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Title: Western Larch Resistance to Douglas-fir Beetle Attack.

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Darrell W. Ross

Live western larch, *Larix occidentalis* Nutt., a tree species resistant to the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, produces the monoterpene 3-carene in higher concentrations compared to Douglas-fir, the preferred host of *D. pseudotsugae* (Reed et al. 1986). The inhibitory effects on attraction to aggregation pheromones and toxicity of 3-carene to *D. pseudotsugae* were investigated.

Inhibition of attraction to aggregation pheromones was demonstrated in natural Douglas-fir beetle populations using baited funnel traps. Toxicity to individual Douglas-fir beetles was confirmed in laboratory bioassays. Douglas-fir beetle aggregation pheromones were used to stimulate attack on mature, live western larch and Douglas-fir, as well as mature, felled western larch and Douglas-fir. Trap catches and bark samples revealed the relative resistance of live western larch to Douglas-fir beetle attack.

The monoterpene 3-carene may extend the beetle colonization phase, allowing tree defenses to more effectively wall off associated fungi as well as stop brood development and feeding. This monoterpene may be a factor in the resistance of live western larch to Douglas-fir beetle attack.

The impairment of olfactory perception and the prevention of aggregation pheromone production using non-host tree volatiles are potential bark beetle management tactics.

Western Larch Resistance to Douglas-fir Beetle Attack

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Tiffany A. Neal, Author

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1 INTRODUCTION

1.1 Introduction

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is an important bark beetle (Coleoptera: Scolytidae) associated with Douglas-fir, *Pseudotsugae menziesii* (Mirb.) Franco, throughout western North America (Furniss and Carolin 1977, Schmitz and Gibson 1996). Most of the time, the beetle breeds in recently dead or dying trees that have little or no defensive capacity (Rudinsky 1966a). However, when populations reach high densities, the beetle is capable of attacking and successfully breeding in healthy, live trees as well. Because it can cause substantial tree mortality during a short time period, the Douglas-fir beetle has the potential to adversely impact forest resource values (Cornelius 1955, Johnson and Belluschi 1969, Furniss et al. 1979). In addition to Douglas-fir, this beetle has been reported to occasionally attack western larch, *Larix occidentalis* Nutt. Although brood production in dead western larch is similar to that in Douglas-fir, successful brood development has not been observed in live western larch (Ross 1967, Reed et al. 1986).

The colonization of host trees by bark beetles includes four phases: dispersal, selection, concentration, and establishment (Wood 1982). Adult beetles emerge from beneath the bark of brood trees, disperse, and locate new host trees within which they mate and reproduce. The concentration phase, during which the beetles bore into the bark, is characterized by mass attack. Sonic signals and a complex pheromone communication system facilitate aggregation of adult beetles and regulate attack spacing (Raffa and Berryman 1980, Ryker 1984).

Bark beetles bore into the bark of a living or recently killed tree and lay their eggs in characteristic galleries constructed in the phloem of the tree. The phloem is excavated during both oviposition and larval development. In live trees, successful brood development is contingent upon the death of the host tissues. All or part of the tree must be killed to insure bark beetle reproductive success due to the ability of active host tree defenses to overcome invading and developing insects (Raffa and Berryman 1980, 1983a). Each beetle generation must breed in a new, living or recently killed tree because the phloem-cambium tissues act only as a temporary habitat, becoming nutritionally unsuitable soon after their death (Lanier 1983).

Host suitability is an important factor influencing beetle population dynamics. The suitability of a host tree involves all factors that affect the chemical environment of the subcortical region the beetles utilize. The chemical composition of the phloem affects the nutritional quality of the breeding substrate and the availability of host tree compounds that serve as pheromone precursors or synergists (Christiansen et al. 1987, Byers 1989). Therefore, it is necessary for the beetles to detect the appropriate host within a variable population of trees. Scolytid life tables illustrate that most mortality occurs during host location and colonization (McCowan and Rudinsky 1958, Reid 1963, Berryman 1973).

A potential element of host-tree resistance to bark beetle attack is the monoterpene composition of conifer resin (Mirov 1961, Hanover and Furniss 1966, Smith 1966a, Hodges et al. 1979). Individual monoterpenes have been shown to be toxic to both adults and immatures of some bark beetle species and to inhibit growth of their associated fungi (Smith 1963, Shrimpton and Whitney 1968, Pitman and Vité 1969, Smith 1972, Coyne and Lott 1976, Raffa and Berryman 1983a, Cook and Hain 1985, Hain and Cook 1985, Cook and Hain 1988). The attractiveness or synergistic effect on pheromones of many oleoresin compounds have been well documented (Heikkenen and Hrutfiord 1965, Rudinsky 1966b, Furniss and Schmitz 1971, Dickens

et al. 1983, 1984, Borden 1989). However, the repellent or inhibitory effects of other monoterpenes on aggregation pheromones have been less well studied and deserve further investigation (Hayes et al. 1994a, 1994b, 1996). The disruption of olfactory perception with non-host tree volatiles may prove to be a feasible alternative for managing bark beetles (Borden 1989).

Reed et al. (1986) identified possible reasons why Douglas-fir beetle broods do not survive in live western larch by comparing certain chemical and physical properties of Douglas-fir and western larch. Western larch has thinner phloem, larger diameter vertical resin ducts, and lower oleoresin exudation pressure. All concentrations of monoterpenes are higher in Douglas-fir than in western larch, except for 3-carene. This monoterpene was the only one present in higher concentrations in western larch oleoresin compared to Douglas-fir. Furthermore, Hanover and Furniss (1966) observed higher concentrations of 3-carene in unattacked Douglas-fir from two geographic regions compared to those attacked by the Douglas-fir beetle. The 3-carene content of the unattacked Douglas-fir was about 25% of that found in western larch measured by Reed et al. (1986). In this case, the higher concentration of 3-carene may, in part, be the cause for the failure of Douglas-fir beetle brood in live western larch.

1.2 Objectives

The overall objective of this study was to evaluate the potential role of 3-carene in the observed resistance of live western larch to Douglas-fir beetle attack.

1.2.1 Laboratory Toxicity of 3-carene

This study assessed the relative toxicity of 3-carene to adult Douglas-fir beetles in the laboratory.

1.2.2 Field Repellency of 3-carene

This study assessed the repellent properties of 3-carene to dispersing Douglas-fir beetles in the field.

1.2.3 Western Larch Resistance to Douglas-fir Beetle Attack

This study compared Douglas-fir beetle aggregation, egg gallery formation and brood production in live and felled western larch and Douglas-fir, and in live Douglas-fir surrounded by 3-carene releasers.

2 LITERATURE REVIEW

2.1 Aggressive Bark Beetle Life Strategies

Most bark beetles are secondary pests, attacking only physiologically weakened or dead trees (Kozlowski 1969). When populations reach high densities, however, certain species, particularly of the genus *Dendroctonus*, are capable of overcoming the defense mechanisms of ordinarily resistant trees. Many bark beetle species are able to detect and respond to periods of declining host vigor. Rapid and highly concentrated beetle attacks can kill healthy, vigorous trees (Johnson and Belluschi 1969, Cobb et al. 1968, Berryman 1972, Furniss et al. 1979, Raffa and Berryman 1980, Wood 1982, Raffa and Berryman 1983a, Berryman 1986, Christiansen et al. 1987).

2.1.1 Bark Beetle Population Density and Food Supply Relationship

Figure 2.1 illustrates the relationships that exist among environmental conditions, food supply, and beetle population density. Most of these relationships can be positive (+) or negative (-) depending upon the relative levels of the various factors. Beetle reproductive success and survival is most strongly affected by the amount of food available per beetle (e). When the input of susceptible host material (d) is constant, the beetle population density determines beetle reproductive success and survival. This is because as the constant supply of susceptible trees becomes available as suitable breeding material (e) the beetle population will increase, resulting in a larger number of beetles per unit area and subsequently the amount of suitable food available per beetle will decrease (f). This produces a (-) negative density-dependent feedback loop (+e $\leftrightarrow$ -f) by which beetles regulate their own population density when there is a constant supply of susceptible trees (McMullen and Atkins 1961, Berryman 1986).

The food supply (quantity of susceptible trees available as host material) is not typically constant. Bark beetle food supply is affected by three basic categories of environmental factors: stand, site, and disturbance (±c, ±b, ±a). First, the food supply is influenced by stand attributes such as abundance of host tree species and overall stand density. In general, beetle population density increases as stand factors such as host tree species abundance, density, and age increase (+c). However, this relationship can be more complicated for future generations. A (-) negative density-dependent feed-back loop exists (+e → -c → -d → -e → -h) where the current beetle population decreases the density of host species; thus, negatively impacts the food supply for future generations (McMullen and Atkins 1961, Berryman 1986). Moreover, site factors and disturbances that weaken or kill trees also increase the amount of food available to beetles. An increase in disturbance increases the food supply (+a). A decrease in site quality can increase the food supply as well (+b). A rapid increase in host material allows the current beetle population to reproduce at a high rate. This results in larger subsequent beetle populations and potentially greater densities of beetle attacks per tree. Consequently, large populations have the potential to attack more resistant trees.

Finally, large populations of aggressive bark beetle species exhibit epidemic behavior characterized by the ability to expand the quantity and distribution of food (+g). Dense beetle populations are able to successfully breed in more vigorous trees that would be able to survive attacks from less dense populations. This generates a positive density-dependent feed-back loop (+g → +d → +e → +g) by which a devastating beetle outbreak can occur (Berryman 1972, Raffa and Berryman 1983a, Berryman 1986, Christiansen et al. 1987).

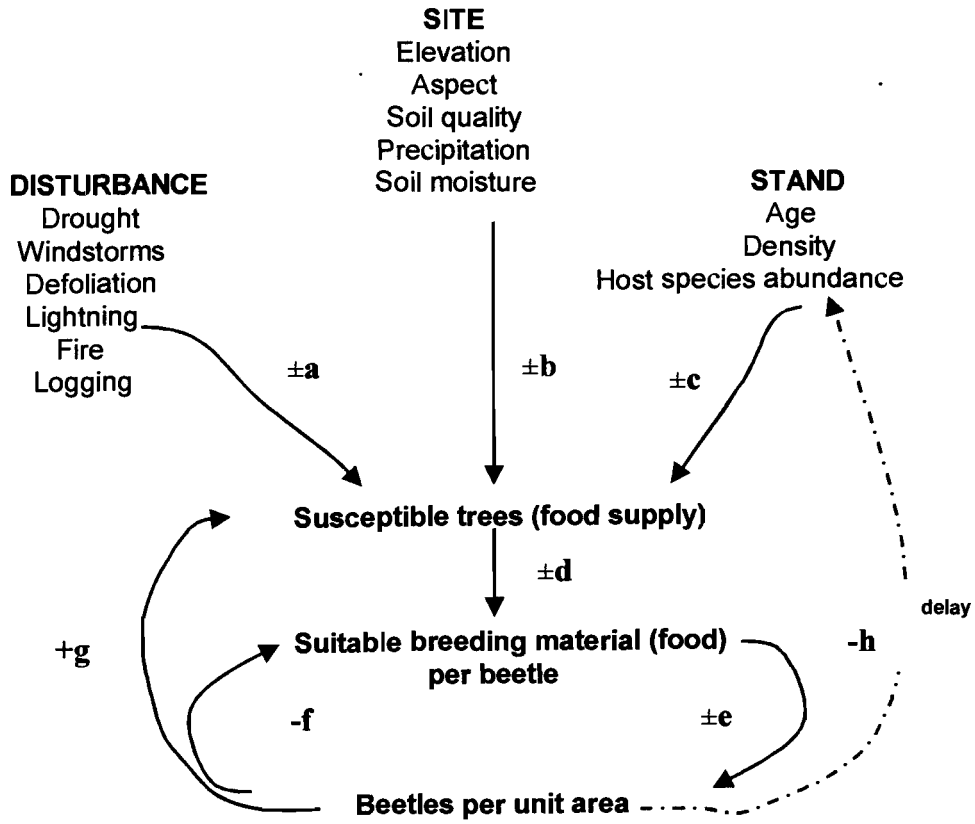


Figure 2.1 Population feedback diagram illustrating the relationships among the environment, the amount of breeding resources available, and the density of the beetle population (Berryman 1986).

2.1.2 Phases of Host Colonization

Host colonization can be partitioned into four phases: dispersal, selection, concentration, and establishment. This series of colonization behaviors has evolved in response to the widely scattered nature of breeding material and to the resistance of host trees to beetle attack. The dispersal phase begins when the beetles emerge from a brood tree. Olfactory cues from host trees and/or attractive pheromones produced

by feeding beetles stimulate the end of the dispersal phase and the beginning of the selection phase. Many scolytid bark beetles apparently require flight exercise and a subsequent decrease in fat content to become sensitive to olfactory cues (Graham 1959, McMullen and Atkins 1962, Rudinsky 1966a, Atkins 1969). This may increase the likelihood that beetles from the same brood tree will not mate.

When beetles become sensitive to olfactory cues they may encounter volatile compounds produced by trees, such as resin odors (Heikkenen and Hrutfiord 1965, Rudinsky 1966a, 1966b). Stimulation to feed on the phloem of a tree may occur before and/or after landing on the tree. Beetles feeding on the phloem of previously unattacked trees are pioneers. Pioneers initiate the concentration phase by releasing attractive pheromone. The release of attractive pheromone induces numerous beetles to attack a tree. Host tree volatile cues and attractive pheromone are released during the concentration phase. Pheromone cues are stronger than those given off by the host tree, yet are shorter lived in the atmosphere (McMullen and Atkins 1962, Rudinsky 1963, Jantz and Rudinsky 1966, Johnson and Bellushci 1969, Rudinsky 1966a, 1966b, Vité and Pitman 1982, Wood 1982).

The establishment phase begins as adult beetles mate and lay eggs beneath the bark. Because attractive pheromones are continually released, the concentration phase overlaps the establishment phase. However, following mating, beetles produce a repellent, antiaggregant pheromone. (Rudinsky 1969, 1973, Rudinsky et al. 1973, Pitman and Vité 1974). This pheromone limits the beetle density within a tree and, thus, decreases the potential for intraspecific competition among larvae as they develop beneath the host tree bark (McMullen and Atkins 1961, Hedden and Gara 1976). The concentration phase is final when an overwhelming amount of antiaggregation pheromone is released as the tree becomes fully colonized (Wood 1982, Payne 1983, Byers 1989).

