.001	ns
Unknown 55	0.031	0.120	**
Unknown 58	0.012	0.075	**
Unknown 60	0.006	0.023	**
Unknown 61	0.0	0.006	*
Unknown 62	0.004	0.017	**
Unknown 64	0.003	0.009	*
Unknown 72	0.005	0.005	ns

Table 4.9. continued.

Compound	Douglas-fir	Western Larch	Significance
Total Monoterpenes	0.515	0.305	**
Total group II unknowns	0.195	0.625	**
Total	0.710	0.930	**

ns nonsignificant
* significant at the 0.05 level
** significant at the 0.01 level

β-pinene, sabinene, myrcene, β-phellandrene, and bornyl acetate had significantly greater concentrations in Douglas-fir, while western larch sapwood had concentrations of 3-carene were nearly seven times greater than that of Douglas-fir. Of the 22 quantitative differences in group II unknowns found, 17 were associated with greater concentrations in western larch. The overall total of monoterpenes and group II unknowns was significantly greater in western larch.

The major monoterpene in sapwood tissue in both species was α-pinene, while β-pinene comprised 9.92% and 8.58% in Douglas-fir and western larch, respectively. With the additional 20.46% component of 3-carene, more than 86% of the total monoterpene fraction in western larch sapwood is accounted for by the three monoterpenes. The mean volatile monoterpene composition of sapwood tissue of both species as a percent of the total monoterpenes is listed in Table B.2.

Discussion

The lack of any current epidemic outbreaks of the Douglas-fir beetle did not allow the comparison of heavily colonized trees with nonattacked trees. The sampling procedures and designs did, however, allow the evaluation of factors which have been previously implicated in beetle selection mechanisms. Tissue sampling time of mid to late May and the climatic conditions experienced during this time were well within the seasonal conditions and period of previously documented beetle spring flight (McGowan and Rudinsky, 1958; Atkins and McMullen, 1960; McMullen and Atkins, 1962). The method of sampling enabled representative, physiological tree conditions which would normally be encountered by an attacking Douglas-fir beetle to be studied.

Previous studies of cortical monoterpenes in several fir species have revealed that the majority of monoterpenes show strong deviations from normal distributions (Zavarin and Snajberk, 1972; Zavarin et al. 1978). This author is aware of few studies which compared the data distribution of volatile components of more than one tissue system per tree. Zavarin et al. (1971) found most monoterpenes of phloem and cortical tissue of subalpine fir to be nonnormally distributed. However, some monoterpene distributions varied between tissues. The finding of this study that only α-pinene and total monoterpenes of phloem tissue did not vary significantly from normal distributions is similar to previously published work. Zavarin and Snajberk (1973) in examining cortical monoterpenes of inland Douglas-fir material also found that only α-pinene and total terpenes exhibited normal distributions. Differences found here in monoterpene distributions between bark, phloem, sapwood, and foliage tissue are suggestive of an independent biosynthetic control system.

The monoterpene concentrations of this study contrast with several previous studies on the leaf oil composition of Douglas-fir. Initial studies by Sweet (1908) showed coastal Douglas-fir foliage to contain camphene and possibly limonene. Schorger (1913), in his investigation of California Douglas-fir foliage reported α-pinene, β-pinene, and borneol predominating. Distillation of foliage of trees from northwestern Washington (Johnson and Cain, 1937) showed terpenes to comprise 75% of all components, which is in approximate agreement with the 90% found in this study. In the coastal source analyzed by Johnson and Cain, β-pinene was the major component while camphene was only a minor component. This is in direct contrast with findings here where camphene was the major component. Guenther (1952) studied the *glauca* variety of Douglas-fir and found the terpenes to consist mainly of pinenes. Of the most recent studies of Douglas-fir foliar terpenes, only those of von Rudloff (1972, 1973, and 1975a) have investigated the Rocky Mountain variety. In these three studies, von Rudloff determined the main monoterpene constituents of Rocky

Mountain variety as santene, tricyclene, α-pinene, camphene, limonene, and bornyl acetate. With the substitution of β-pinene for tricyclene, these six monoterpenes were also identified in this study as being the major components in Douglas-fir foliage. The present study also confirmed that monoterpenes of leaf oil of young and old trees of the same population are quite similar both qualitatively and quantitatively. These similar conclusions are of particular interest because von Rudloff's studies on Douglas-fir foliage were conducted during the recommended dormant season whereas this study was conducted just prior to bud break in the spring.

There were no previous studies of western larch foliage terpenes with which to compare results obtained here. Because larch has the deciduous habit there is the question of whether the optimal sampling time for such species is the same as the late fall and winter recommended for other conifers (Pauly and von Rudloff, 1971; von Rudloff, 1972, 1974, 1975a,b; Hunt and von Rudloff, 1974). These and other authors (Squillace, 1976) discuss the use and limitations of gas chromatography in the analysis of terpenes, especially in chemosystematic studies.

Monoterpene synthesis in foliage of western larch occurs shortly after leaf flush. Concentrations in new foliage of western larch comparable to those found in year old Douglas-fir foliage were found for a major monoterpene α-pinene and the minor monoterpenes myrcene and terpinolene.

In addition, even greater concentrations of sabinene and 3-carene were found in new foliage of western larch.

In Douglas-fir bark tissue analysis, the finding of the majority of significantly greater concentrations in young trees is reflected in the comparison of the sample position. Bark of younger trees and upper sampling positions is relatively thin and has numerous resin blisters as compared with the upper bark of mature trees and lower bole samples in general. Resin blisters increase the quantity of resin on a microliter per g dry weight basis.

Closer analysis of the composition of bark on a percent total monoterpene basis reveals that not only does sample position affect absolute quantities of monoterpenes but it also affects the relative composition within the monoterpene fraction. Comparison of upper sample positions with lower positions revealed α-pinene and β-pinene dropped by roughly 30% and 50%, respectively, while camphor and terpinen-4-ol increased by factors of 3 and 4, respectively. Position differences found here in Douglas-fir var. glauca are in direct contrast with findings of Zavarin and Snajberk (1965). In their previous investigation of the influence of height on monoterpene composition of bark blisters of Douglas-fir var. menziesii, Zavarin and Snajberk found the monoterpene percentages to be very consistent across sample heights. But in their study only a single tree was observed and the samples were taken over a total height differential of only nine feet.

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In contrast with bark tissue and despite several quantitative differences associated with sample position, the mean composition of individual monoterpenes as percent of total phloem and sapwood monoterpenes was constant in both species.

The greater total concentrations of monoterpenes and unknowns in sapwood in western larch may be related to the significantly larger mean area of individual resin ducts in the outer sapwood of western larch (Chapter 2).

A comparison of the monoterpene composition as a percent of total monoterpenes of sapwood tissue distillate and xylem oleoresin samples as reported in Chapter 3 showed very little difference between each method (Table B.6). In future analyses, if the purpose of investigation is to only derive the percent composition of monoterpenes on a total monoterpene basis, it would be easier and quicker to sample xylem oleoresin.

The relatively high concentration of 3-carene found in vapors produced by core sections and in xylem oleoresin in western larch (Chapter 3) provided additional evidence supporting the hypothesis that high 3-carene concentrations are associated with unsuccessful attacks of the Douglas-fir beetle. The four western larch tissue distillations showed 3-carene to be the second most abundant monoterpene behind α-pinene in all but bark tissue where it ranked in a tie for third. The ratio of individual tissue content of 3-carene in western larch to that in Douglas-fir foliage, phloem, and

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sapwood tissues is 53, 15, and 7, respectively. However, the ratio was only 0.7 in bark tissue.