Beetle brood success is contingent upon the death of the entire tree or the inhabited portion of the tree. Typically, the death of a tree requires a sufficient number of beetle attacks and associated fungal colonization spread over the bole to overcome tree defenses (Wood 1982). Whether an attack is successful or not depends upon the relationship between the number of beetle attacks spread over the tree and the capacity of the tree under attack to defend against attacking beetles (McMullen and Atkins 1961, Raffa and Berryman 1983a, Christiansen et al. 1987).

2.2 Bark Beetle Associations With Pathogenic Fungi

Bark beetles have many different microorganisms inside and on the surface of their bodies. About 100 species of microorganisms have been found to be associated with bark beetles, but only a few of the 38 genera of fungal associates are considered to be important symbionts (Whitney 1982).

The relationships among bark beetles and associated microorganisms are more than casual. The primary benefit the fungi receive from the beetle is facilitation in dispersal, inoculation, and penetration into fresh substrate. Antagonistic relationships between microorganisms and bark beetles have been observed. Observations include inhibition of bark beetle mating, inhibition of oviposition, and poor brood development most likely due to decreases in the nutritional quality and/or quantity of phloem available (Barras 1970). Mutualistic relationships with bark beetles have been observed as well. Some microorganisms enhance nutritional quality or quantity of the phloem available to beetles. Other microorganisms produce beetle pheromones, assist beetles in overcoming host tree defenses, and finally, assist beetles in killing host tree tissues.

The blue-stain fungi commonly associated with most bark beetle species produce spores and hyphae or mycelia. The spores become available as food for some beetle species. The hyphae or mycelia invade host sapwood and phloem adjacent to beetle

galleries, thus killing tree tissues beyond the immediate vicinity of the attacking beetles. Research suggests that some species of blue-stain fungi carried by bark beetles are highly pathogenic and contribute to tree death (Solheim 1988, Hortvedt and Solheim 1991, Solheim 1993). Other research suggests blue-stain fungi may not be required for bark beetle success (Barras 1970).

2.3 Tree Defense Mechanisms

Tree death due to bark beetle attack is different from tree death due to defoliation, debarking, felling, or other causes. Bark beetle-caused mortality begins when beetles enter the phloem, inoculating fungi that cause tissue death near the entrance holes. This is followed by termination of translocation, thus the crown of the tree soon becomes water stressed, vital functions become impaired or cease, and the tree rapidly dies (Whitney 1982). Bark beetle success in live trees depends on their ability to overcome host defenses (Berryman 1972, Raffa and Berryman 1983a).

2.3.1 Insect Attack Avoidance, Repellence, and Triggered Target Defense

Conceptually, conifers may defend against insect attack in three basic ways (Berryman 1972, Christiansen et al. 1987). First, avoidance of or escape from insect attack may take place through physical or chemical means. This mode of defense expends the least amount of energy. Second, insects attempting to bore into a tree may be repelled by physical or chemical means. Finally, physical and chemical means of host tree defense can occur following insect boring or feeding beneath the bark. This mode of conifer defense expends the greatest amount of energy.

Most long-lived conifers avoid insect attack by incorporating persistent, complex chemicals into tree tissues as they are produced. This mode of defense is commonly

referred to as a qualitative means of chemical defense. The production of such defensive chemical formulations requires a substantial amount of energy. However, this mode of defense does not need to be triggered by insect attack.

Many conifers also produce reserves of defensive chemicals that can be directed to tree tissues under insect attack. This is commonly referred to as a quantitative means of chemical defense. In the case of bark beetle attack, a tree must mobilize defensive chemicals directly to the site of attack (Berryman 1986). Conifers actively defend themselves from bark beetle and fungal attack by employing two forms of defense: preformed resin flowing from severed resin ducts and an induced wound response (Reid et al. 1967, Berryman 1972).

The first triggered form of conifer defense can thwart bark beetle attack by the flow of resin from ducts severed by the mandibles of boring beetles. Resin ducts exist within a system of spaces between structural cells of the phloem and xylem. The storage capacity of the duct system and the viscosity of the resin determine the character of exuding resin. This primary, quantitative defense system is characterized by the total flow of resin, the rate of resin flow, the rate of resin crystalization, viscosity, and pressure of the resin exudation. The environmental conditions and vigor of the tree can influence these attributes of resin flow (Vité and Wood 1961, Raffa and Berryman 1983a, Christiansen et al. 1987).

The second triggered form of conifer defense can surround bark beetles in toxic compounds. The induced wound response is a hypersensitive reaction in tissues neighboring a wound. It restricts the invading insect or pathogen by accumulating toxic or inhibitory compounds such as terpenes (Berryman 1972). The surrounding tissues become impregnated with resinous terpenoid and phenolic compounds that deprive invaders of nutrients and can be highly toxic to adults, eggs, larvae, and fungi. This is a graded, localized reaction that physically and chemically isolates an attack and contains fungal growth (Miller 1950, Reid et al. 1967, Berryman 1969).

2.3.2 Conifer Bark Beetle Attack Threshold

Each tree has an attack threshold above which it cannot resist individual attacks. An increasing number of beetle attacks can deplete a tree of its defensive capacity. The joint action by the bark beetle and its associated fungi can deplete host tree defensive resources resulting in successful colonization; thus, tree death. In order to successfully resist attack and contain fungal growth, an accumulation of resinous material is required.

The induced response can have notable adverse effects on the entire tree. The regions surrounding reaction zones can be depleted of carbohydrates because the synthesis of terpenes and phenols in reaction zones requires large amounts of energy. Stored carbohydrates in adjacent tissues cannot be mobilized fast enough during attacks to contribute to the induced response (Reid et al. 1967, Christiansen et al. 1987).

The capacity of trees to endure bark beetle attack and fungal growth depends on the quantity of carbohydrates available for induced defense reactions. Tree defenses require large amounts of energy, however, they are especially important when the consequences of successful insect attack are tree death. The qualitative chemical defense strategy requires more energy to be available to the tree as it develops tissues that will avoid or repel attack. In contrast, the quantitative chemical defense requires more energy to be available to the tree while it is under attack.

The production of sufficient quantities of defensive chemicals is dependent upon the energy reserves (starches and sugars) of the tree. The success of both the qualitative and quantitative defense mechanisms can be restricted by environmental conditions that create other physiological requirements for energy as well as by the size of the canopy or its photosynthetic efficiency (Christiansen et al. 1987). Trees are more

vulnerable to bark beetle attack when experiencing stress due to light, moisture, and nutrient deficiencies, disease, or old age.

2.3.3 Counter-Offensive Adaptation by Insects

The short life span, high reproductive potential and genetic variability of insects affords them the ability to evolve offensive tactics more rapidly than trees are able to evolve new defensive tactics. Insects that rely on a single plant for food are continually exposed to the static defenses of that plant. Thus, in the case of specialist herbivores, defenses such as preformed resin exudation and the hypersensitive reaction seem to be vulnerable to counter-offensive adaptation by insects. Many insects possess intricate biochemical mechanisms for altering plant defensive chemicals in order to make use of them. Insects frequently detoxify plant defensive chemicals and make use of the end products for nutrition, defense, or reproductive strategies. For example, bark beetles oxidize certain tree terpenes and use the end products as aggregation pheromones (Byers 1989).

Much of the defensive physiology of forest trees and the offensive behavior of insects is not understood. More research is needed to further elucidate the relationships between trees and herbivorous insects. A better understanding of these interactions will eventually result in a better ability to manage insect pest populations by improving defensive capacities of forest trees (Berryman 1986).

2.4 Host Selection

Knowledge of host tree selection is imperative to understanding bark beetle behavior and to developing bark beetle management tactics (Miller and Keen 1960).

Traditional pest management tactics, such as application of toxic chemicals, are not easily applied to bark beetles due to the protective subcortical environment within which they dwell and feed. Hence, successful bark beetle management tactics have focused on vulnerable aspects of bark beetle life strategies.

Selection of a suitable host is the first barrier that emerging adult beetles encounter (Dethier 1970). This requires the ability to locate and discriminate host trees. Intense selective pressures have driven bark beetles to develop complex host selection behavior during the dispersal and selection phases of attack (Raffa and Berryman 1980). Mass-attack will be subverted if flying bark beetles are unable to perceive or locate the source of aggregant pheromones and/or attractive host tree volatiles. Many investigators have studied bark beetle host tree selection (Rudinsky and Vité 1956, Atkins 1959, 1960, 1961, Vité and Gara 1961, Vité and Rudinsky 1961, Vité and Wood 1961, Moeck et al. 1962, Wood 1972, Borden 1977, Moeck et al. 1978).

2.4.1 Processes of Host Selection

There are four behavioral processes involved in the detection of a suitable host. First, host habitat must be located. This may involve visual and/or olfactory cues within a stand. Second, searching beetles detect visual, olfactory, and gustatory cues from individual potential host trees. Pioneer beetles may rely on chemical cues from trees whereas later arriving beetles may rely more on pheromones than host tree compounds. Adult beetles select a host tree to bore into during the third phase. Lastly, the beetles determine the suitability of the host. Pheromones and characteristics of the defensive capacity of the host tree, such as resin properties, are important during this phase (Payne 1983). The ratio of pheromones to host tree volatiles may be particularly important to beetles searching for a host tree (Raffa and Berryman 1983a).

2.4.2 Primary Attraction

Host seeking bark beetles encounter many trees in the forest, including non-host trees, however, only certain trees may be mass-attacked (Raffa and Berryman 1980, 1983a, 1983b). The predominant hypothesis is that host selection is mainly the result of beetle attraction to specific chemical and/or visual cues (Tunset et al. 1993). A substantial amount of research has been devoted to discovering primary attractants of tree-killing bark beetles.

Heikkenen and Hrutfiord (1965) explored primary attractant for the Douglas-fir beetle. They found adult beetles display positive and negative motor responses to select volatile chemicals of Douglas-fir and these motor responses vary with the composition, concentration, and ratios of the volatile compounds. In addition, Rudinsky (1966a) established that pioneering female Douglas-fir beetles are attracted to prospective hosts by particular terpenes (alpha-pinene, limonene, and camphene). Dickens et al. (1983) tested the antennal olfactory responses of male and female Douglas-fir beetles to host terpenes. They found that males and females both respond, but females (the pioneering sex in the case of the Douglas-fir beetle) have a heightened sensitivity to particular host odors.

Considering the number of trees, including non-host trees that host seeking bark beetles encounter, very few are mass-attacked (Wood 1976, Raffa and Berryman 1980, Moeck et al. 1981, Raffa and Berryman 1983a). The primary attraction hypothesis suggests that beetles are able to detect a suitable host in flight, but does not exclude the possibility that host tree selection is influenced more by the perceived unsuitability of a potential host. In contrast to, or in addition to the primary attraction hypothesis, host selection may be a function of a lack of repellency (Heikkenen and Hrutfiord 1965, Cobb et al. 1968). Mass attack and excessive build up of bark beetle populations may be prevented if these repellent type of phenomena can be exploited by humans.

There are chemical and physical reasons that some trees are not mass-attacked. The trees that are not mass-attacked may chemically or physically repel beetles or they may lack chemicals required to stimulate feeding or may lack precursors required to synthesize essential pheromones (Borden 1989).

Attempting to identify a single sensory mode employed for host finding is inadequate. All of the senses of a bark beetle are engaged at some point while searching for a host tree. Even though the chemosensory system appears to predominate in insect-host tree interaction, this sensory system does not act alone. Host tree signals and other ambient stimuli are integrated by the nervous system of the bark beetle before performance organs are activated; thus, inducing appropriate host selection behaviors. The many details involved in flight response and host selection behaviors makes bark beetles very discriminating. The ability of bark beetles to incorporate their many senses allows them to determine the suitability of a potential host tree and this affords bark beetles their success in affecting host trees (Payne 1983).

2.4.3 Host Tree Resistance Effects on Host Tree Selection

Potentially, some trees emit chemical cues that reveal their unsuitability or their lack of repellent cues may make them acceptable. As Furniss and Schmitz (1971) pointed out, certain monoterpenes will elicit one of three possible host selection responses: first, attract a bark beetle species and/or make their aggregation pheromones more attractive; second, elicit no response from a bark beetle species; and third, repel a bark beetle species.

The function of qualitative defense compounds is to lessen attack or brood success. However, these compounds may have little or no effect on adapted bark beetle species. Some compounds that can be highly toxic are actually used as signaling cues that assist adapted bark beetles in host selection. Some bark beetles require host

defensive compounds as precursors to pheromones required for successful attack. Consequently, some species will not attack unless certain host olfactory cues such as resin odors are present (Payne 1979).

Dendroctonus bark beetles routinely detoxify alpha-pinene and myrcene by increasing their water solubility (hydroxylation or oxidation). Only a small change is needed to convert these exogenous precursors into a pheromone. From both an energetic and evolutionary standpoint, the products resulting from the detoxification of tree defensive chemicals have always been logical pheromones (Byers 1989). Quantitatively, the production of these bark beetle pheromones may be subject to the availability of host tree monoterpene precursors. Gas-liquid chromatograph analyses have shown that quantitative differences in pheromones produced by pioneering female beetles are related to host tree condition (Vité and Pitman 1968). The ability of trees to evolve different monoterpene compositions could, in part, decrease beetle success because variation in tree defensive compounds could translate to greater resistance for trees with insufficient quantities of precursors essential to beetle chemical communication (Smith 1964, 1969, Sturgeon 1979).

Host trees with tissues high in concentrations of toxic monoterpenes may be resistant to certain scolytids. There is a large array of host tree compounds that may serve as toxins, repellents or feeding deterrents. Defense compounds have received particular attention in research with aggressive bark beetles. The preformed monoterpene concentrations found in resin and tree tissues do not appear to be directly involved in host tree survival. However, the induced concentrations of monoterpenes following beetle attack are directly involved in host resistance. While most conifers share the same preformed, qualitative monoterpenes, they differ in concentrations of induced, quantitative monoterpenes. This difference among conifers may be a factor in differing host tree resistance to beetle attack. More resistant conifers may be able to increase the concentrations of the most toxic and repellent monoterpenes (Wright et al. 1979, Raffa and Berryman 1983a).

Raffa and Berryman (1983a) established that the level of terpene exposure may influence attraction of beetles succeeding pioneers in a few ways. First, host compounds will interact with pheromones causing different beetle responses to pheromones according to different terpene exposure conditions. Second, host tree monoterpenes may compete with aggregation pheromone molecules for antennal receptor sites (Dickens and Payne 1977). For instance, Payne (1975) observed that two monoterpenes, α-pinene and 3-carene, share the same antennal receptor site with frontalin, an aggregation pheromone employed by a few *Dendroctonus* species including the Douglas-fir beetle. Once this receptor site has been saturated with either α-pinene or 3-carene molecules, the beetle may not respond to frontalin.

2.4.4 Bark Beetle Olfactory Manipulation

Disrupting the orientation of bark beetles to host trees through olfactory manipulation is a useful approach to bark beetle management. Attractive chemicals are most frequently employed as a means of managing bark beetles. According to Borden (1989), three strategies of pest management through pheromone based olfactory manipulation have led to effective operational pest management programs. Olfactory manipulation can be applied in the following three ways: baiting man-made traps with attractive pheromones, baiting trap-trees or downed trees with attractive pheromones; and protecting vulnerable trees from attack by applying antiaggregation pheromones. Pheromone based management tactics can prevent large numbers of beetles from coming into contact with host trees by either trapping-out numerous host-searching beetles or interfering with bark beetle aggregant pheromone production.

Both aggregation and antiaggregation pheromones have been used together to manage some bark beetle populations. Ross and Daterman (1994) applied both aggregant and antiaggregant pheromones to an area inhabited by a Douglas-fir beetle population.

Their objective was to prevent or reduce beetle infestation of high-risk stands using aggregation pheromones to trap adult beetles and MCH (3-methylcyclohex-2-en-1-one) to protect high-value trees from attack. Subsequent studies led to the development of an operational treatment using MCH to protect high-valued stands at high-risk of infestation (Ross and Daterman 1995, Ross et al. 2001). Hedden and Pitman (1978) applied both aggregant (frontalin and seudenol) and antiaggregant (MCH) pheromones to an area inhabited by a Douglas-fir beetle population. Their objective was to prevent tree death and beetle reproduction by dispersing the beetle population among host trees at attack densities below the threshold required to overcome host tree defenses. More recent research has demonstrated the potential for using pheromone-baited traps as one component of integrated Douglas-fir beetle management programs (Ross and Daterman 1997, Dodds et al. 2000, Dodds and Ross 2002).

2.4.5 Tree Volatile Chemicals and Bark Beetle Host Specificity

Many bark beetle pheromones have been discovered and, when used appropriately, are very effective bark beetle population management tools. However, the use of host tree chemicals to manipulate bark beetle populations has received less attention. A host produced compound, 4-allylanisole, repels the southern pine beetle, *Dendroctonus frontalis* Zimmerman, similar to verbenone, the antiaggregant pheromone produced by the southern pine beetle (Hayes et al. 1994a). However, the ratio of female to male beetles that were repelled by 4-allylanisole was large compared to verbenone. The females of this bark beetle species are the pioneering sex. Thus, the greater capacity of 4-allylanisole to repel female beetles potentially makes it a more favorable repellent than verbenone.

Pitman and Vité (1970) suggest that host volatiles preserve host specificity. Frontalin is an important component of the chemical communication system of many

Dendroctonus species that colonize different conifer genera, e.g. *D. frontalis, D. pseudotsugae, D. rufipennis, D. simplex,* and *D. brevicomis.* However, within the same stand, *D. pseudotsugae* can differentiate an infested Douglas-fir tree from a ponderosa pine infested by *D. brevicomis.* Although secondary components of the aggregation pheromones are involved in species specific attraction, host odors may also play a role. The case of *D. brevicomis* and *D. pseudotsugae* serves as an example. Landing and feeding by *D. brevicomis* is induced by 3-carene which is found in minute quantities in Douglas-fir and appears to be unattractive to *D. pseudotsugae* (Hanover and Furniss 1966, Vité and Pitman 1969, Furniss and Schmitz 1971). The eastern larch beetle, *D. simplex,* also employs frontalin as an aggregant pheromone, yet Prendergast (1991) reported that 3-carene synergized the attractiveness of seudenol to the eastern larch beetle. Moreover, camphene, absent or present in minute quantities in ponderosa pine (Mirov 1961), can similarly induce landing and feeding of *D. pseudotsugae* while remaining unattractive to *D. brevicomis.* The same tree monoterpene has contrasting effects on the host selection behavior of different species of *Dendroctonus.*

2.4.6 Unattractiveness of 3-carene

The role of 3-carene as a repellent or inhibitor of the response of the Douglas-fir beetle to the aggregation pheromone, frontalin, has been reported by Furniss and Schmitz (1971). They found the combination of frontalin and 3-carene to be least attractive to the Douglas-fir beetle compared to all other combinations of frontalin and individual monoterpenes found in Douglas-fir

Reed et al. (1986) compared the chemical and physical properties of Douglas-fir and western larch (a conifer species resistant to the Douglas-fir beetle). They found that larch had a significantly higher concentration of the monoterpene, 3-carene,

compared to Douglas-fir. The potential role that 3-carene may play in the resistance of live western larch to Douglas-fir beetle attack has not been studied.

Hanover and Furniss (1966) found, within two geographic locations, higher concentrations of 3-carene in unattacked Douglas-fir compared to Douglas-fir attacked by the Douglas-fir beetle. Possibly, toxic or inhibitory effects of 3-carene would have contributed to the resistance of these individual Douglas-fir to the Douglas-fir beetle. Similarly, Smith (1977) theorized that *P. ponderosa* with high concentrations of limonene may be resistant to *D. brevicomis*. Inhibitory or repellent compounds that come from host trees may play an important role in bark beetle host selection.

3 TOXICITY AND REPELLENCY OF THE MONOTERPENE, 3-CARENE, TO THE DOUGLAS-FIR BEETLE (*DENDROCTONUS PSEUDOTSUGAE*)

3.1 Introduction

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is found in western North America associated with its primary host, Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Schmitz and Gibson 1996). Under favorable conditions, this beetle can reach densities high enough to kill large numbers of Douglas-fir trees, adversely impacting forest resource values (Johnson and Belluschi 1969, Furniss et al. 1979). The host selection and colonization behavior of the Douglas-fir beetle is regulated by complex chemical and sonic communication systems that involve both pheromones and host tree volatiles, such as monoterpenes (Raffa and Berryman 1980, Ryker 1984). Heikkenen and Hrutfiord (1965) studied primary attractants of the Douglas-fir beetle. They found that adult beetles displayed both positive and negative responses to selected volatile chemicals of Douglas-fir, and these responses varied with the composition, concentration, and ratios of the volatile compounds.

The monoterpenes of Douglas-fir include α-pinene, camphene, β-pinene, myrcene, 3-carene, and limonene (Hanover and Furniss 1966). Alpha-pinene is the most abundant and camphene and 3-carene are found in minute amounts. Hiekkenen and Hrutfiord (1965) reported that α-pinene attracts Douglas-fir beetles. The field response of female produced pheromones (McMullen and Atkins 1962, Rudinsky 1966a) and other host volatiles (Rudinsky 1966b) have also been documented. Pitman and Vité (1970) identified frontalin from female Douglas-fir beetles and

reported the attractiveness of synthetic frontalin under field conditions. Furniss and Schmitz (1971) conducted an experiment to identify the most attractive materials for future use in monitoring and controlling Douglas-fir beetle populations. They found frontalin to be more attractive when combined with certain host tree monoterpenes. Frontalin combined with Douglas-fir resin was the most attractive followed by frontalin combined with α-pinene.

Considering the number of trees, including non-host trees that dispersing bark beetles encounter, very few are mass-attacked (Wood 1976, Raffa and Berryman 1980, Moeck et al. 1981, Raffa and Berryman 1983a). In addition to Douglas-fir, the Douglas-fir beetle has been reported to occasionally attack western larch, *Larix occidentalis* Nutt. Although brood production in dead western larch is similar to that in Douglas-fir, successful brood development has not been observed in live western larch (Ross 1967, Reed et al. 1986). A previous study that compared the chemical and physical properties of larch and Douglas-fir found that larch had a significantly higher concentration of the monoterpene, 3-carene, compared to Douglas-fir (Reed et al. 1986). The potential role that 3-carene may play in the resistance of live western larch to Douglas-fir beetle attack has not been studied.

We conducted this study to test the hypothesis that the higher concentration of 3-carene in western larch contributes to its resistance to attack by the Douglas-fir beetle. The objectives of this study were to assess the relative toxicity of 3-carene to adult Douglas-fir beetles and to determine whether 3-carene interferes with the attraction of dispersing Douglas-fir beetles to aggregation pheromones under field conditions.

3.2 Methods

3.2.1 Toxicity Bioassay

Adult Douglas-fir beetles used in laboratory bioassays were collected within a clear-cut in the Wenatchee National Forest, Washington (approximately 47° N, 120° W) on 17 July 1996 and 7 July 1997 and within a clear-cut in the Okanogan National Forest, Washington (approximately 48° N, 119° W) on 13 May 1998. At each location, six multiple-funnel traps were baited with polyvinychloride formulations of frontalin and seudenol releasing 1.5 mg/day and 0.9 mg/day, repsectively, at 24°C.

Beetles were removed from the traps within one hour of being caught. Beetles were placed in paper cartons with crumpled paper towels and held in a cooler with ice until transported to the laboratory. Beetles were held for up to five days at 3°C in the laboratory until used in a test. The beetles were sorted by sex and apparently unhealthy beetles were discarded. An insufficient number of females were trapped during July 1996 and 1997, thus only males were tested at those times. Female beetles were tested following the May 1998 collection date.

Each beetle was placed in a ½ dram vial and 16 vials were positioned upright in a 300 ml sealed glass jar (Figure 3.1). For male beetles, three terpene treatments and a water control were tested for each collection date using methods similar to those of Raffa et al. (1985) and Cook and Hain (1988). The terpenes tested for toxicity to adult Douglas-fir beetles included (±) α-pinene, myrcene, and 3-carene. In addition, limonene was tested with beetles collected on 13 May 1998. All terpenes were purchased from Aldrich Chemical Company, Milwaukee, WI. The chemical purities of α-pinene, myrcene, 3-carene, and limonene were 98%, 89%, 90%, and 97%, respectively. 3-carene contained about 5% 2-carene. Each treatment was replicated

in three jars and each jar contained 16 isolated beetles. Ten µl of a terpene or water were placed on the inside wall of each jar, yielding a concentration of about 33 ppm. The jars were immediately sealed and observed for at least 3 days. All jars were held at room temperature throughout the study. Light and dark periods varied, but the beetles experienced at least 8 hours of light each day. The numbers of live and dead beetles in each jar were recorded at 0, 3, 5, 11, 19, 24, 36, 44, 48, 60, and 72 hours after the jars were sealed.

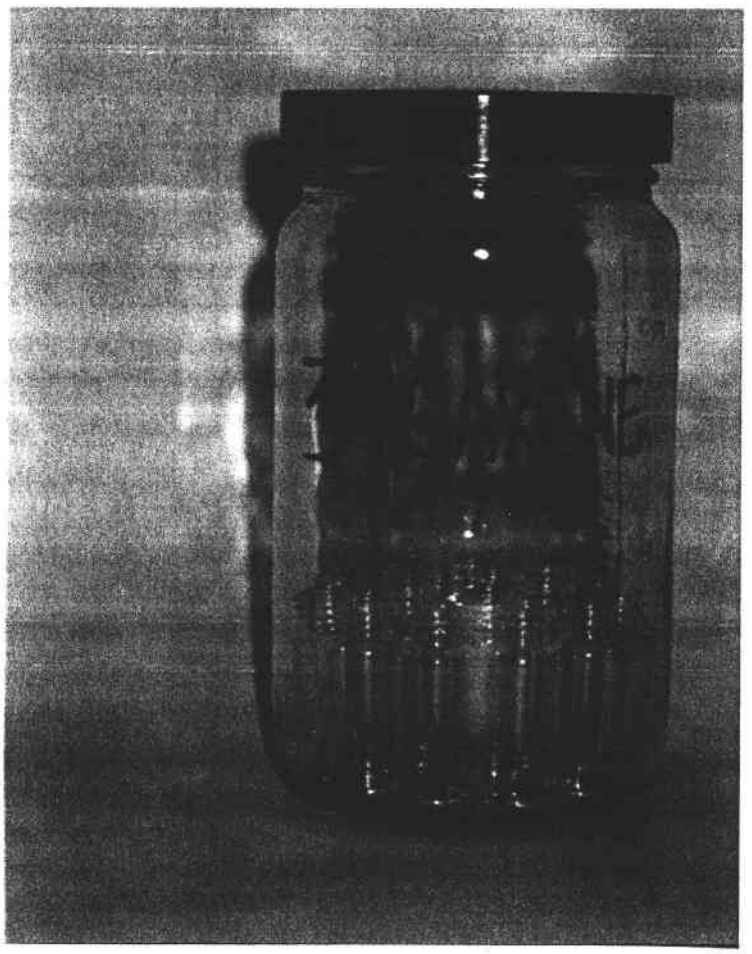


Figure 3.1 Glass jar holding vials containing beetles exposed to 3-carene.

3.2.2 Repellency Field Test

The effects of the monoterpenes 3-carene and α-pinene on attraction of beetles to frontalin were field tested 3 times, once on the Wallowa Whitman National Forest, in eastern Oregon (approximately 45° N,118° W) and twice on the Wenatchee National Forest, in central Washington (approximately 47° N, 120° W). This study used a randomized complete block design with six replications and three treatments. The treatment baits were placed in 16 unit multiple-funnel traps. Treatments were: 1) frontalin and α-pinene, 2) frontalin, α-pinene, and 3-carene, and 3) frontalin and 3-carene. Frontalin was formulated in polyvinylchloride to release at 1.5 mg/day at 24°C. The terpenes were formulated as 5 ml of α-pinene or 3-carene in 30 ml polyethylene bottles. Release rates of α-pinene and 3-carene were 24 mg/day and 40 mg/day at 24°C, respectively. The chemical purities of frontalin, α-pinene, and 3-carene were 89%, 98%, and 90%, respectively.