The findings of this study further support the repellent hypothesis of 3-carene. The content of 3-carene in the bark tissue of western larch is apparently not above the critical repellent threshold. Thus the beetle will proceed to attack a western larch as if it were a Douglas-fir. Attack fails in western larch when the beetle encounters the phloem tissue where levels of 3-carene far surpass the critical threshold. Similar observations have been made by Elkington and Wood (1980) who showed that *Ips paraconfusus* selects host ponderosa pine (*Pinus ponderosa*) over white fir (*Abies concolor*) only after boring into the phloem.

Further studies of phloem monoterpenes should be undertaken. Specific attention should be given to the level of 3-carene over time with respect to felling, initial attack dates, and colonization success.

CHAPTER 5

A COMPARISON OF PHLOEM NUTRIENTS AND MOISTURE CONTENT OF TWO HOSTS OF THE DOUGLAS-FIR BEETLE

Abstract

Phloem tissues of Douglas-fir and western larch were analyzed for ethanol soluble reducing and nonreducing sugars, starch, crude protein, lipid and moisture content. Comparisons within age and crown classes of Douglas-fir revealed codominant trees had greater concentrations of reducing and total soluble sugars than did suppressed/intermediate class trees while younger trees had higher concentrations of crude proteins than did older trees. Lower bole phloem had greater concentrations of lipids, higher moisture content, and smaller concentrations of crude protein than samples from the upper bole. Douglas-fir trees which had previously resisted bark beetle attack had higher lipid concentrations and lower phloem moisture content than did trees which were not attacked. Mature codominant western larch trees had greater concentrations of nonreducing sugars, total soluble sugars, starch, crude protein and phloem moisture content than mature codominant Douglas-fir. High phloem moisture content was implicated in Douglas-fir beetle resistance. Independent beetle resistance mechanisms were implicated to exist within each species.

Introduction

The Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopk.) infests Douglas-fir (*Pseudotsuga menziesii* (Beissn.) Franco) throughout the species range in Western United States, British Columbia, and Mexico. The Douglas-fir beetle has been reported to occasionally attack western larch (*Larix occidentalis* Nutt.), western hemlock (*Tsuga heterophylla* (Rafn.) Sarg.), and grand fir (*Abies grandis* (Dougl.) Lind.) (Rudinsky, 1963). The beetle produces broods in felled western larch but fails to produce broods in living larch (Furniss and Orr, 1978). Infestations usually occur in Douglas-fir trees damaged by wind, snow, fire, root disease or defoliation (Kimmey and Furniss, 1943; Johnson and Belluschi, 1969; Ciesla, *et al*., 1971; Furniss, 1962, 1965).

The causes of susceptibility to attack appear complex and individual trees vary greatly in their resistance. Previous work has developed information about the factors associated with host tree susceptibility and resistance with the goal of determining risk classifications for entire stands (Furniss, Livingston, and McGregor, 1981). The underlying cause(s) or mechanism(s) of resistance to the Douglas-fir beetle are unknown. In particular, the reasons for immunity of immature, live Douglas-fir and live western larch of all ages are not known but are important for future forest protection. Likewise, knowledge about resistance of some mature Douglas-fir would find immediate application in

Douglas-fir management.

The Douglas-fir beetle devotes the short time in which the beetle is outside of the host tree to searching for a new suitable source of phloem tissue. Bark beetles rely on tactile (Shephard, 1965; Elkington and Wood, 1980), visual (Gara, Vite, and Cramer, 1965), olfactory (Person, 1931; Rudinsky, 1966,a,b,c; Jantz and Rudinsky, 1966; Chapman 1963, 1967; Bennett and Borden, 1971; Furniss and Schmitz, 1971; Werner, 1972; Heikkenen, 1977), gustatory (Baker and Norris, 1967; Hynmum and Berryman, 1980), and auditory (Rudinsky and Ryker, 1976; Ryker, 1984) stimuli in the host selection process. Host finding behavior of the Douglas-fir beetle has been described by various authors: Johnson and Pettinger (1961), McMullen and Atkins (1962), and Rudinsky (1963, 1966c).

There are many complex interactions between host trees and phytophagous insects (Hanover, 1975). Substances on which these insects feed are directly related to host susceptibility (Thorsteinson, 1960; Beck and Reese, 1976) and host resistance (Beck, 1965). Person (1931) hypothesized that carbohydrates may be an important factor in beetle nutrition and in host attraction. Thorsteinson (1960) also stated that sugars appear to be significant factors in the regulatory mechanisms of phytophagous insects. According to the hypothesis of Waring and Pitman (1980) low levels of carbohydrates are associated with low vigor and induce susceptibility to beetle attack.

The role of lipids as insect stimulatory substances has been reviewed by Seigler (1983) and investigated by others (Thomas and Hertel, 1969; Thomas, 1972; Parkerson and Whitmore, 1972). Thomas and White (1971) found both neutral and polar lipids of inner bark of loblolly pine to evoke positive feeding responses in the pales weevil, *Hylobius pales*, with neutral lipids giving a greater response. Their studies also showed that feeding responses to neutral lipids were synergized by mono- and disaccharides.

Crude protein or insoluble proteins have received little attention in studies of host susceptibility to insect attack. Hodges and Lorio (1969) found that stressed trees exhibited no significant change in the amino-nitrogen content which is readily used by the attacking southern pine beetle (*Dendroctonus frontalis* Zimmermann).

Berryman (1974) has suggested that bark beetle development is adversely effected by high phloem moisture content. Webb and Franklin (1978) found reduced survival of southern pine beetle larvae in high moisture content phloem and no significant difference in phloem moisture content between sampling heights in shortleaf and loblolly pines.

The purpose of this study was to investigate Douglas-fir beetle stimuli which may impart or be related to the observed host resistance or lack of successful brood production in Douglas-fir and western larch. Factors selected for these analyses were phloem carbohydrate, crude protein, lipid, and moisture content.

Materials and Methods

In May, 1981, 48 Douglas-fir and 16 western larch trees growing together in an uneven-aged second growth stand were selected for study. The stand was located 12.8 km southwest of Elk River, in the St. Joe National Forest, Clearwater County, Idaho, at an elevation of 800 m. Associated tree species in the stand were grand fir (*Abies grandis* (Dougl.) Lindl.), ponderosa pine (*Pinus ponderosa* Laws.), western white pine (*P. monticola* Dougl.), and western red cedar (*Thuja plicata* Donn.).

Trees for this study were selected on the basis of age, crown class and previous attack history. Each day during a two week period randomly chosen pairs of trees from each of two crown classes, codominant and suppressed/intermediate, and two age categories, young and mature, were selected for felling and sampling. Before felling, each tree was measured for diameter at breast height (DBH). After felling, total tree height and live crown length were measured. Two inner bark phloem samples were removed from each tree at a lower position of 3.0-3.5 m up the bole and an upper position of approximately 25% of the distance into the live crown in an acropetal direction. Strips of inner phloem, 12 cm wide and 15-20 cm in length (length varied with the diameter of the main stem at sampling position) were easily separated from the outer bark and xylem. Phloem sections were placed in polyethylene bags, sealed, and put into portable ice coolers. They were later weighed and kept at -17^{o}C until analysis.

Field observations of all felled trees were made three weeks after sampling. The total number of frass piles visible on the felled tree (not including underside) were recorded. From these data a measure of relative attack rate based on the number of attacks and the surface area of each tree was calculated.

Concentrations of carbohydrates, crude protein, lipid extracts, and phloem moisture content were compared for: 1. crown and age classes within Douglas-fir, 2. attack history within Douglas-fir, and 3. species differences. The first comparison involved four groups, each composed of ten Douglas-fir of the following categories: 1. mature/ codominant, 2. young/codominant, 3. mature/suppressed or intermediate, and 4. young/suppressed or intermediate. Trees in this comparison were felled and sampled as described above. The experimental design used for analysis of data for crude protein, lipid extracts, and phloem moisture content was a 2x2x2 factorial split-split plot with the main plots (age classes) arranged in a completely randomized design. The reducing sugars, nonreducing sugars, and the starch content from the lower sampling positions only were analyzed as a 2x2 factorial split-plot with the main plots (age classes) arranged in a completely randomized design.

The second comparison was made between two groups of four Douglas-fir trees each. One group of four mature, codominant trees was labeled "resistant" since they had survived attacks from a localized Douglas-fir beetle outbreak in 1971. These trees were identified by resin pockets in the outer sapwood produced by unsuccessful beetle attacks. A second group of Douglas-fir included four mature, codominant trees which showed no sign of ever being attacked. These trees were felled and sampled as described above. The four previously unsuccessfully attacked trees were sampled at the lower position only and were not felled but left standing for further study of their apparent resistance. The two groups of trees were analyzed using a completely randomized design.

The third comparison was made between 14 mature, codominant Douglas-fir (ten from the first comparison and the four previously unattacked from the second main comparison above) and 16 mature, codominant western larch. These two groups were felled and sampled as described above and analyzed as a 2x2 split-plot design with the main plots (species) arranged in a completely randomized design.

Carbohydrate concentrations were analyzed in three replicate inner phloem subsamples from each lower bole sample. The analysis provided quantification of the ethanol soluble reducing sugars, ethanol soluble nonreducing sugars and the ethanol insoluble starch content.

One g samples of each frozen replicate were divided approximately in half and the weight of each half was recorded to the nearest mg. Half of the sample was dried to a constant weight at 105°C for determination of moisture content. The other half-gram sample was macerated with a razor blade and placed in a blender with 125 ml of 80% ethanol and blended for two minutes at high speed. The solution was then allowed to stand for ten minutes before filtering through a Whatman No. 1 filter. The blender rinse, 25 ml of 80% ethanol was then filtered and added to the original filtrate. The precipitate was washed with 20 ml of anhydrous diethyl ether, filtered again, and used in the analysis of the ethanol insoluble starch which will be described later.

The ethanol extract was evaporated at room temperature under an air stream (12-14 hours) to a volume of 15 ml. Treatment with 150 mg polyvinylpyrrolidone (PVP) for 15 to 30 minutes to remove phenolic contaminants, as described by Parkerson and Whitmore (1972) resulted in no difference between PVP treated and untreated extracts so the PVP step was eliminated. The 15 ml ethanol extract was then filtered through a Millipore GS 0.22 micron filter under vacuum and then diluted to 20.0 ml with 80% ethanol.

Portions of this ethanol extract were then diluted 50/50 and analyzed for reducing sugars before and after acid hydrolysis using a modified method of Ward and Johnson (1960) and Nelson's colorimetric modification of Somogyi's method (Hodge and Hofreiter, 1962) for reducing sugars. A standard series of glucose solutions were run in tandem with each reducing sugar analysis to construct a standard curve.

Phloem sample absorbances were measured at 500 nm on a Beckman double beam spectrophotometer, converted to glucose mg equivalents, and the results expressed as amount of reducing sugar present on a percent dry weight basis. Known amounts of glucose were added to some ethanol extracts to verify absence of interference from other extract components.

Diluted ethanol extract (5.0 ml) was hydrolyzed with 54% HCl for 30 minutes in a 70°C water bath. The extracts were then cooled in an ice bath for 15 minutes, neutralized with 25% NaOH, and rediluted with distilled water to 25.0 ml. A 1.0 ml sample of this hydrolyzed extract was then analyzed by the colorimetric method with sucrose standards as described above.

Nonhydrolyzed ethanol extract analyzed with the colorimetric test represents the reducing sugar portion of phloem. Acid hydrolyzed ethanol extract represents the total ethanol soluble sugar portion of the phloem. The difference between the hydrolyzed and the nonhydrolyzed ethanol extracts is the nonreducing sugar portion of phloem and was reported as percent content on a dry weight basis.

Analysis of ethanol insoluble starch was performed on the original triplicate precipitates by a modified method of Smith, Paulsen, and Raguse, (1964). A 0.1 g sample of this precipitate was placed in a 200 mm test tube with 35.0 ml of 0.4N H_2SO_4. The test tubes, fitted with small condensers, were placed in a 100°C water bath for 1.0 hour. The hot acid and precipitate were filtered through Whatman No. 42 filter under vacuum. The filtrate was cooled in an ice bath for 15 minutes, neutralized with 25% NaOH, and diluted to 40.0 ml with distilled water. A 1.0 ml portion was then removed for the colorimetric test and determination of glucose mg equivalents from standards as described above for acid hydrolyzed and nonhydrolyzed ethanol extracts.

Total nitrogen/crude protein content of the inner phloem samples was determined by an automated total protein nitrogen block digestor and automatic analyzer method (Wall and Gehrke, 1975). Phloem samples were oven dried at 70°C for 48 hours to a constant weight. The dried samples were then ground to pass through a 40 mesh screen in a Wiley mill. Triplicate half-gram samples from both the lower bole and crown locations of each tree were weighed to the nearest 0.1 mg before analysis. The samples were digested in 4.0 ml of concentrated sulfuric acid at 385°C for 2.5 hours with a potassium sulfate/copper sulfate catalyst in calibrated 50.00 ml graduated digestion flasks. After digestion the samples were diluted with distilled, deionized water, allowed to cool 30 minutes, diluted again with water to exactly 50.00 ml and then analyzed. Standards of 100 ppm and 200 ppm nitrogen (5.8681g of $(NH4)_2SO_4$ in 250.0 ml H_2O is equivalent to 100 ppm nitrogen) were run simultaneously for detector calibration. From the standards the total percent nitrogen content was determined on a phloem dry weight basis. Percent crude protein content (dry weight basis) was calculated by multiplying percent nitrogen content by a factor of 6.25.

Lipid content of the inner phloem tissues was analyzed by the Goldfisch ether extraction method (Association of Official Agricultural Chemists, 1965). Triplicate phloem samples from both the upper and lower sampling positions were oven dried to constant weight at 70°C, ground to pass through a 40 mesh Wiley mill screen, and weighed to nearest 0.1 mg before extraction with anhydrous diethyl ether. The results were calculated as lipid content expressed as percent of the phloem dry weight.

Four replicate 1.0-1.5 g samples of phloem tissue from each position were weighed to the nearest mg and then dried at 105°C to constant weight. Phloem moisture content was expressed as total moisture present in the fresh weight as a percent of the total oven dry weight.

Results

Field measurements

Field measurements of tree characteristics of age, height, DBH, number of attacks, and attack rate are presented in Table 5.1.

Carbohydrate analysis

Mean percent of the carbohydrates analyzed as reducing sugars, nonreducing sugars, and starch in the first comparison of age and crown classification within Douglas-fir inner phloem are given in Table 5.2. Analysis of

Table 5.1. Field measurements of tree characteristics and beetle attack rates of Douglas-fir and western larch.

Tree Group	n	$\bar{x}$ age	$\bar{x}$ height (m)	$\bar{x}$ DBH (cm.)	$\bar{x}$ No. Beetle attacks	$\bar{x}$ Attack rate (no./m^2)
		-Douglas-fir-				
Young Codom.	10	54	26.2	35.6	88.6	5.30
Young Supp./Int.	10	51	20.1	19.3	23.3	2.86
Mature Codom.	10	88	32.3	48.0	205.1	7.72
Mature Supp./Int.	10	83	22.9	26.9	75.9	6.45
Mature Codom.	14	91	32.9	53.1	217.0	7.43
		-Western Larch-				
Mature Codom.	16	111	34.7	41.9	13.8	0.53

Table 5.2. Sugar and starch content of phloem tissue of two age and two crown classes of Douglas-fir.