Traps were placed a minimum of 40 m apart in clearcuts. Traps were placed in the field on 16 June 1996 on the Wallowa-Whitman National Forest and on 22 July and 12 August 1997 in the Wentachee National Forest. Trap catches were collected two weeks following installation. The total number of beetles collected from each trap was recorded. The sex of up to one hundred beetles from each sample was determined (Jantz and Johnsey 1964). The total number of 3 species of predators (*Thanasimus undatulus* (Say), *Enoclerus sphegeus* F., and *Temnochila chlorodia* (Mann.)) were recorded as well.

3.2.3 Data Analysis

3.2.3.1 Toxicity Bioassay

Adult Douglas-fir beetle mortality was determined by counting the number of dead beetles among the 16 beetles exposed to each treatment in the laboratory. Beetles within controls began to die after 72 hours of confinement. Thus, statistical analysis was conducted on mortality at 72 hours past initial exposure. Only male beetles were observed for collection dates 17 July 1996 and 7 July 1997. Observations of male and female beetles were pooled for collection date 13 May 1998. A regression model was applied to these data. This model included a term for error and terms describing the effects of treatment, test dates, and replicates. This analysis provided the odds of mortality when beetles are exposed to a terpene treatment in comparison to water, the control.

$$\text{logit}(\pi) = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_3 + \beta_4\chi_4 + \beta_5\chi_5 + \beta_6\chi_6 + \beta_7\chi_7 + \beta_8\chi_8 + \beta_9\chi_9 + \beta_{10}\chi_{10} + \beta_{11}\chi_{11}$$

where π = treatment mean expressed as a proportion of probability the beetle was dead.

$\chi_1 = 1$ if treatment = limonene, 0 if otherwise.

$\chi_2 = 1$ if treatment = 3-carene, 0 if otherwise.

$\chi_3 = 1$ if treatment = alpha-pinene, 0 if otherwise.

$\chi_4 = 1$ if treatment = myrcene, 0 if otherwise.

$\chi_5 = 1$ if treatment = water, 0 if otherwise.

$\chi_6 = 1$ if replicate = 1, 0 if otherwise.

$\chi_7 = 1$ if replicate = 2, 0 if otherwise.

$\chi_8 = 1$ if replicate = 3, 0 if otherwise.

$\chi_9 = 1$ if date = 7/17/96, 0 if otherwise.

$\chi_{10} = 1$ if date = 7/7/97, 0 if otherwise.

$\chi_{11} = 1$ if date = 5/13/98, 0 if otherwise.

3.2.3.2 Repellency Field Test

The numbers of trapped Douglas-fir beetles and predators were analyzed using a Poisson log-linear regression model that accounted for error due to trap collection date and block as well as differences in attractiveness between the trap treatments.

$$\ln(y_i) = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_3 + \beta_4\chi_4 + \beta_5\chi_5 + \beta_6\chi_6 + \beta_7\chi_7 + \beta_8\chi_8 + \beta_9\chi_9 + \beta_{10}\chi_{10} + \beta_{11}\chi_{11} + \beta_{12}\chi_{12} + \beta_{13}\chi_{13}$$

where y_i = number of Douglas-fir beetles in the i^{th} observation.

χ_1 = 1 if treatment was frontalin + alpha-pinene, 0 if otherwise.

χ_2 = 1 if treatment was frontalin + alpha-pinene + 3-carene, 0 if otherwise.

χ_3 = 1 if treatment was frontalin and 3-carene, 0 if otherwise.

χ_4 = 1 if sex = male, 0 if female.

χ_5 = 1 if replicate = 1, 0 if otherwise.

χ_6 = 1 if replicate = 2, 0 if otherwise.

χ_7 = 1 if replicate = 3, 0 if otherwise.

χ_8 = 1 if replicate = 4, 0 if otherwise.

χ_9 = 1 if replicate = 5, 0 if otherwise.

χ_{10}= 1 if replicate = 6, 0 if otherwise.

χ_{11} = 1 if date = 6/16/96, 0 if otherwise.

χ_{12} = 1 if date = 7/22/97, 0 if otherwise.

χ_{13} = 1 if date = 8/12/97, 0 if otherwise.

3.3 Results

3.3.1 Toxicity Bioassay

Exposure to any of the 4 terpenes that were tested resulted in significantly higher mortality compared to the water control. The following odds of mortality result from 72 hours of exposure to each treatment. The odds of dying were 15 times greater for beetles exposed to 3-carene than for those exposed to water (*P*-value = 0.0001, 95% CI = 7 to 41). Exposure to limonene also resulted in odds of dying 15 times greater than water (*P*-value = 0.0001, 95% CI = 3 to 16). The odds of mortality for beetles exposed to myrcene were 6 times greater than beetles exposed to water (*P*-value = 0.0001, 95% CI = 3 to 16). The odds of dying were 3 times greater for beetles exposed to α-pinene than for those exposed to water (*P*-value = 0.04, 95% CI = 1 to 7) (Figure 3.2). The mean numbers of dead beetles per replicate at 48 and 72 hours past initial exposure to each terpene dosage for all three trial dates are presented in Figure 3.3.

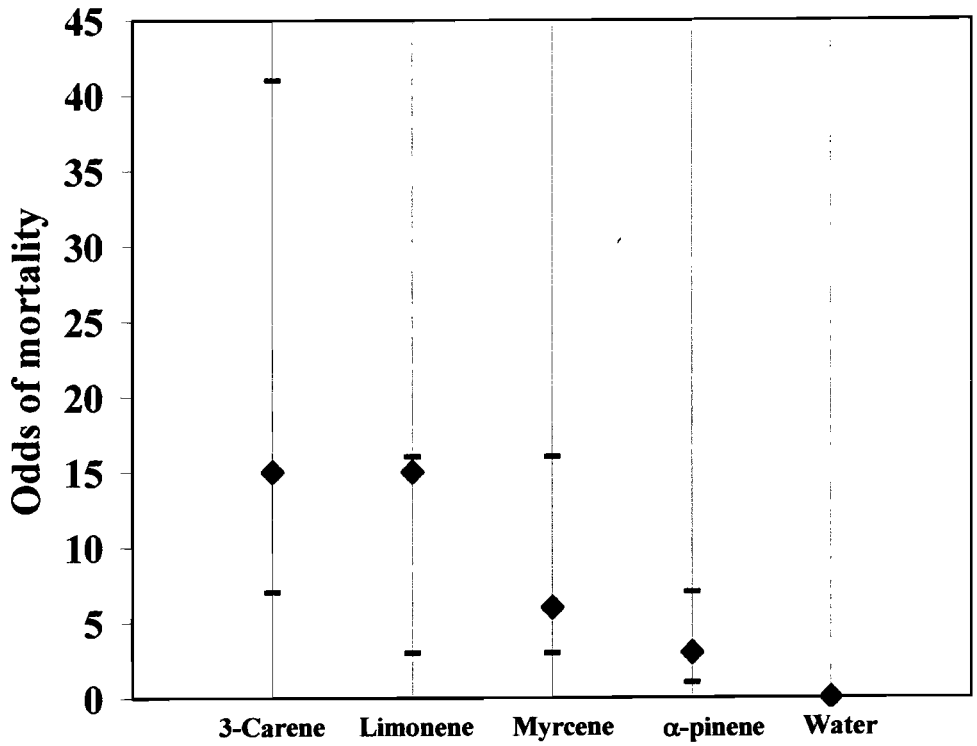
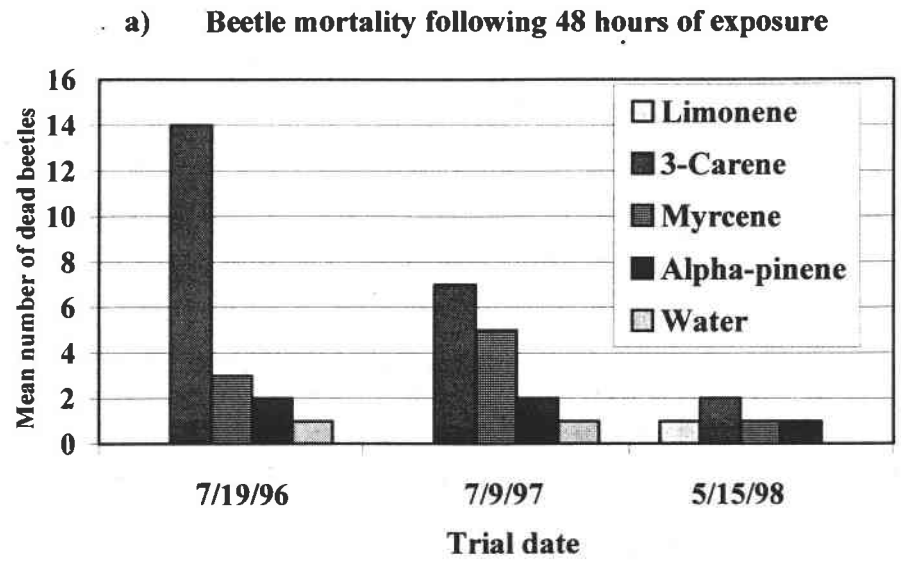


Figure 3.2 The odds of mortality, relative to water, following 72 hours of exposure to terpene treatments. Confidence intervals are plotted as lines above and below the data points. Binary logistic regression was used to calculate these odds.



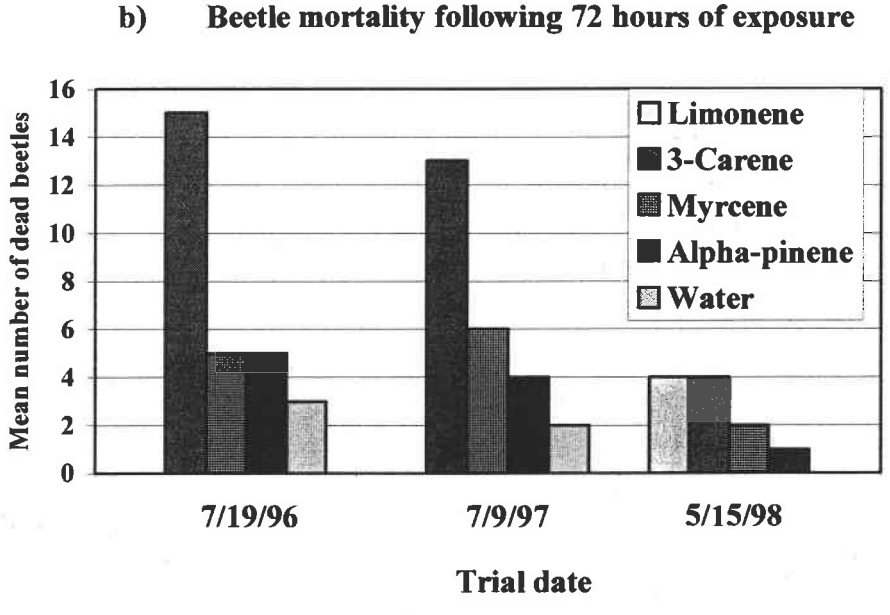


Figure 3.3 Mean numbers of dead Douglas-fir beetles out of sixteen exposed to each treatment following a) 48 and b) 72 hours of exposure. The mean numbers of dead Douglas-fir beetles for the water control on 5/15/98 were zero. Limonene was only tested in 1998.

3.3.2 Repellency Field Test

Lures containing 3-carene attracted significantly fewer Douglas-fir beetles than those without 3-carene (Figure 3.4). Traps baited with 3-carene in addition to α-pinene and frontalin caught only 30% as many Douglas-fir beetles as traps baited with only alpha-pinene and frontalin (*P*-value = 0.0001, 95% CI = 100% to 16%). Traps that were baited with frontalin and 3-carene caught 8% as many Douglas-fir beetles as traps baited with α-pinene and frontalin (*P*-value = 0.0001, 95% CI = 20% to 3%). Sex ratios of Douglas-fir beetles were not affected by treatments (*P*-value > 0.05). Although captures were low, the total numbers of coleopteran predators were not significantly affected by the treatments (*P*-value > 0.05).

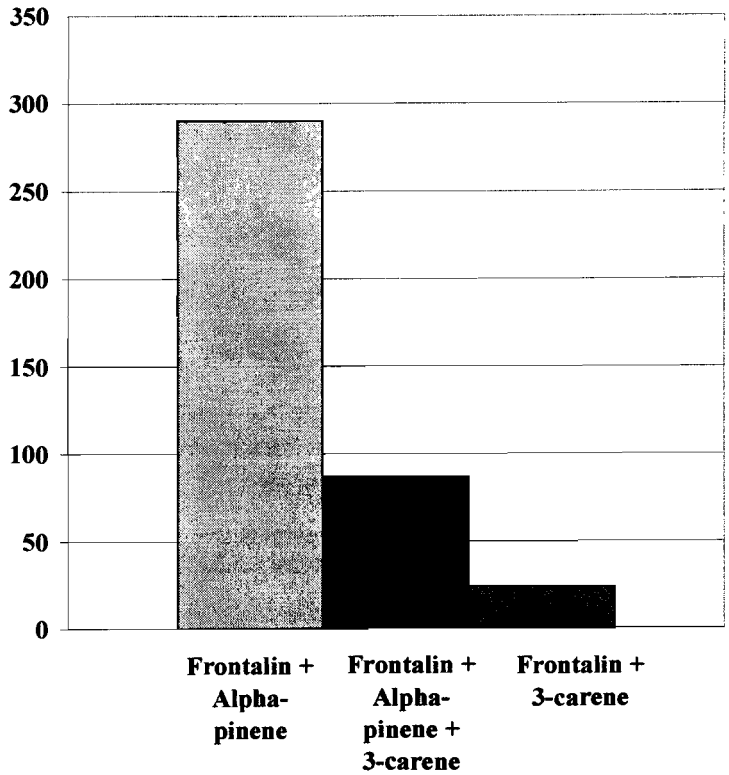


Figure 3.4 Total numbers of Douglas-fir beetles caught in traps baited with frontalin in combinations with α-pinene and 3-carene.

3.4 Discussion

3.4.1 3-Carene Toxicity

The vapors of limonene and 3-carene at 33ppm were most toxic to Douglas-fir beetles at 72 hours of exposure. Limonene was tested during the third trial only and was as toxic as 3-carene. The relative toxicity of monoterpenes at 33 ppm for 72 h to male Douglas-fir beetles, in decreasing order of toxicity, was 3-carene = limonene > myrcene > α-pinene (Figure 3.2). There was significant variation in mortality among the three trial dates (Figure 3.3). However, the amount of mortality recorded for the control groups varied among dates as well.