	-Age Class-			-Crown Class-		
Phloem Trait	Mature	Young	Sig.	Codom.	Supp./ Int.	Sig.
	---Mean % dry weight (±SE)---					
Reducing	9.0(0.8)	9.7(0.7)	ns	11.1(0.6)	7.6(0.7)	**
Nonreducing	7.1(0.5)	6.9(0.6)	ns	7.0(0.6)	7.0(0.5)	ns
Total Soluble Sugar[a/]	16.1(0.8)	16.6(1.1)	ns	18.1(0.8)	14.6(0.8)	**
Starch	15.4(0.7)	16.8(0.6)	ns	15.4(0.8)	16.8(0.5)	ns

[a/] Reducing sugar + nonreducing sugar
* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

variance revealed significant differences associated with crown classes in reducing and total soluble sugars. Codominant Douglas-fir trees had 45.7% and 24.3% greater amounts of reducing sugars and total soluble sugars, respectively, than did the suppressed/intermediate Douglas-firs. No significant age x crown class interactions were detected in any of the carbohydrate categories.

No significant differences in carbohydrate levels were detected in unattacked and previously attacked "resistant" Douglas-fir from the second comparison, (Table 5.3) although, a rather high F-value was obtained for starch.

The third comparison of mature, codominant Douglas-fir and mature, codominant western larch is reported in Table 5.4. Western larch had 69.4%, 24.3%, and 46.9% greater amounts of nonreducing sugars, total soluble sugar and starch components, respectively, than Douglas-fir.

Crude protein, lipid, and moisture content analysis

Young Douglas-fir contained 23.8% more crude protein in phloem than mature Douglas-fir (Table 5.5). The Douglas-fir comparison of crude protein, lipid, and moisture content, revealed significant differences associated with sample position. Phloem from the upper bole had 36.3% more crude protein than phloem from the lower bole. An opposite trend was exhibited in lipid content where 16.9% more lipid was found in the lower bole. Also, moisture content of phloem was 11.0% higher in the lower bole. The analysis of variance of the three factors of age, crown class, and

Table 5.3. Inner phloem sugar and starch content of previously attacked and unattacked Douglas-fir.

Phloem Trait	Unattacked	Previously attacked	Significance
	---Mean % dry weight (±SE)---		
Reducing sugar	9.1 (0.6)	7.9 (0.9)	ns
Nonreducing sugar	6.8 (1.1)	7.5 (0.5)	ns
Total soluble sugar[a]	15.9 (1.3)	15.4 (1.3)	ns
Starch	13.2 (0.5)	9.4 (1.3)	ns

[a] Reducing sugar + nonreducing sugar
* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Table 5.4. Inner phloem sugar and starch content of mature codominant Douglas-fir and western larch.

Phloem Trait	Douglas-fir	Western larch	Significance
	---Mean % dry weight (±SE)---		
Reducing sugar	10.5 (0.7)	10.0 (0.8)	ns
Nonreducing sugar	6.8 (0.6)	11.5 (1.0)	**
Total soluble sugar[a]	17.3 (0.8)	21.5 (1.3)	*
Starch	14.3 (0.9)	20.8 (0.5)	**

[a] Reducing sugar + nonreducing sugar
* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

Table 5.5. Inner phloem crude protein, lipid, and moisture content of two age classes, two crown classes, and two sampling positions within Douglas-fir.

Phloem Trait	-Age Class- Young	Mature	Sig.	-Crown Class- Codom.	Supp./ Int.	Sig.	-Sample Position- Upper	Lower	Sig.
				---Mean % dry weight (±SE)---					
Crude protein	2.1(0.1)	1.7(0.0)	**	1.9(0.1)	1.8(0.1)	ns	2.1(0.1)	1.6(0.1)	**
Lipid	2.4(0.1)	2.3(0.1)	ns	2.4(0.1)	2.4(0.1)	ns	2.2(0.1)	2.5(0.1)	**
Moisture	116.3(3.2)	104.6(3.8)	ns	106.1(3.6)	114.7(3.5)	ns	104.9(4.1)	115.9(2.0)	*

* significant at 0.01 level
** significant at 0.05 level
ns nonsignificant

sample position produced one significant (at the 0.01 level) age x position interaction for crude protein. Young upper bole samples had 36.3%, 50.9%, and 64.2% more crude protein than mature upper, young lower, and mature lower sampling positions, respectively.

Previously attacked "resistant" trees differed significantly from unattacked trees in lipid and moisture content (Table 5.6). The "resistant" trees had 49.9% more lipid and 37.2% less phloem moisture.

The species comparison of crude protein, lipid, and moisture content (Table 5.7) revealed that mature codominant western larch had 24.1% more crude protein and 36.1% higher moisture content averaged over sample positions than did Douglas-fir. Results of sampling position with both species combined, revealed samples taken from the lower crown area

Table 5.6. Inner phloem crude protein, lipid, and moisture content of previously attacked and unattacked Douglas-fir.

Phloem Trait	Unattacked	Attacked	Significance
	---Mean % dry weight (±SE)---		
Crude protein	1.5 (0.1)	1.4 (0.2)	ns
Lipid	1.6 (0.1)	2.4 (0.1)	**
Moisture	109.1 (4.7)	71.9 (5.1)	**

* significant at 0.05 level
** significant at 0.01 level
ns nonsignificant

Table 5.7. Inner phloem crude protein, lipid, and moisture content of mature, codominant Douglas-fir and western larch.

Phloem Trait	-Species- Douglas-fir	Western larch	Sig.	-Position- Upper	Lower	Sig.
	---Mean % dry weight (±SE)---					
Crude protein	1.7(0.1)	2.1(0.1)	**	2.0(0.1)	1.8(0.1)	**
Lipid	2.1(0.0)	2.0(0.1)	ns	2.2(0.1)	1.9(0.1)	ns
Moisture	99.6(4.8)	135.8(7.5)	**	93.4(5.0)	144.4(6.1)	**

* significant at the 0.05 level
** significant at the 0.01 level
ns nonsignificant

had 12.8% more crude protein than samples from the lower bole and lower bole samples had 51.0% higher moisture content. From the analysis of variance one significant species by sampling position interaction was found in relation to phloem moisture content. Lower bole samples of western larch contained 69.7%, 56.3%, and 85.7% more moisture than did upper bole western larch, lower bole Douglas-fir, and upper bole samples of Douglas-fir, respectively.

Simple correlations were calculated for carbohydrate contents measured within Douglas-fir and western larch. Significant positive correlations were found between total soluble sugar and reducing sugar in both Douglas-fir ($r=.80$, $p\leq.001$) and western larch ($r=.64$, $p\leq.008$). Positive correlations were also found between total soluble sugars and nonreducing sugars in both species (Douglas-fir, r=.60, p≤.001; western larch, r=.76, p≤.001).

Correlations between phloem characteristics and relative attack rate are presented in Table 5.8. The strongest relationships exist between starch concentrations and attack rate (r=-.46, p≤.01) for Douglas-fir. For western larch only reducing sugars were significantly negatively correlated with rate (r=-.58, p≤.05) of attack.

Table 5.8. Correlations of phloem traits and relative attack rates within Douglas-fir and western larch.

Phloem Trait	-Species- Douglas-fir (n=44)	Western Larch (n=16)
Reducing sugar	-.10	-.58*
Nonreducingsugar	.04	.30
Total soluble sugar[a/]	-.06	-.14
Starch	-.44**	.28
Crude protein-lower	.04	.06
Crude protein-upper	-.37**	-.04
Lipid-lower	.00	.08
Lipid-upper	-.07	.29
Phloem moisture-lower	-.11	.32
Phloem moisture-upper	-.17	.28

[a/] reducing sugar + nonreducing sugar
* significant at the 0.05 level
** significant at the 0.01 level

Discussion

At the time of this study, no epidemic outbreaks of the Douglas-fir beetle were available to observe attacks in standing live trees. The study was however, undertaken at the time of year normal beetle flight and attack occur.

Thus, the physiological condition of the trees studied were representative of potential hosts encountered by the beetle.