The toxicity bioassay involved concentrations of limonene, myrcene, and α-pinene that are either present in the constitutive phloem of Douglas-fir trees or occur following an induced wound response (Hanover and Furniss 1966). Likewise, the bioassay concentration of 3-carene is present or induced within the phloem of western larch (Reed et al. 1986). The data suggest 3-carene and limonene are toxic to male adult Douglas-fir beetles. Consequently, Douglas-fir beetles will be adversely affected by tunneling within the phloem of trees with elevated concentrations of 3-carene and limonene. Beetles may respond to a toxic subcortical environment by excavating galleries more slowly or by retreating from the tree. These types of responses would allow the tree defenses to target a smaller area of wounded tissue and thus more effectively contain any colonization. This study suggests that the relatively high concentrations of 3-carene found in western larch (Reed et al. 1986), in part, could impair Douglas-fir beetle colonization.

The toxicity of 3-carene to the Douglas-fir beetle has not previously been reported. Furniss and Schmitz (1971) reported inhibitory effects of 3-carene on beetle response to aggregation pheromone and, presumably, beetle exposure to inhibitory terpenes may increase the odds of beetle mortality. The toxicity of limonene to adult Douglas-fir beetles is consistent with reports of limonene toxicity to several *Dendroctonus* species (Coyne and Lott 1976, Hodges et al. 1979). Smith (1964) reported the toxicity of monoterpene vapors from ponderosa pine, *Pinus ponderosae* Dougl. Ex Laws, to the western pine beetle, *Dendroctonus brevicomis* LeConte. In that case, the decreasing order of toxicity was limonene > 3-carene > myrcene > β-pinene. Limonene was one of the most toxic host tree monoterpenes to the fir engraver, *Scolytus ventralis* LeConte (Raffa et al. 1985), the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coyne and Lott 1976, Hodges et al. 1979), the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coyne and Lott 1976, Hodges et al. 1979), the spruce beetle, *Dendroctonus rufipennis* (Kirby), and the eastern larch beetle, *Dendroctonus simplex* LeConte (Werner 1995). Additionally, myrcene appears to be relatively less toxic, but more so than alpha-pinene. This is also consistent with other reports (Smith 1965, 1966b, 1972, Coyne and Lott 1976, Hodges et al. 1979, Raffa and Berryman 1983b, Raffa et al. 1985, Werner 1994b, 1995).

Different species of *Dendroctonus* react differently to resin vapors from host and non-host tree species (Smith 1963). Generally, bark beetle species can survive in an atmosphere permeated by host tree resin vapors, but not necessarily in one permeated by non-host tree resin vapors. Resin vapor is potentially an important factor involved in host tree recognition by bark beetles and host tree resistance against bark beetles

The monoterpene composition of conifer resin appears to be an important characteristic of host resistance to bark beetles (Hanover and Furniss 1966, Hodges et al. 1979). Because of the relatively high toxicity of 3-carene to the Douglas-fir beetle, the higher concentrations of this terpene in western larch compared to

Douglas-fir (Reed et al. 1986) may be a factor contributing to the resistance of live larch to Douglas-fir beetle infestation.

3.4.2 3-Carene Repellency

There were significant differences in trap catches between treatments consisting of different combinations of frontalin, α-pinene, and 3-carene. The relative attractiveness of each treatment, in decreasing order, was frontalin + alpha-pinene > 3-carene + frontalin + alpha-pinene > 3-carene + frontalin (Figure 3.4). Sex ratios of Douglas-fir beetles were not affected by the terpenes. Although captures were low, coleopteran predators did not appear to be affected by the terpenes.

The results from the field test of 3-carene are consistent with the laboratory bioassays. The results suggest 3-carene interferes with the attractiveness of α-pinene and frontalin. The potential role of 3-carene as a repellent or inhibitor of the response of the Douglas-fir beetle to frontalin was reported by Furniss and Schmitz (1971). They found the combination of frontalin and 3-carene to be least attractive to the Douglas-fir beetle compared to all other combinations of frontalin and individual monoterpenes found in Douglas-fir.

Frontalin is a component of the pheromone system of many *Dendroctonus* species that colonize conifers of different genera, e.g. *D. frontalis, D. pseudotsugae, D. rufipennis, D. simplex,* and *D. brevicomis*. However, within the same stand, *D. pseudotsugae* can differentiate an infested Douglas-fir tree from a ponderosa pine infested by *D. brevicomis.* Although secondary components of the aggregation pheromones are involved in species specific attraction, host odors may also play a role. The case of *D. brevicomis* and *D. pseudotsugae* serves as an example. Landing and feeding by *D. brevicomis* is induced by 3-carene which is found in minute quantities in Douglas-fir and appears to be unattractive to *D. pseudotsugae* (Hanover and Furniss 1966, Vité and Pitman 1969, Furniss and Schmitz 1971). The eastern

larch beetle, *D. simplex*, also employs frontalin as an aggregant pheromone, yet Prendergast (1991) reported that 3-carene synergized the attractiveness of seudenol to the eastern larch beetle. Moreoever, camphene, absent or present in minute quantities in ponderosa pine (Mirov 1961), can similarly induce landing and feeding of *D. pseudotsugae* while remaining unattractive to *D. brevicomis.* The same tree monoterpene has contrasting effects on the host selection behavior of different species of *Dendroctonus.*

Certain monoterpenes attract particular bark beetles and/or make aggregation pheromones more attractive (Furniss and Schmitz 1971). Other monoterpenes may not elicit a response from some bark beetles or may have a repellent effect (Tunset et al. 1993). Host trees with tissues high in concentration of toxic monoterpenes may be resistant to certain scolytids. Hanover and Furniss (1966) found, within two geographic locations, higher concentrations of 3-carene in unattacked Douglas-fir compared to Douglas-fir attacked by the Douglas-fir beetle. Possibly, the toxic and inhibitory effects of 3-carene contributed to the resistance of Douglas-fir with higher 3-carene concentrations. Similarly, Smith (1977) theorized that *P. ponderosa* with high concentrations of limonene may be resistant to *D. brevicomis.* Inhibitory or repellent compounds that come from potential host trees may play an important role in bark beetle host selection.

The results from this study suggest 3-carene is unattractive in the sense that it interferes with the attractiveness of frontalin and α-pinene. In order to conclude that 3-carene inhibits Douglas-fir beetle attraction to frontalin, the odds of beetle capture for baits of frontalin alone would need to be compared to baits of frontalin combined with 3-carene.

4 WESTERN LARCH (*LARIX OCCIDENTALIS*) RESISTANCE TO DOUGLAS-FIR BEETLE (*DENDROCTONUS PSEUDOTSUGAE*) COLONIZATION

4.1 Introduction

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is found in western North America within the range of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, (Schmitz and Gibson 1996). Under favorable conditions, the Douglas-fir beetle can reach densities high enough to kill large numbers of Douglas-fir trees adversely impacting forest resource values (Johnson and Belluschi 1969, Furniss et al. 1979). Considering the number of trees, including non-host trees that dispersing bark beetles encounter, very few are mass-attacked (Wood 1976, Raffa and Berryman 1980, Moeck et al. 1981, Raffa and Berryman 1983a). The host selection and colonization behavior of the Douglas-fir beetle is regulated by a complex chemical and sonic communication system that involves both pheromones and host tree volatiles, such as monoterpenes (Rudinsky 1966a, Ryker 1984). There are chemical and physical reasons that some trees are not mass-attacked. Some trees may repel beetles, lack phagostimulants, inhibit the release of aggregation pheromones, or be deficient in pheromone precursors (Borden 1989).

Raffa and Berryman (1983a) established that the level of terpene exposure may influence pioneer beetle generated attraction in several ways. First, host compounds interacting with pheromones will elicit different responses under different conditions. Second, volatile monoterpenes may compete with aggregation pheromone molecules for antennal receptor sites. Payne (1975), studying *Dendroctonus frontalis*

Zimmerman and *Dendroctonus brevicomis* Hopkins, determined that two monoterpenes, α-pinene and 3-carene, share the same antennal receptor site with frontalin, an aggregation pheromone employed by several *Dendroctonus* species, including the Douglas-fir beetle. Once this receptor site has been saturated with monoterpene molecules, the beetle may not respond to frontalin.

While most conifers share the same qualitative monoterpenes, they differ quantitatively. The quantity of toxic monterpenes within individual trees and among tree species may be a factor in differing host tree resistance to beetle attack. The preformed monoterpenes may be less important for within-tree survival than induced monoterpenes following beetle attack. Resistant trees may be able to increase concentrations of the most toxic and repellent monoterpenes more than less resistant trees (Wright et al. 1979, Raffa and Berryman 1982, Raffa and Berryman 1983a).

There is a large array of host tree compounds that may serve as toxins, repellents or feeding deterrents. However, these compounds may have little or no affect on adapted bark beetle species. Some compounds that can be highly toxic are actually used as signaling cues that assist adapted bark beetle species in host selection. Some bark beetles have developed associations with symbionts or have evolved mechanisms of detoxifying or converting these compounds into pheromones used to their own benefit. In some cases, host defensive compounds are required as precursors to pheromones. Consequently, some species will not attack unless host olfactory cues such as resin odors are present (Payne 1979).

Defensive compounds have received particular attention in research with aggressive bark beetles. Gas-liquid chromatograph analyses have shown that quantitative differences in pheromones produced by pioneering female beetles are related to host tree condition (Vité and Pitman 1968). Pioneering beetles that detect differences in the quantity and quality of tree defensive chemicals would have a greater chance of inducing a mass-attack following release of aggregation pheromones.

Dendroctonus spp. bark beetles routinely detoxify α-pinene and myrcene by increasing their water solubility (hydroxylation or oxidation). Only a small change is needed to convert exogenous precursors into pheromones. From both an energetic and evolutionary standpoint, the products resulting from the detoxification of tree defensive chemicals are logical pheromones (Byers 1989).

The attractiveness or synergistic characteristic of many oleoresin compounds have been well documented (Heikkenen and Hrutfiord 1965, Rudinsky 1966b, Furniss and Schmitz 1971, Dickens et al. 1983, 1984). However, the repellent or aggregant inhibitory effects of other monoterpenes have been studied less and deserve further investigation (Hayes et al. 1994, 1996). The subversion of olfactory perception with non-host tree volatiles is a potential bark beetle management tactic (Borden 1985).

The Douglas-fir beetle has been reported to occasionally attack western larch, *Larix occidentalis* Nutt. Although brood production in dead western larch is similar to that in Douglas-fir, successful brood development has not been observed in live western larch (Ross 1967, Reed et al. 1986). However, there are no published data that document these differences in suitability of Douglas-fir and western larch as hosts for the Douglas-fir beetle. Live western larch has a significantly higher concentration of the monoterpene, 3-carene, compared to Douglas-fir (Reed et al. 1986). In an earlier study, 3-carene was shown to have a relatively high toxicity to adult Douglas-fir beetles and to interfere with attraction of dispersing beetles to known pheromone components (Chapter 3). This study compared the suitability of western larch and Douglas-fir as hosts for the Douglas-fir beetle and investigated the role of 3-carene in resistance to the Douglas-fir beetle under field conditions.

4.2 Methods

4.2.1 Installation

This study was installed on 13-16 May 1998 on the Okanogan National Forest, Washington (48° N, 119° W). A 1997 aerial sketch map (USDA Forest Service Region 6) showing trees infested in 1996 indicated light Douglas-fir beetle activity in the study area. Several recently downed Douglas-fir trees, less than 1.6 km from the study area, were found to contain healthy brood.

A randomized complete block design was used with each of five treatments represented in each block. Three adjacent blocks were established within each of two study sites. The two sites were approximately 3.2 km apart between the center points of each site. Stands at both sites were composed primarily of sawtimber size Douglas-fir and western larch. Englemann spruce, *Picea engelmannii* Parry ex Engelm., and lodgepole pine, *Pinus contorta* Dougl. Ex Loud., were much smaller components of the stands. One site was less dense than the other as a result of thinning two years earlier. Table 4.1 summarizes some stand characteristics for all six blocks.

Table 4.1 Mean (standard error) characteristics of stands used to study host suitability and the role of 3-carene in western larch resistance to the Douglas-fir beetle.

Site	Block	SDI	Trees per Hectare	Diameter at Breast Hieght (cm)	Basal Area (m^2/ha)	% Basal Area of Douglas-fir	% Basal Area of Western Larch
Unthinned	1	220 (10)	555 (125)	27.0 (2.5)	27.9 (0.3)	75.0 (3)	24 (3)
Unthinned	2	184 (27)	559 (201)	26.0 (3.6)	21.9 (4.0)	67.0 (9)	33 (10)
Unthinned	3	165 (8)	754 (161)	18.4 (1.6)	18.2 (1.1)	56.0 (12)	26 (11)
Thinned	4	121 (29)	642 (402)	23.9 (4.6)	13.9 (3.1)	76.0 (7)	24 (7)
Thinned	5	85 (15)	362 (101)	21.2 (3.7)	9.7 (1.9)	71.0 (7)	25 (8)
Thinned	6	124 (14)	384 (121)	24.4 (2.4)	15.1 (1.5)	58.0 (15)	22 (6)

Treatments were represented by the following five types of host trees: 1) live western larch; 2) felled western larch; 3) live Douglas-fir; 4) felled Douglas-fir; and 5) live Douglas-fir encircled by 3-carene releasers. Treated trees were selected based on similar appearance within species, DBH $\geq$ 34 cm, and lack of obvious defects (aside from light mistletoe infection). At each site, experimental trees were approximately 150 m apart. Each tree was baited with 30 mg frontalin and 15 mg seudenol formulated in polyvinylchloride (PVC) releasing at 1.5 mg/day and 0.9 mg/day at 24°C, respectively. Baits were secured to treatment trees at 2 meters above the root collar. Baits were taken off of the trees after beetle colonization was initiated. The live Douglas-fir encircled by 3-carene releasers was surrounded by twelve polyethylene bottles containing 5 ml of 3-carene each. The dispensers were equally spaced around the baited tree, secured on saplings or snags at 1-2 meters off the ground and 4-5 meters from the base of the tree. The polyethylene bottles released 3-carene at a rate of 40 mg/day at 24°C.

Diameter at the base, diameter at breast height, and total height were measured on each experimental tree to account for possible confounding variables. These measurements are summarized in table 4.2.

Table 4.2 Mean (standard error) characteristics of experimental trees used to study host suitability and the role of 3-carene in western larch resistance to the Douglas-fir beetle.

Species	Treatment(s)	Diameter at Breast Height (cm)	Diameter at Base (cm)	Total Height (m)
Western Larch	Live	41.1 (2.4)	60.5 (2.0)	29.6 (1.2)
Western Larch	Felled	39.6 (1.3)	50.0 (2.5)	27.1 (1.3)
Douglas-fir	Live and Live surrounded by 3-carene releasers	45.0 (3.1)	59.5 (2.8)	27.6 (1.8)
Douglas-fir	Felled	42.9 (1.0)	52.6 (1.1)	26.0 (1.3)

4.2.2 Response Variables

Arriving and colonizing Douglas-fir beetles were sampled to determine relative attraction, egg gallery construction, and brood production for each treatment. Flight intercept traps were used to sample adult beetles attracted to the baited trees (Figures 4.1 and 4.2). Bark samples were collected to estimate egg gallery construction as well as brood production (Figure 4.3). These data were compared among treatments to assess the degree of western larch resistance and the effect of 3-carene exposure on the success of Douglas-fir beetle colonization.

Figure 4.1 Flight intercept traps used to sample Douglas-fir beetles attracted to the baited live trees.

Figure 4.2 Flight intercept traps used to sample Douglas-fir beetles attracted to the baited felled trees.

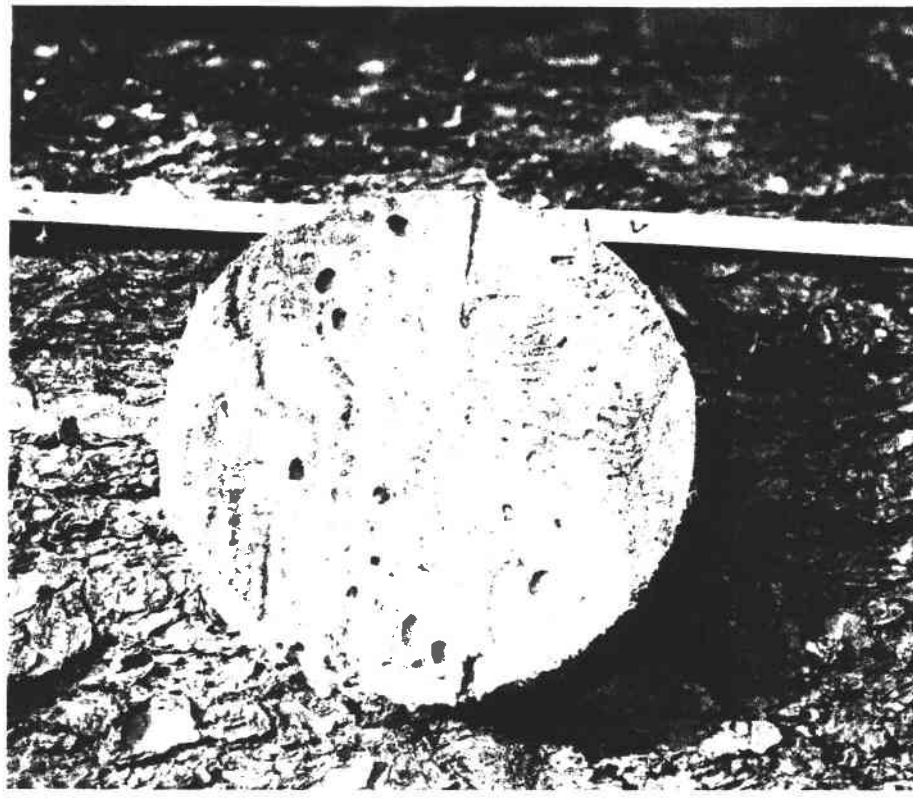


Figure 4.3 Example of 100 cm^2 bark sample used to count the number of Douglas-fir beetle attack sites, egg galleries, eggs hatched, brood, predator brood, wood borer brood, and percentage of area fed upon by wood borers.

4.2.2.1 Aggregation

Four flight intercept traps, each with a 30x30 cm trapping surface, were secured onto treated trees at the time that the study was installed and trees were baited (Figures 4.1 and 4.2). A 2.5 x 2.5 cm piece of plastic impregnated with 2,2-dichlorovinyl dimethyl phosphate (DDVP) was placed in each collection cup to kill trapped insects. Two traps were secured on opposite sides of the tree at 9 meters from the base of the tree. In addition, two traps were secured directly below those at 4.5 meters from the base. Traps were emptied on 24 May 1998 and 13 June 1998. Trap samples were stored in a freezer until processed. The total numbers of Douglas-fir beetles and 3

species of bark beetle predators (*Thanasimus undatulus* (Say), *Enoclerus sphegeus* F., and *Temnochila chlorodia* (Mann.)) were counted. The sex was determined for up to one hundred Douglas-fir beetles from each sample (Jantz and Johnsey 1964).

4.2.2.2 Colonization and Brood Production

Bark samples (Figure 4.3) were collected on 16 August 1998 after Douglas-fir beetle flight had ended. One hundred cm^2 circular bark samples were removed using an electric drill and hole saw. Bark samples were stored in separate plastic bags inside a freezer until dissected. Two bark samples were taken from the following four positions on the bole: 10.5 m, 4.5 m, 2.7 m, and 1.2 m from the base of the tree.

The number of attack sites, the number of egg galleries, and the number of successful egg galleries on each bark sample were counted. Numbers of eggs that hatched and the number of live Douglas-fir beetle brood of various life stages were counted for each bark sample. Numbers of selected predacious insects and wood borers of various life stages were recorded for each bark sample. The percentage of bark area that had been fed upon by wood boring beetles (Coleoptera: Buprestidae and Cerambycidae) was estimated in order to account for any effect that interspecies competition may have had on colonization and/or brood production. A sheet of acetate with a circular grid of 100 one cm^2 squares was placed over each bark sample and the number of squares falling onto the areas fed upon by wood borers was recorded.

4.2.3 Experimental Design and Analyses

Each response variable was analyzed individually and the error due to the effect of study site and block was tested. There was no significant error introduced as a result of the differences between the two study sites. Thus, differences between the six blocks were included in each regression model that was applied to each treatment

response measured. There were significant differences between sample heights for live tree responses. Thus, sampling height was included in each model used to test for treatment differences between live trees. The distribution of responses for felled tree treatments was much larger than the distribution of responses for live tree treatments. These large differences resulted in unreliable comparisons between felled and live tree treatment responses for most measurements. For this reason, felled and live trees were not compared for most responses and the total length of egg galleries per bark sample was not analyzed.

All responses except the number of successful egg galleries were analyzed using Poisson regression. Because the number of successful egg galleries depends on the total number of egg galleries excavated, a binomial linear regression model was applied to estimate the odds of egg gallery success. The binomial regression model provided the relative odds of egg gallery success of one treatment versus the others.

The attack densities for each treatment were averaged and converted into the number of attacks per meter squared to provide a basis for comparing the observed number of attacks per meter squared to attack densities reported in the literature.

4.3 Results

4.3.1 Aggregation

Traps mounted on live trees caught significantly more Douglas-fir beetles than traps mounted on felled trees (P=0.0001). Live tree trap catches were extremely variable. There were no significant differences between any live tree treatments. Some variation may have been due to differences in attraction to traps mounted on the upper portion of the bole versus the lower portion of the bole. An estimated 50% fewer

Douglas-fir beetles were caught in traps at 4.5 meters from the base of live trees compared to those at 9 m (95% CI = 30% to 100%), although the difference was not statistically significant (*P*-value = 0.06). Traps at 4.5 m caught 32% of the number caught with traps at 9 m from the base of felled trees (*P*-value = 0.0035, 95% CI = 14 to 66%). Predator catches were low, and there were no significant differences among treatments (*P*-value = 0.08). Douglas-fir beetle sex ratios were not significantly different among treatments either (*P*-value = 0.836).

4.3.2 Colonization and Brood Production

The mean numbers of attacks, egg galleries, successful egg galleries, and unsuccessful egg galleries within a 100 cm^2 bark sample are presented in Figure 4.4. Live western larch sustained 70% fewer attacks compared to live Douglas-fir (*P*-value = 0.0001, 95% CI = 49% to 84%). Felled western larch sustained 49% fewer attacks compared to felled Douglas-fir (*P*-value = 0.0045, 95% CI = 20% to 88%). There were 52% fewer attacks on live Douglas-fir surrounded by 3-carene releasers compared to those without 3-carene releasers (*P*-value = 0.003, 95% CI = 27% to 71%). There was no significant difference in number of attack sites between live western larch and live Douglas-fir that had been surrounded by 3-carene releasers (*P*-value = 0.1402).

The average number of attacks per square meter of bark surface for each treatment were 34/m^2 for live western larch, 112/m^2 for live Douglas-fir, 48/m^2 for felled western larch, 93/m^2 for felled Douglas-fir, and 54/m^2 for live Douglas-fir surrounded by 3-carene releasers.

Adult beetles excavated 71% fewer egg galleries in live western larch compared to live Douglas-fir (*P*-value = 0.0001, 95% CI = 54% to 83%). An estimated 25% fewer egg galleries were counted in felled western larch compared to felled Douglas-fir (95% CI = 0% to 45%), although the difference was not statistically significant (*P*-

value = 0.059). There were 30% fewer egg galleries in live Douglas-fir surrounded by 3-carene releasers compared to live Douglas-fir without 3-carene releasers (*P*-value = 0.05, 95% CI = 1% to 53%). Adult beetles excavated 41% fewer egg galleries in live western larch compared to live Douglas-fir surrounded by 3-carene (*P*-value = 0.0007, 95% CI = 24% to 68%).

The odds of successful galleries formed in live western larch was only 1% of that in live Douglas-fir (*P*-value = 0.0001, 95% CI = 0% to 5%). All egg galleries formed in felled trees were successful. The odds of egg gallery success in live Douglas-fir surrounded by 3-carene releasers was 21% of that in live Douglas-fir without 3-carene releasers(*P*-value = 0.0012, 95% CI = 8% to 52%).

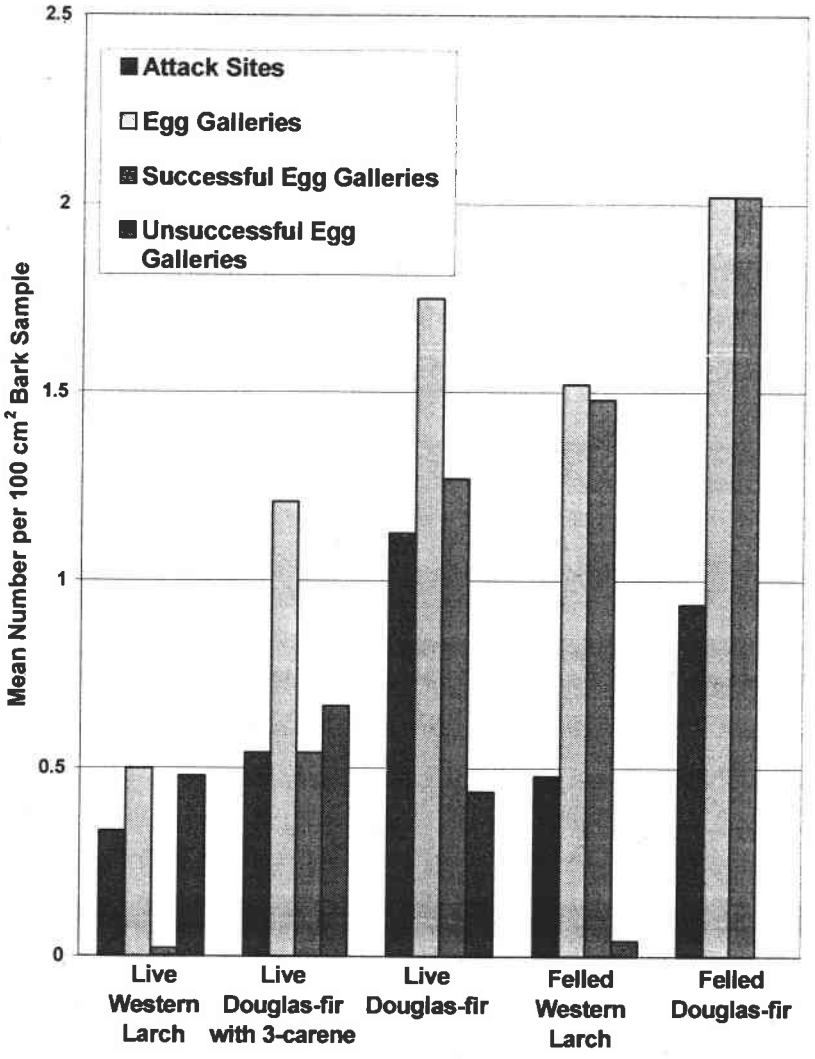


Figure 4.4 Douglas-fir beetle attacks and egg gallery construction in live and felled western larch and Douglas-fir and Douglas-fir surrounded by 3-carene releasers.

The mean length of successful egg galleries, numbers of eggs hatched, live Douglas-fir beetle brood ,and natural enemies found within a 100 cm^2 bark sample are presented in Figure 4.5. No eggs hatched within live western larch. There were 32% fewer eggs that hatched within felled western larch compared to felled Douglas-fir (*P*-value = 0.051, 95% CI = 11% to 48%). An estimated 27% fewer eggs hatched in Douglas-fir surrounded by 3-carene compared to live Douglas-fir, but this difference was not statistically significant (*P*-value = 0.16). There was 97% less brood produced in baited live western larch compared to baited live Douglas-fir (*P*-value = 0.0001, 95% CI = 0% to 88%). The number of Douglas-fir beetle brood was not significantly different between felled western larch and felled Douglas-fir (*P*-value = 0.7377). No significant differences were detected in the number of brood produced between live Douglas-fir surrounded by 3-carene and those not surrounded by 3-carene (*P*-value = 0.6).