Concentrations of reducing sugars in the phloem were similar both between and within species with the only significant differences occurring within crown classes of Douglas-fir. Codominant trees which have larger total leaf areas may tend to have greater amounts of reducing sugars available as products from hydrolyzed starch pools. The significant difference in total soluble sugars between crown classes of Douglas-fir is directly related to the difference found in reducing sugars for two reasons. First, the levels of nonreducing sugars are almost identical in both crown classes and, second, the quantitative measure of total soluble sugars is the algebraic sum of reducing and nonreducing sugars. The species difference can be attributed to the significantly higher levels of nonreducing sugars in western larch. The algebraic relationship can also account for the significant positive correlations exhibited in both species between reducing or nonreducing sugars and the total soluble sugar content.

Levels of reducing and nonreducing sugars and starch in Douglas-fir and western larch are very similar to values found in freshly felled loblolly pines during mid winter (Barras and Hodges, 1969) and higher than those found in lodgepole pine during early June (Cole, Guymon, and Jensen, 1981). The starch concentrations found in this study are higher than those found in Douglas-fir during and after bud burst (Webb, 1980). From seasonal studies of starch levels in mature Douglas-fir (Webb, 1980), Douglas-fir seedlings (Krueger and Trappe, 1967), and in foliage and twigs of Abies balsemea (Little, 1970, 1974), highest concentrations are found just before bud burst followed directly by a decrease in amounts. Some of the variation of higher values observed here from lower values observed elsewhere could be explained by the fact that the Douglas-fir in this study had not broken bud. Despite bud burst and needle expansion having occurred in western larch, starch levels were significantly higher in western larch than in Douglas-fir. Because no samples were taken previous to bud burst, no phenological patterns of starch reduction are available for western larch.

Waring and Pitman (1980) proposed that low carbohydrate levels lead to limited stemwood production and susceptibility to beetle attack but this theory is not supported by the data here. Western larch has higher carbohydrate reserves than Douglas-fir but lower stemwood production (Chapter 2) and lower susceptibility to beetle attack. Higher carbohydrate and the low attack rates for western larch might better be explained by an alternate theory of Thorsteinson (1960) based on Beck's (1956) finding that high levels of sucrose had a negative effect on the aggregation of Pyrausta nubilalis in corn. The optimum concentrations of sucrose or combination of sugars sensed by the Douglas-fir beetle may be surpassed in western larch.

Thus, high levels of carbohydrates in western larch may account for its low rate of attack and subsequent failure of brood production.

Some Douglas-fir were observed to be attacked within 48 hours after being felled. This is not uncommon as Douglas-fir beetles have been observed in freshly fallen material within a matter of minutes after being felled (Johnson and Belluschi, 1969). No western larch either standing or felled were attacked during the two week sampling period. However, three weeks after the last sampling date, all felled western larch had been attacked. One young Douglas-fir of the suppressed/intermediate crown class which was the smallest tree in terms of height and DBH, was the only tree that did not receive at least one attack.

The effect of felling a living tree could have a large impact on its carbohydrate levels. Over the period of four weeks respiration and other degradative processes would decrease starch and sugar pools to tolerable levels thereby disposing the tree to beetle colonization.

The method used for crude protein analysis allowed quantification of total nitrogen. Several classes of nitrogen containing compounds in addition to simple and conjugated proteins, such as amides, amino acids, ammonium salts and nitrogenous glycosides are also included in this "crude" determination. As was the case for total soluble carbohydrates crude protein levels are significantly higher in western larch than in Douglas-fir. The higher concentrations of crude protein in western larch may be unrelated to vigor since quantitative vigor ratings were not significantly different between western larch and Douglas-fir (Chapter 2).

Nitrogen is a very mobile element and is translocated as organic nitrogen from phloem to growing shoots in the spring. Past studies on nitrogen content in Douglas-fir seedlings have shown that concentrations decrease in both mature and new shoots (Krueger, 1967) and in tops and roots (Krueger and Trappe, 1967) during spring growth. Other species including lodgepole pine (Cole, et al., 1981) and loblolly pine (Nelson, et al., 1970) have similar seasonal patterns of decreasing nitrogen in the inner bark. Even though bud burst had occurred in western larch and not in Douglas-fir, the results for Douglas-fir and western larch are phenologically comparable. Shoot elongation which would signal the onset of decreasing nitrogen values in the bark had not begun in western larch.

Phloem lipid levels of mature Douglas-fir and western larch were similar to those found in 33-year-old eastern white pine by Pakerson and Whitmore, 1972. (3.0% in May to 0.4% in mid July). In this study the lipid content did not vary significantly between species and the correlation analysis showed no relationship with attack rate.

Phloem moisture content is potentially the most rapidly fluctuating characteristic measured in this study. Reduced phloem moisture may also explain the observed higher brood survival rate in felled trees versus standing live trees (Furniss, 1962). The determination of average phloem moisture content from samples taken throughout the day at random times provided an evaluation of average diurnal fluctuations within and between species in relation to beetle encounter. The significantly higher moisture content observed in western larch may partially explain the observed unsuccessful brood development in live larch. The higher levels of phloem moisture in lower compared with upper samples of Douglas-fir might also be related to the observed preference for midbole attack (Furniss, 1962). This study shows a strong sampling position effect in both Douglas-fir and western larch. Further conclusions on attack patterns cannot be made from this study since distribution of attacks was not recorded.

The lack of significant consistent correlations in both species between phloem factors and attack rate suggests independent resistance mechanisms are involved in the two species. Although independent, the mechanisms may be based on a set of common factors and depend on specific quantities or synergistic combinations of factors. Further studies are warranted in relation to changes in phloem carbohydrate and moisture contents prior to felling and up to date of attack. Attention to sampling position and within-tree patterns of attack should also be given in future investigations of host tree susceptibility to the Douglas-fir beetle.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

In the preceeding chapters, results of physiological, anatomical, and biochemical comparisons both within age and crown classes of Douglas-fir and between Douglas-fir and western larch revealed patterns of Douglas-fir beetle attack associated with physiological and biochemical traits of the host. Some resistance mechanisms commonly associated with conifers are not exhibited in the Douglas-fir beetle/host interactions. The results also suggest the resistance mechanism associated with live immune western larch are independent of the resistance mechanisms associated with age and crown classes within Douglas-fir.

The lack of any appreciable oleoresin flow or pressure in all western larch would rule out association of oleoresin pressure (i.e. "pitching-out") with Douglas-fir beetle resistance in western larch. However, the same conclusion cannot be made in relation to Douglas-fir and beetle attack since varying oleoresin exudation pressures were measured in Douglas-fir. Measurement of Douglas-fir oleoresin pressure in epidemic outbreak areas in relation to attacks by the beetle should be investigated further.

The Douglas-fir beetle may favor thicker phloem as suggested by the significant positive correlation found between attack rate and phloem thickness in Douglas-fir. Phloem tissue was significantly thinner in mature, codominant western larch and may be associated with the low beetle attack rates. However, phloem thickness cannot explain the difference in immunity of standing, live western larch versus the susceptibility of felled western larch.

Another phloem attribute which could explain beetle attack preferences is phloem moisture. Western larch phloem exhibited a significantly higher moisture content than that found in Douglas-fir. Previous studies of bark beetles have shown that larval development is hindered by high phloem moisture content. Future studies should monitor phloem moisture of western larch from the initial time of felling to the first sign of successful beetle attack. The lower phloem moisture levels in Douglas-fir are apparently not a deterrent since successful attacks have been observed almost immediately following felling.

A significant negative correlation was found between Douglas-fir bark moisture content and attack rate. Significantly higher moisture levels were also found in younger Douglas-fir than in the preferred mature Douglas-fir. Bark moisture is probably not associated with western larch resistance since significantly lower moisture levels were found in mature, codominant western larch. Results also suggest that bark moisture content in Douglas-fir could effect initial attack response but not larval development. Additional observations of bark moisture and initial patterns of attack success within Douglas-fir age classes should be taken to test the hypothesized relationship.

Resin duct density did not differ between the two species and within Douglas-fir it differed only between crown classifications. Individual duct area was positively correlated with attack rate in Douglas-fir but not in western larch which possesses resin duct areas which are significantly larger than those of Douglas-fir. This would indicate that the resistance of western larch may be associated with the quantity and composition of xylem oleoresin.

The described method of volatile analysis by steam distillation gave reproducible results and was suitable for studies of volatile biochemicals associated with insect resistance and could potentially be used for chemosystematic studies. The majority of the tissue volatiles in this study showed non-normal distributions. Thus in future studies particular attention to data distribution should be given in choosing methods of data analysis.

From the analyses of xylem oleoresin and individual tissues of foliage, sapwood, phloem, and bark, a repellent hypothesis was developed involving volatile terpenes. Much greater concentrations of 3-carene were present in western larch xylem oleoresin, foliage, sapwood, and phloem relative to Douglas-fir tissues. In contrast the majority of other individual monoterpenes were present in significantly greater concentrations in Douglas-fir than in the corresponding tissues of western larch. Thus, 3-carene has the potential of being used as a criterion for indirect selection in tree improvement strategies of insect resistance in Douglas-fir.

Further investigation of biochemical changes of tissue monoterpene content over time in relation to felling date should be undertaken with special attention given to the concentration of 3-carene. Studies should also include periodic sampling of volatiles in air immediately surrounding standing and felled trees. This would be accomplished by drawing specific volumes of air through a column containing an activated packing material. Other investigations of monoterpene concentration should include localized response of phloem tissue before and after wounding and before and after inoculation with fungi which are associated with the Douglas-fir beetle. Bioassays of beetle olfactory response to phloem tissue of western larch and Douglas-fir along with 3-carene standards should also be performed. Future studies should be replicated over different locations to investigate geographic variability within each tree species. The sampling should also be carried out in areas of epidemic outbreaks of the Douglas-fir beetle and not in areas exposed to synthetic pheromones which induce beetle attack and confound natural beetle tree selection.

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APPENDICES

APPENDIX A

Table A.1. Mean xylem oleoresin monoterpene composition as percent total monoterpene of two age and two crown classes of Douglas-fir.

	-Age Class-		-Crown Class-	
Monoterpene	Young	Mature	Codominant	Suppressed/ Intermediate
	---Percent Monoterpene---			
α-pinene	77.26	74.44	75.25	76.54
Camphene	1.73	1.32	1.53	1.49
β-pinene	9.06	9.43	9.13	9.37
Sabinene	0.83	1.01	0.88	1.01
Myrcene	1.15	0.98	1.19	0.97
3-Carene	0.96	1.66	1.78	0.80
Limonene	3.40	4.83	4.34	3.85
β-phellandrene	4.39	4.23	4.28	4.34
γ-terpinene	0.06	trace	0.03	0.03
Terpinolene	0.45	0.74	0.75	0.42
Camphor	0.03	trace	0.03	trace
Borneol	trace	0.20	trace	0.21
Terpinen-4-ol	0.03	0.24	0.03	0.21
α-terpineol	trace	0.14	0.03	0.10
Unknown 10	0.10	0.07	0.13	0.03
Unknown 12	0.03	0.14	0.03	0.14
Citronellol	trace	0.07	0.03	0.03
Unknown 13	trace	0.03	trace	trace
Bornyl acetate	0.51	0.47	0.56	0.45

Table A.2. Mean xylem oleoresin monoterpene composition as percent total monoterpene of previously attacked and unattacked mature, codominant Douglas-fir.

Monoterpene	Unattacked	Attacked
	---Percent Monoterpene---	
α-pinene	73.16	72.21
Camphene	1.43	1.17
β-pinene	9.78	11.46
Sabinene	0.78	0.71
Myrcene	2.36	2.15
3-Carene	0.70	1.01
Limonene	6.02	4.73
β-phellandrene	4.80	5.71
γ-terpinene	0.10	0.15
Terpinolene	0.39	0.37
Unknown 10	0.10	0.06
Camphor	trace	trace
Borneol	trace	trace
Terpinen-4-ol	trace	trace
α-terpineol	trace	trace
Citronellol	0.03	0.03
Bornyl acetate	0.34	0.25

Table A.3. Mean xylem oleoresin monoterpene composition as percent total monoterpene of mature, codominant Douglas-fir and western larch.

Monoterpene	Douglas-fir	Western Larch
	---Percent	Monoterpene---
α-pinene	72.43	59.21
Camphene	1.24	1.47
β-pinene	10.24	5.62
Sabinene	0.93	0.0
Myrcene	1.56	0.61
3-Carene	1.79	25.25
Limonene	5.20	3.90
β-phellandrene	5.14	0.66
γ-terpinene	0.03	0.40
Terpinolene	0.83	1.52
Camphor	trace	0.05
Borneol	trace	0.10
Terpinen-4-ol	0.03	0.25
α-terpineol	0.03	0.20
Unknown 10	0.10	0.25
Unknown 12	0.03	0.40
Citronellol	0.06	0.05
Unknown 13	0.03	trace
Bornyl acetate	0.32	0.10

Table A.4. Mean xylem oleoresin resin acid composition as percent total resin acid of two age and two crown classes of Douglas-fir.

Resin Acid	-Age Class- Young	-Age Class- Mature	-Crown Class- Codominant	-Crown Class- Suppressed/ Intermediate
	---Percent Resin Acids---			
Unknown 102	0.23	0.27	0.38	0.15
Unknown 103	0.70	0.88	0.83	0.76
Unknown 104	0.50	0.61	0.52	0.56
Unknown 105	0.17	0.36	0.17	0.35
Unknown 106	2.09	1.34	1.66	1.73
Unknown 108	0.07	0.03	trace	0.09
Unknown 109	0.30	0.18	0.03	0.41
Unknown 110	0.10	0.12	0.07	0.15
Unknown 112	0.10	0.09	0.07	0.12
Unknown 114	0.56	0.55	0.42	0.65
Unknown 115	0.10	0.09	0.14	0.06
Unknown 117	0.23	0.33	0.31	0.26
Unknown 118	0.56	0.58	0.59	0.56
Unknown 119	0.0	0.06	0.03	0.03
Unknown 120	0.50	0.36	0.24	0.62
Unknown 121	0.27	0.12	0.14	0.23
Unknown 122	0.46	0.33	0.45	0.35
Unknown 123	0.83	0.64	0.69	0.70
Unknown 124	0.07	0.06	0.10	0.03
Unknown 126	0.20	0.12	0.24	0.06
Unknown 127	0.13	0.03	trace	0.15
Unknown 128	2.12	1.67	2.32	1.41
Unknown 129	0.20	0.24	0.17	0.26
Unknown 130	1.00	0.58	0.73	0.79
Sandaracopimarate	2.19	2.25	2.08	2.35
Levopimarate + Palustrate	30.23	29.73	30.59	29.50
Isopimarate	20.94	23.83	22.63	22.34
Unknown 131	1.53	1.64	1.63	1.53
Dehydroabietate + Strobate	6.20	5.90	4.50	7.40
Unknown 132	0.76	0.70	0.83	0.68
Abietate	10.95	10.55	11.04	10.51
Unknown 133	1.13	1.03	1.21	0.97
Neoabietate	13.74	14.22	14.29	13.77
Unknown 134	0.83	0.58	0.90	0.50

Table A.5. Mean xylem oleoresin resin acid composition as percent total resin acid of previously attacked and unattacked, mature, codominant Douglas-fir.