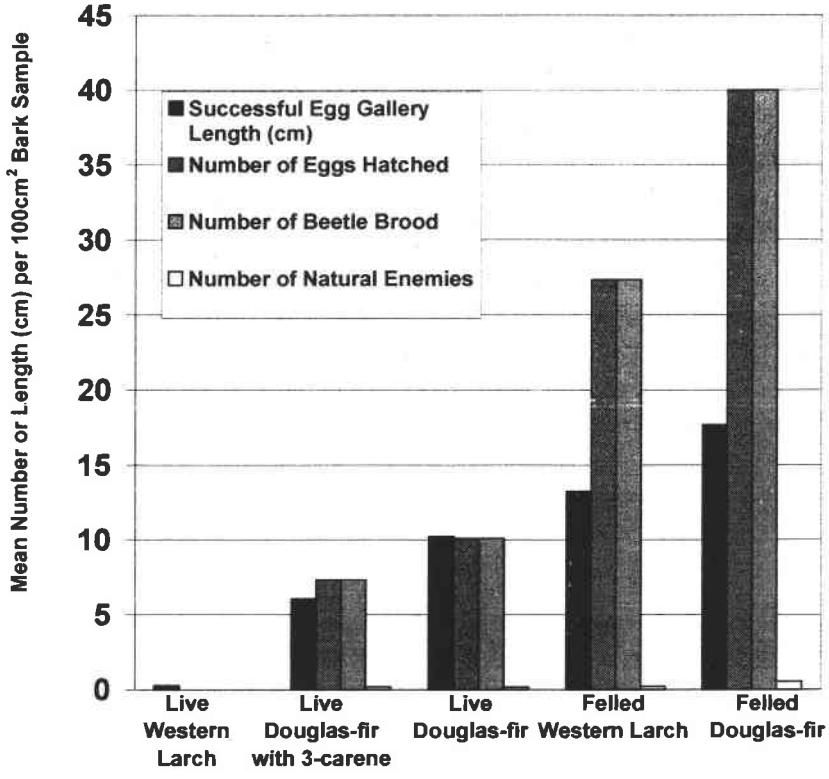


Figure 4.5 Douglas-fir beetle brood production and natural enemy densities in live and felled western larch and Douglas-fir and Douglas-fir surrounded by 3-carene releasers. The natural enemies present included *Thanasimus undatulus* (Say) and *Coeloides brunneri* Viereck.

The number of predators and parasitoids at various life stages was low. Some *Thanasimus undatulus* (Say) (Coleoptera: Cleridae) larvae and *Coeloides brunneri* Viereck (Hymenoptera: Braconidae) cocoons were found within bark samples. However, no differences were detected among treatments. Over three times (319%) the amount of wood borer feeding occurred in felled western larch compared to felled Douglas-fir (*P*-value = 0.0001, 95% CI = 191% to 559%).

4.3.3 Within Tree Spatial Variation

The relative numbers of attack sites and egg galleries at each sampling height are presented in Figure 4.6. There was no significant interaction between treatment and sampling height. There were significant differences among sampling heights for numbers of attacks, egg galleries, successful egg galleries, and unsuccessful egg galleries for all live tree treatments (P-values < 0.05). The middle portion of the bole had the largest number of attacks, egg galleries, and successful egg galleries. The amount of beetle activity for each sampling height, in decreasing order, was 4.5m > 2.7m > 1.2m > 9 m.

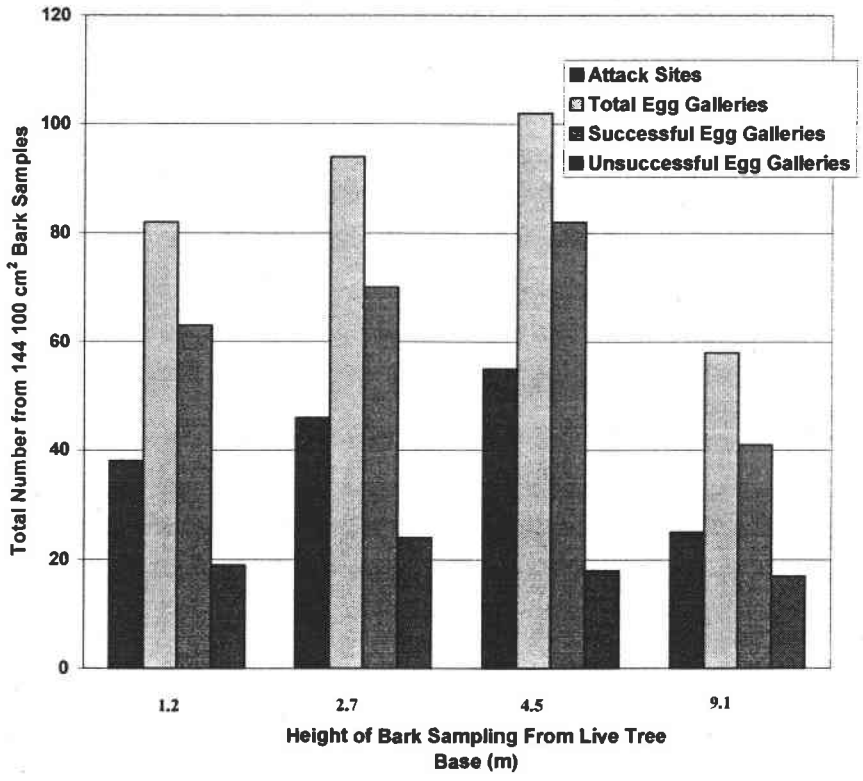


Figure 4.6 Douglas-fir beetle attacks and egg gallery construction in live western larch and Douglas-fir and Douglas-fir surrounded by 3-carene releasers for each bark sampling height.

4.4 Discussion

4.4.1 Aggregation

There are a number of possible reasons for the lack of significant differences in trap catches of Douglas-fir beetles and predators, and Douglas-fir beetle sex ratios among treatments. The lack of differences may be a result of the large variation among trap catches and the relatively small sample size. Also, the trap used in this study was a new design and several problems arose which may have affected trap catches. The traps on live trees were suspended from cords and this allowed them to move somewhat in windy conditions. As a result, the trapping surface of some traps was not always flat against the tree. In addition, pitch exuding from attacked trees flowed into collection cups blocking the drainage openings. This caused some traps on live trees to collect water and possibly spill in windy conditions. The exposure of traps to the wind varied among sites and may have contributed to the variation among trap catches for live trees. Traps on felled trees were also prone to blockage of the drainage openings due to contact between the forest floor and the collection cup. Because of these problems there were a number of missing and partial samples that reduced the chances of detecting treatment differences.

4.4.2 Colonization and Brood Production

4.4.2.1 Live Tree Treatments

There were significantly fewer Douglas-fir beetle attacks on both live western larch and live Douglas-fir surrounded by 3-carene compared to live Douglas-fir, although

all trees were baited identically. Since there were no differences in the numbers of beetles attracted to these trees, beetles attracted to live larch or Douglas- fir surrounded by 3-carene releasers must have been less likely to initiate an attack than those attracted to live Douglas-fir. Host location does not always result in host acceptance (Payne 1983). Douglas-fir beetles attracted to western larch may not have initiated attacks due to the lack of subsequent cues required for host acceptance or due to the presence of repellent volatile cues (Reed et al. 1986). The dramatic difference in attack densities for baited live western larch compared to live Douglas-fir may also involve a lack of aggregation pheromone exudation from initial attacks.

It is unlikely that the lower number of attack sites on live Douglas-fir surrounded by 3-carene releasers was due to a lack of aggregation pheromone released from initial attacks. Aggregation pheromones were undoubtedly released when Douglas-fir beetles attacked live Douglas-fir trees. However, Douglas-fir beetles may have been behaviorally disorientated in a few ways.

First, a low pheromone to host volatile ratio could have resulted. Such a ratio could result from a low attack density as well as an increased concentration of volatiles caused by the presence of 3-carene releasers. However, the presence of pheromones should be attractive regardless of the pheromone to host volatile ratio. If the level of attractiveness of the pheromones is reduced by a low pheromone to host volatile ratio then the attack density may be affected. Host tree death depends upon the attack density (Raffa and Berryman 1983a). An attack density too low to surpass the defensive capacity of the tree can result in disruption of bark beetle chemical communication by the host tree volatile chemicals.

Second, adult beetles may have become behaviorally disoriented due to a lack of antennal receptor responses to the aggregation baits placed on live Douglas-fir surrounded by 3-carene. Different antennal receptor sites have specific affinities for certain chemicals. Once antennal receptor sites are occupied by one kind of molecule, other chemicals may go undetected. Payne (1975) found that 3-carene

shares some of the same antennal receptor sites with frontalin. A 48% decrease in response to frontalin by Douglas-fir beetles following saturation of those sites with 3-carene has been reported (Payne and Dickens 1976, Dickens et al. 1983).

The lack of significant differences between attacks on live western larch and Douglas-fir surrounded by 3-carene suggests that these two treatments were similarly unattractive to adult Douglas-fir beetles, although it does not prove that the mechanism responsible for the observed behavior was the same in both cases.

According to Hedden and Pitman (1978) the defensive capacity of a Douglas-fir tree will be exhausted upon receiving between 40 and 60 Douglas-fir beetle attacks per m^2. McMullen and Atkins (1961) found an optimal Douglas-fir beetle brood production at 43-86 attacks per m^2, above which intraspecific competition may result. In this study, live western larch sustained an average of only 33 attacks per m^2. The likely result is a decrease in Douglas-fir beetle brood production and potentially host tree survival. In contrast, live Douglas-fir sustained an average of 112 attacks per m^2; thus, a decrease in beetle reproduction due to competition among brood may have occurred in live Douglas-fir. Live Douglas-fir surrounded by 3-carene releasers sustained an average of 54 attacks per m^2, within the previously cited ranges of attack density for overcoming host tree defensive capacity and optimal brood production.

The number of egg galleries excavated by adult Douglas-fir beetles, number of eggs hatched, and brood produced followed similar trends as described for attack density. Fewer egg galleries were excavated in live western larch and in live Douglas-fir surrounded by 3-carene compared to live Douglas-fir. However, the number of egg galleries excavated in live western larch was half that observed in live Douglas-fir surrounded by 3-carene. The lack of a significant difference in attack sites between live western larch and Douglas-fir surrounded by 3-carene releasers suggests that the primary affect of the 3-carene releasers was to interfere with location of the host tree. Once a Douglas-fir surrounded by 3-carene was located, there was a higher probability of the beetle excavating a gallery compared to live western larch that were

located by beetles. Not surprisingly, releasing 3-carene into the atmosphere around a Douglas-fir tree seemed to have little, if any direct affect on egg gallery excavation. Most egg galleries excavated in live western larch resulted in secondary resinosis and no eggs hatched.

Within Douglas-fir surrounded by 3-carene, 30% fewer egg galleries were excavated than in live Douglas-fir. The success of these egg galleries was one fifth that of live Douglas-fir. However, there appeared to be plenty of resources for the larvae that made it past the danger of egg gallery resinosis. The number of eggs hatched and brood production were not significantly different between Douglas-fir surrounded by 3-carene and Douglas-fir without 3-carene releasers.

The lack of optimal egg gallery formation, in live western larch and Douglas-fir surrounded by 3-carene could be partially attributed to the low attack densities. At suboptimal attack densities, defensive capacity of the tree is likely not depleted rapidly enough to prevent secondary resinosis and subsequent toxicity to eggs (Hedden and Pitman 1978). A direct relationship between eggs hatched and attack density has been described for *Scolytus ventralis* LeConte and *D. ponderosae* (Berryman and Ashraf 1970, Dudley 1971). Once eggs have been deposited, the success or failure of beetle development is not affected by adult beetle behavior.

4.4.2.2 Felled Tree Treatments

Felled western larch was attacked half as much as felled Douglas-fir (Figure 4.4). There were 25% fewer egg galleries excavated by adult Douglas-fir beetles within felled western larch compared to felled Douglas-fir. However, this difference was not statistically significant. All egg galleries in felled trees were successful (Figures 4.4 and 4.5). The numbers of eggs hatched and brood produced were not significantly different between felled western larch and felled Douglas-fir. In this case, host defense responses are not present or are minimal and concentrations of 3-carene in

felled trees likely decrease over time. Thus, only the quality and quantity of host nutrients as well as insect factors influence beetle reproductive success. The thinner phloem and bark of western larch may contribute to the slightly lower suitability and attractiveness of felled western larch compared to felled Douglas-fir.

4.4.2.3 Insect Competitors

There was three times as much wood borer feeding in the phloem of the felled western larch compared to felled Douglas-fir. This interspecific competition may have prevented adult Douglas-fir beetles from constructing as many galleries within felled western larch.

4.4.2.4 Natural Enemies

The number of predacious insects was not different among treatments. However, no predators were found under the bark of live western larch. The largest number of predators was found beneath the bark of felled Douglas-fir which also had the largest number of successful Douglas-fir beetle egg galleries. Predacious insects may not have been attracted to live western larch for similar reasons adult Douglas-fir beetles were not or the lack of predator attraction may be due to the lack of Douglas-fir beetle activity. Predacious insects may be able to detect the presence of high densities of Douglas-fir beetles. For this reason, predatory insects may have been attracted to felled Douglas-fir the most. Similarly, predacious insects may arrive and leave from a tree once detecting low numbers of Douglas-fir beetles. For this reason, predacious insects may have been absent from live western larch.

4.4.3 Within Tree Spatial Variation

Figure 4.6 illustrates the differences among live tree bark sampling height for the numbers of attacks, egg galleries, successful egg galleries, and unsuccessful egg galleries. Apparently, the upper and lower most portions of the bole are not as attractive and are less suitable for egg gallery construction than the middle portion. Secondary resinosis was observed for many of the egg galleries formed at 9 and 1.2 meters from the base of the tree. Johnson and Belluschi (1969) reported the most successful attacks were within the middle portion of the bole. This pattern of attack spacing is consistent with other reports (Furniss 1962, Hedden and Gara 1976).