Resin Acid	Attacked	Unattacked
	---Percent Resin Acids---	
Unknown 102	0.27	0.90
Unknown 103	0.96	0.27
Unknown 104	0.50	0.39
Unknown 105	1.33	1.54
Unknown 106	1.01	1.11
Unknown 108	0.87	0.09
Unknown 109	0.50	0.45
Unknown 113	0.0	0.06
Unknown 114	0.69	0.42
Unknown 115	0.41	0.30
Unknown 117	0.78	0.60
Unknown 118	1.42	1.20
Unknown 120	0.96	0.36
Unknown 121	0.37	0.21
Unknown 122	0.55	0.36
Unknown 123	0.87	0.78
Unknown 124	0.0	0.09
Unknown 126	0.32	0.0
Unknown 127	0.27	0.24
Unknown 128	1.42	1.39
Unknown 129	1.51	0.0
Unknown 130	0.87	0.24
Sandaracopimarate	2.11	2.20
Levopimarate + Palustrate	25.45	28.25
Isopimarate	22.79	24.07
Unknown 131	2.75	1.84
Dehydroabietate + Strobate	4.49	5.21
Unknown 132	0.64	0.75
Abietate	8.97	10.33
Unknown 133	1.69	1.20
Neoabietate	13.41	14.34
Unknown 134	1.83	0.78

Table A.6. Mean xylem oleoresin resin acid composition as percent total resin acid of mature, codominant Douglas-fir and western larch.

Resin Acid	Douglas-fir	Western Larch
	---Percent Resin Acids---	
Unknown 102	0.34	0.40
Unknown 103	0.87	0.62
Unknown 104	0.48	0.34
Unknown 105	0.48	1.76
Unknown 106	1.43	0.09
Unknown 108	0.17	0.49
Unknown 109	0.17	0.34
Unknown 110	0.10	0.77
Unknown 112	0.07	0.31
Unknown 113	trace	0.53
Unknown 114	0.44	0.99
Unknown 115	0.17	1.51
Unknown 116	0.0	0.56
Unknown 117	0.41	3.06
Unknown 118	0.75	6.36
Unknown 119	0.07	0.74
Unknown 120	0.34	0.49
Unknown 121	0.17	0.96
Unknown 122	0.44	0.43
Unknown 123	0.75	0.40
Unknown 124	0.03	0.0
Unknown 126	0.20	0.25
Unknown 127	0.07	0.46
Unknown 128	1.91	14.58
Unknown 129	0.58	0.0
Unknown 130	0.68	0.83
Pimarate + Communate	0.0	2.01
Sandaracopimarate	2.11	1.64
Levopimarate + Palustrate	29.33	13.59
Isopimarate	23.70	21.07
Unknown 131	1.88	1.42
Dehydroabietate + Strobate	4.64	2.07
Unknown 132	0.75	0.68
Abietate	10.30	8.84
Unknown 133	1.19	1.67
Neoabietate	13.98	8.46
Unknown 134	0.95	1.27

Table A.7. Mean percent volatile monoterpene composition as percent total volatile monoterpenes produced by core sections of two age and two crown classes of Douglas-fir.

Monoterpene	-Age Class- Young	Mature	-Crown Class- Codominant	Suppressed/ Intermediate
	--- Percent Volatile Monoterpenes---			
Unknown 2	1.34	0.80	0.89	1.22
Unknown 4	1.21	0.61	0.56	1.27
α-pinene	83.15	84.04	84.19	83.02
Unknown 5	0.22	0.0	0.16	0.0
Camphene	5.19	3.08	2.50	6.44
β-pinene	4.80	5.31	5.62	3.80
3-Carene	0.36	0.64	0.46	0.59
Sabinene	0.02	0.04	0.03	0.04
Myrcene	0.92	1.88	1.97	0.80
Limonene	1.92	2.34	2.07	2.29
β-phellandrene	0.83	1.23	1.48	0.50
γ-terpinene	0.01	0.02	0.02	0.01
Terpinolene	0.02	0.06	0.05	0.03

Table A.8. Mean percent volatile monoterpenes as percent total volatile monoterpenes produced by core sections of mature, codominant Douglas-fir and western larch.

Monoterpene	Douglas-fir	Western Larch
	---Percent Volatile Monoterpenes---	
Unknown 2	0.58	1.61
Unknown 4	0.17	10.15
α-pinene	83.48	63.71
Camphene	2.62	4.09
β-pinene	6.35	3.28
3-Carene	0.37	14.32
Sabinene	0.07	0.0
Myrcene	2.23	0.47
Limonene	2.43	2.29
β-phellandrene	1.63	0.0
γ-terpinene	0.03	0.0
Terpinolene	0.05	0.02

APPENDIX B

Table B.1. Mean volatile monoterpene composition as percent total monoterpenes of foliage tissue from two age and two crown classes of Douglas-fir.

Monoterpene	-Age Class- Young	Mature	-Crown Class- Codominant	Suppressed/ Intermediate
	---Percent Monoterpene---			
Santene	5.40	5.74	5.34	5.73
Tricyclene	2.49	2.53	2.56	2.48
α-pinene	16.21	15.72	15.97	16.01
Camphene	31.71	29.85	31.12	30.73
β-pinene	7.47	9.53	8.42	8.42
Sabinene	0.21	1.46	0.13	0.52
Myrcene	1.42	0.51	1.49	1.39
3-Carene	0.21	0.16	0.13	0.26
Limonene	6.31	6.64	6.57	6.40
β-phellandrene	0.97	0.73	0.81	0.75
γ-terpinene	1.05	0.89	0.68	1.18
Terpinolene	1.18	1.17	0.91	1.37
Camphor	0.21	0.32	0.26	0.26
Borneol	0.37	0.48	0.45	0.36
Terpinen-4-ol	0.37	0.63	0.32	0.62
α-terpineol	0.71	0.92	0.91	0.72
Citronellol	0.63	0.67	0.74	0.59
Bornyl acetate	23.08	22.05	23.19	22.21

Table B.2. Mean volatile monoterpene composition as percent total monoterpenes of foliage, bark, phloem, and sapwood tissues of mature, codominant Douglas-fir and western larch.

	-Foliage-		-Bark-		-Phloem-		-Sapwood-	
Monoterpene	Douglas-fir	Western Larch	Douglas-fir	Western Larch	Douglas-fir	Western Larch	Douglas-fir	Western Larch
				---Percent Monoterpene---				
Santene	5.65	0.0	0.34	trace	1.45	0.68	0.0	0.0
Tricyclene	2.40	0.0	0.0	0.0	0.0	0.0	0.0	0.0
α-pinene	15.23	44.83	47.36	27.40	57.00	50.33	69.46	57.43
Camphene	30.43	5.25	2.73	3.18	1.93	2.03	1.17	1.65
β-pinene	8.80	13.25	6.30	7.64	8.21	6.08	9.92	8.58
Sabinene	0.14	0.80	0.68	trace	0.48	0.0	1.56	0.33
Myrcene	1.56	6.23	1.19	0.64	3.38	1.35	1.95	1.32
3-Carene	0.08	14.15	3.07	7.64	1.93	20.27	1.75	20.46
Limonene	6.66	2.76	8.86	5.10	7.26	6.76	5.06	5.94
β-phellandrene	0.76	1.25	2.73	1.27	4.83	1.35	5.25	1.32
γ-terpinene	0.76	1.25	0.68	0.64	0.97	1.01	0.78	0.66
Terpinolene	0.87	2.66	1.70	2.55	1.45	2.03	1.36	1.65
Unknown 6	0.0	0.0	0.51	1.27	1.45	1.01	0.0	0.0
Unknown 7	0.0	0.0	0.85	1.27	0.0	0.0	0.0	0.0
Camphor	0.29	0.09	3.07	5.73	0.48	trace	trace	trace
Borneol	0.35	0.18	2.39	5.73	0.48	0.34	trace	trace
Terpinen-4-ol	0.43	0.36	4.94	5.10	1.45	0.34	0.19	trace
α-terpineol	0.78	0.89	5.28	10.83	1.93	0.68	0.19	trace
Unknown 10	0.0	0.0	1.53	3.18	1.45	2.70	0.39	0.33
Unknown 12	0.0	0.0	1.19	2.55	1.45	1.35	0.39	0.33
Citronellol	0.84	0.80	1.19	2.55	1.45	1.35	0.19	trace
Piperitone	0.0	0.0	1.02	3.18	0.0	0.0	0.0	0.0
Bornyl acetate	23.97	5.25	2.39	2.55	0.97	0.34	0.39	trace

Table B.3. Mean volatile monoterpene composition as percent total monoterpenes of bark tissue from two age classes, two crown classes, and two sample positions within Douglas-fir.

	-Age Class-		-Crown Class-		-Position-	
Monoterpene	Young	Mature	Codominant	Suppressed/ Intermediate	Upper	Lower
			---Percent Monoterpene---			
Santene	0.13	0.23	0.18	0.16	0.16	0.19
α-pinene	60.73	52.45	60.02	55.84	62.22	43.41
Camphene	2.15	2.67	2.32	2.44	2.17	3.15
β-pinene	4.76	5.81	5.08	5.21	5.80	2.97
Sabinene	1.57	0.70	0.89	1.58	1.41	0.74
Myrcene	1.57	1.28	1.69	1.26	1.68	0.74
3-Carene	1.11	2.44	1.34	1.74	1.52	1.86
Limonene	6.98	9.07	7.57	7.89	8.24	6.12
β-phellandrene	2.28	2.09	2.14	2.21	2.38	1.67
γ-terpinene	0.78	0.70	0.53	0.95	0.70	0.93
Terpinolene	1.50	1.51	1.16	1.81	1.19	2.60
Unknown 6	0.46	0.47	0.62	0.39	0.54	0.19
Unknown 7	0.33	0.58	0.45	0.39	0.16	1.48
Camphor	1.89	2.09	2.32	1.74	1.41	4.08
Borneol	1.11	2.33	1.34	1.81	0.98	3.71
Terpinen-4-ol	3.98	5.23	3.11	5.60	2.66	10.58
α-terpineol	2.87	3.72	3.28	3.08	2.28	6.12
Unknown 10	0.65	1.05	0.71	0.87	0.60	1.48
Unknown 12	1.30	1.16	1.25	1.17	0.32	2.60
Citronellol	1.04	1.05	0.62	1.34	0.87	1.48
Piperitone	0.46	0.93	0.71	0.47	0.49	1.11
Bornyl acetate	2.35	2.44	2.67	2.05	2.22	2.78

Table B.4. Mean volatile monoterpene composition as percent total monoterpenes of phloem tissue of two age classes, two crown classes, and two sample positions within Douglas-fir.

Monoterpene	-Age Class- Young	-Age Class- Mature	-Crown Class- Codominant	-Crown Class- Suppressed/ Intermediate	-Position- Upper	-Position- Lower
			---Percent Monoterpene---			
Santene	1.05	1.57	1.05	1.56	0.93	2.40
α-pinene	63.16	57.59	60.21	59.91	61.68	58.68
Camphene	2.11	2.09	2.09	2.08	2.34	1.80
β-pinene	6.32	6.81	6.81	6.26	7.01	5.99
Sabinene	0.53	0.52	0.52	0.52	0.93	trace
Myrcene	1.58	3.14	2.62	2.08	2.34	2.40
3-Carene	1.05	2.62	1.57	2.08	1.87	1.20
Limonene	6.84	7.34	6.28	7.82	6.54	7.78
β-phellandrene	3.68	4.20	4.20	3.65	3.74	4.19
γ-terpinene	1.05	1.05	1.05	1.56	1.40	0.60
Terpinolene	1.58	1.57	1.57	2.08	1.87	1.20
Unknown 6	1.05	1.57	1.05	1.04	1.87	0.60
Camphor	trace	0.52	0.52	trace	trace	0.60
Borneol	trace	0.52	0.52	0.52	0.47	trace
Terpinen-4-ol	1.05	1.57	1.57	1.04	1.40	1.20
α-terpineol	1.58	1.57	1.57	1.56	1.87	1.20
Unknown 10	2.63	2.09	2.09	2.60	1.87	3.58
Unknown 12	2.11	2.09	2.09	2.08	0.47	3.58
Citronellol	1.05	0.52	1.05	0.52	0.47	1.20
Bornyl acetate	1.58	1.05	1.57	1.04	0.93	1.80

Table B.5. Mean volatile monoterpene composition as percent total monoterpenes of sapwood tissue from two age classes, two crown classes, and two sample positions within Douglas-fir.

	-Age Class-		-Crown Class-		-Position-	
Monoterpene	Young	Mature	Codominant	Suppressed/ Intermediate	Upper	Lower
	---Percent Monoterpene---					
α-pinene	72.37	72.07	71.12	73.61	69.86	74.69
Camphene	1.64	1.58	1.36	1.58	1.16	2.01
β-pinene	8.55	7.88	9.27	7.12	8.11	8.27
Sabinene	1.97	1.58	1.63	1.86	2.90	0.50
Myrcene	1.64	2.24	1.91	2.11	2.03	2.01
3-Carene	0.99	1.80	1.91	0.79	1.74	1.00
Limonene	4.28	5.38	4.10	5.54	4.34	5.26
β-phellandrene	4.28	3.81	4.64	3.18	3.77	4.01
γ-terpinene	0.66	0.68	0.54	0.79	1.16	0.25
Terpinolene	1.64	1.12	1.36	1.32	2.03	0.50
Camphor	trace	trace	trace	trace	trace	trace
Borneol	trace	trace	trace	trace	0.29	trace
Terpinen-4-ol	0.33	0.23	0.27	0.26	0.29	trace
α-terpineol	0.33	0.23	0.27	0.26	0.58	trace
Unknown 10	0.33	0.47	0.54	0.53	0.58	0.25
Unknown 12	0.33	0.23	0.27	0.26	0.58	0.25
Citronellol	0.33	0.23	0.27	0.26	0.29	0.50
Bornyl acetate	0.33	0.45	0.54	0.53	0.29	0.50

Table B.6. Comparison of composition of xylem oleoresin and distillate of sapwood tissue monoterpenes as percent of total monoterpenes.

Monoterpene	Douglas-fir Xylem Oleoresin n=40	Douglas-fir Sapwood Distillate n=40	Douglas-fir Xylem Oleoresin n=14	Douglas-fir Sapwood Distillate n=14	Western Larch Xylem Oleoresin n=16	Western Larch Sapwood Distillate n=16
α-pinene	75.87	72.29	72.43	69.46	59.21	57.43
Camphene	1.52	1.54	1.24	1.17	1.47	1.65
β-pinene	9.25	8.21	10.24	9.92	5.62	8.58
Sabinene	0.93	1.76	0.93	1.56	0.0	0.33
Myrcene	1.07	1.98	1.56	1.95	0.61	1.32
3-carene	1.30	1.37	1.79	1.75	25.25	20.46
Limonene	4.11	4.83	5.20	5.06	3.90	5.94
β-phellandrene	4.31	3.98	5.14	5.25	0.66	1.32
γ-terpinene	0.04	0.67	0.03	0.78	0.40	0.66
Terpinolene	0.59	1.36	0.83	1.36	1.52	1.65
Camphor	0.02	trace	trace	trace	0.05	trace
Borneol	0.10	trace	trace	trace	0.10	trace
Terpinen-4-ol	0.13	0.27	0.03	0.19	0.25	trace
α-terpineol	0.07	0.27	0.03	0.19	0.20	trace
Unknown 10	0.08	0.47	0.10	0.39	0.25	0.33
Unknown 12	0.09	0.27	0.03	0.39	0.40	0.33
Citronellol	0.04	0.27	0.06	0.19	0.05	trace
Unknown 13	trace	0.0	0.03	0.0	trace	0.0
Bornyl acetate	0.50	0.47	0.32	0.39	0.10	trace