4.4.4 Future Research

Resin vapor is potentially an important factor involved in host tree recognition by bark beetles and host tree resistance against bark beetles. Species of *Dendroctonus* react differently to resin vapors from host and non-host tree species (Smith 1963). Generally, bark beetle species can survive in an atmosphere permeated by host tree resin vapors, but not necessarily in one permeated by non-host tree resin vapors. The monoterpene composition of conifer resin appears to be an important characteristic of host resistance to bark beetles (Hanover and Furniss 1966, Hodges et al. 1979). The monoterpenes α-pinene, camphene, beta-pinene, myrcene, 3-carene, and limonene are components of Douglas-fir oleoresin (Hanover and Furniss 1966). α-pinene is the most abundant and camphene and 3-carene are found in minute amounts. Reed et al. (1986) reported that Douglas-fir had higher concentrations of all oleoresin volatiles compared to western larch except western larch had a higher concentration of 3-carene. Because of the relatively high toxicity of 3-carene to the Douglas-fir beetle (Chapter 3), the higher concentrations of this terpene in western larch compared to Douglas-fir may be a factor contributing to the resistance of live larch to Douglas-fir beetle infestation.

An early study comparing monoterpene concentrations of attacked and unattacked Douglas-fir in two geographic locations reported that unattacked Douglas-fir had higher concentrations of 3-carene (Hanover and Furniss 1966). Those elevated 3-carene concentrations are referred to by Reed et al., (1986) as being 25% that of 3-carene concentrations found in western larch. Other differences between species that may contribute to lower attraction or attack density include larger diameter vertical resin ducts, a higher level of moisture in the phloem, and a thinner phloem layer. Reed et al. (1986) measured the oleoresin exudation pressure of western larch and found that there was none; therefore, this trait is not addressed as a factor in the resistance of western larch to Douglas-fir beetle attack.

BIBLIOGRAPHY

1. Atkins, M.D. 1959. A study of the flight of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. I. Flight preparation and response. Can. Entomol. 91, 258-291.

2. Atkins, M.D. 1960. A study of the flight of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. II. Flight movements. Can. Entomol. 92, 941-954.

3. Atkins, M.D. 1961. A study of the flight of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. III. Flight capacity. Can. Entomol. 98, 285-288.

4. Atkins, M.D. 1966. Laboratory studies on the behavior of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. Can. Entomol. 98, 953-991.

5. Atkins, M.D. 1969. Lipid loss with flight on the Douglas-fir beetle. Can. Entomol. 101, 164-165.

6. Barras, S.J. 1970. Antagonism between *Dendroctonus frontalis* and the fungus *Ceratocystis minor*. Ann. Entomol. Soc. Amer. 63, 1187-1190.

7. Berryman, A.A. 1969. Response of *Abies grandis* to attack by *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. 101, 1033-1041.

8. Berryman, A.A. 1972. Resistance of conifers to invasion of bark beetle-fungus associations. BioScience. 22, 598-602.

9. Berryman, A.A. 1973. Population dynamics of the fir engraver *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. 105, 1465-1488.

10. Berryman, A.A. 1986. Forest insects: Principles and practice of population management. Plenum Press, New York.

11. Berryman, A.A.; Ashraf, M. 1970. Effect of *Abies grandis* resin on the attack behavior and brood survival of *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. 102, 1229-1236.

12. Borden, J.H. 1977. Behavioural responses of Coleoptera to pheromones, allomones and kairomones, in: Chemical control of insect behavior: Theory and application. Eds. H.H. Shorey and J.J. McKelvey Jr. Wiley. New York, New York. pp 169-198.

13. Borden, J.H. 1982. Aggregation pheromones, in: Bark Beetles in North American Conifers. Eds. J.B. Mitton and K.B. Sturgeon. University of Texas Press, Austin, TX pp 74-139.

14. Borden, J.H. 1989. Semiochemicals and bark beetle populations: exploitation of natural phenomena by pest management strategist. Holarctic Ecology. 12, 501-510.

15. Byers, J.A. 1988. Novel diffusion-dilution method for release of semiochemicals: Testing pheromone component ratios on western pine beetle. J. Chem. Ecol. 14, 199-212.

16. Byers, J.A.. 1989. Chemical ecology of bark beetles. Experientia. 45, 271-283.

17. Carde, R.T.; Baker, T.C. 1984. Sexual communication with pheromones, in: Chemical Ecology of Insects. Eds. W. J. Bell and R.T. Carde. Sinauer Assoc. Sunderland, Mass. pp 355-383.

18. Christiansen, E.; Waring, R.H.; Berryman, A.A. 1987. Resistance of conifers to bark beetle attack: Searching for general relationships. For. Ecol. Manage. 22, 89-106.

19. Cobb, J.W., Jr.; Wood D.L.; Stark, R.W.; Parmeter, J.R., Jr. 1968. IV. Theory on the relationships between oxidant injury and bark beetle infestation. Hilgardia. 39, 141-152.

20. Cook, S.B.; Hain, F.P. 1985. Qualitative examination of the hypersensitive response of loblolly pine, *Pinus taeda* L., inoculated with two fungal associates of the southern pine beetle, *Dendroctonus frontalis*. Environ. Entomol. 14, 396-400.

21. Cook, S.B.; Hain, F.P. 1986. Defensive mechanisms of loblolly and shortleaf pine against attack by the southern pine beetle, *Dendroctonus frontalis* Zimmerman, and its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. J. Chem. Ecol. 12, 1397-1406.

22. Cook, S.B.; Hain, F.P. 1988. Wound response of loblolly and shortleaf pine attacked or reattacked by *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae) or its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. Can. J. For. Res. 18, 33-37.

23. Cornelius, R.O. 1955. How forest pests upset management plans in the Douglas-fir region. J. For. 53, 711-713.

24. Coyne, J.F.; Lott, L.H. 1976. Toxicity of substances in pine oleoresin to southern pine beetle. J. Ga. Entomol. Soc. 11, 301-305.

25. Dethier, V. 1970. Chemical interactions between plants and insects, in: Chemical ecology. Eds. E. Sonderheimer and J.B. Simeone. Academic Press. New York, New York. pp 83-102.

26. Dickens, J.D.; Gutmann, A.; Payne, T.L.; Ryker, L.C.; Rudinsky, J.A. 1983. Antennal olfactory responsiveness of Douglas-fir beetle to pheromones and host odors. J. Chem. Ecol. 9, 1383-1395.

27. Dickens, J.C.; Payne, T.L. 1977. Bark beetle olfaction: pheromone receptor system in *Dendroctonus frontalis.* J. Insect. Physiol. 23, 481-489.

28. Dickens, J.C.; Payne, T.L.; Ryker, L.C.; Rudinsky, J.A. 1984. Single cell response of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae), to pheromones and host odors. J. Chem. Ecol. 10, 583-600.

29. Dodds, K.J.; Ross, D.W.; Daterman, G.E. 2000. A Comparison of trap trees for capturing Douglas-fir beetle, *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). Entomol. Soc. Brit. Columbia. 97, 33-38.

30. Dodds, K.J; Ross, D.W. 2002. Sampling range and range of attraction of Dendroctonus pseudotsugae pheromone-baited traps. Can Entomol. 134, 343-355.

31. Dudley, C.O. 1971. A sampling design for egg and first instar larval populations of the western pine beetle, *Dendroctonus brevicomis.* Can. Entomol. 103, 1291-1313.

32. Fargo, W.S.; Coulson, R.N.; Pulley, P.E.; Pope, D.N.; Kelley, C.L. 1978. Spatial and temporal patterns of within-tree colonization by *Dendroctonus frontalis* (Coleoptera: Scolytidae). Can Entomol. 110, 1213-1232.

33. Furniss, R.L. 1962. Infestation patterns of Douglas-fir beetles in standing and windthrown trees in southern Idaho. J. Econ. Entmol. 55, 486-491.

34. Furniss, R.L; Carolin, V.M. 1977. Western forest insects. USDA For. Serv. Miscell. Publ. 1339.

35. Furniss, M.M.; McGregor, M.D.; Foiles, M.W.; Partridge, A.D. 1979. Chronology and characteristics of a Douglas-fir beetle outbreak in northern Idaho. USDA For. Serv. Gen. Tech. Rep. INT-59.

36. Furniss, M.M.; Schmitz, R.F. 1971. Comparative attraction of Douglas-fir beetles to frontalin and tree volatiles. USDA For. Serv. Res. Paper INT-96.

37. Graham, K. 1959. Release by flight exercise of a chemotropic response from photopositive domination in a scolytid beetle. Nature. 184, 283-284.

38. Hain, F.P.; Cook, S.B.; Matson, P.A.; Wilson, K.G. 1985. Factors contributing to southern pine beetle host resistance, in: Proceedings, Integrated Pest Management Research Symposium, 15-18 April 1985. Eds. S.J. Branham and R.C. Thatcher. USDA For. Ser. Gen. Tech. Rep. SO-56. pp. 154-160.

39. Hanover, J.W.; Furniss, M.M. 1966. Monoterpene concentration in Douglas-fir in relation to geographic location and resistance to attack by the Douglas-fir beetle. USDA For. Serv. Res. Pap. NC- 6.

40. Hayes, J.L.; Ingram, L.L., Jr.; Strom, B.L.; Roton, L.M.; Boyette, M.W., Walsh, M.T. 1994a. Identification of a host compound and it's practical applications: 4-allylanisole as a bark beetle repellent, in: Southern station chemical sciences meeting proceedings, Starksville, MS, February 1-2, Ed. J. Vozzo.

41. Hayes, J.L.; Meeker, J.R.; Foltz, J.L.; Strom, B.L. 1996. Suppression of bark beetles and protection of pines in the urban environment: a case study. J. of Arboriculture. 22, 67-74.

42. Hayes, J.L.; Strom, B.L.; Roton, L.M.; Ingram, L.L., Jr. 1994b. Repellent properties of the host compound 4-allylanisole to the southern pine beetle. J. Chem. Ecol. 20, 1595-1615.

43. Hedden, R.L.; Gara, R.I. 1976. Spatial attack pattern of a western Washington Douglas-fir beetle population. For. Sci. 22, 100-102.

44. Hedden, R.L.; Pitman, G.B. 1978. Attack density regulation: a new approach to the use of pheromones in Douglas-fir beetle population management. J. Econ. Entomol. 71, 633-637.

45. Heikkenen, H.J.; Hrutfiord, B.F. 1965. *Dendroctonus pseudotsugae*: A hypothesis regarding its primary attractant. Science. 150, 1457-1459.

46. Hodges, J.D.; Elam, W.W.; Watson, W.F.; Nebeker, T.E. 1979. Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks. Can. Entomol. 111, 889-896.

47. Horntvedt, R.; Solheim, H. 1991. Pathogenicity of *Ophiostoma polonicum* to Norway spruce: the effect of isolate age and inoculum dose. Medd. Nor. Inst. Skogforsk. 44, 1-11.

48. Jantz, O.K.; Johnsey, R.L. 1964. Determination of sex of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hoptkins (Coleoptera: Scolytidae). Can. Entomol. 96, 1327-1329.

49. Jantz, O.K.; Rudinsky, J.A. 1966. Studies of the olfactory behavior of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. Oregon State Univ. Agric. Exp. Sta. Bull. 94, 1-38.

50. Johnson, N.E. 1967. The influence of temperature and moisture on the overwintering mortality of the Douglas-fir beetle, *Dendroctonus pseudotsugae* in western Washington (Coleoptera: Scolytidae). Ann. Entomol. Soc. Amer. 60, 199-204.

51. Johnson, N.E.; Belluschi, P.G. 1969. Host-finding behavior of the Douglas-fir beetle. J. For. 67, 290-295.

52. Kozlowski, T.T. 1969. Tree physiology and forest pests. J. For. 69, 118-123.

53. Lanier, G.N. 1983. Integration of visual stimuli, host oderants, and pheromones by bark beetles and weevils in locating and colonizing host trees, in: Herbivorous insects: host-seeking behavior and mechanisms. Ed. S. Ahmad. Academis Press Inc. New York, New York. pp 161-170.

54. McCowan, V.F.; Rudinsky, J.A. 1958. Biological studies on the Douglas-fir bark beetle, Millicoma Forest, Coos Bay, Oregon. Weyerhaeuser Timber Co., For. Res. Note 14, 21.

55. McMullen, .H.; Atkins, M.D. 1961. Intraspecific competition as a factor in the natural control of the Douglas-fir beetle. For. Sci. 7, 197-203.

56. McMullen, .H.; Atkins, M.D. 1962. On the flight and host selection of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae). Can. Entomol. 94, 1309-1325.

57. Miller, J.M. 1950. Resistance of pine hybrid to the pine reproduction weevil. USDA For. Serv..For. Res. Note 68, 17.

58. Miller, J.M.; Keen, F.P. 1960. Biology and control of the western pine beetle. Misc. Publ. USDA. No. 800. pp 381.

59. Mirov. N.T. 1961. Compositions of gum turpentines. Pacific Southwest Forest and Range Exp. Sta. USDA, For. Serv. Tech. Bull. 1239.

60. Moeck, H.A. 1978. Field test for the primary attraction of the spruce beetle. Environ. Can. For. Serv. Bi-mon. Res. Notes. 34, 8.

61. Moeck, H.A., Wood, D.L.; Lindahl, K.Q., Jr. 1981. Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. J. Chem Ecol. 7, 49-83.

62. Payne, T.L. 1975. Bark beetle olfaction. III. Antennal olfactory responses of *Dendroctonus frontalis* Zimm. and *D. brevicomis* LeConte (Coleoptera: Scolytidae) to aggregation pheromones and host tree hydrocarbons. J. Chem. Ecol. 1, 233-242.

63. Payne, T.L. 1979. Pheromone and host odor perception, in: Neurotoxicology of insecticides and pheromones. Ed. by T. Narahashi. Plenum Press. New York, New York. pp 27-58.

64. Payne, T.L. 1983. Nature of insect and host tree interactions. Z. ang. Ent. 96, 105-109.

65. Payne, T.L.; Dickens, J.C. 1976. Adaptation to determine receptor system specificity in insect olfactory communication. J. Insect. Physiol. 22, 1567-1572.

66. Pitman, G.B.; Vité, J.P. 1969. Aggregation behavior of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in response to chemical messengers. Can. Entomol. 101, 143-149.

67. Pitman, G.B.; Vité, J.P. 1970. Field Response of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) to synthetic frontalin. Ann. Ent. Soc. Am. 63, 661-664.

68. Prendergast, B.F. 1991. The chemical ecology of the eastern larch beetle, *Dendroctonus simplex* (Kirby). Ph.D. dissertation, State University of New York, Syracuse.

69. Raffa, K.F.; Berryman, A.A. 1980. Flight responses and host selection by bark beetles, in: Dispersal of forest insects: evaluation theory and management implications. Eds. Berryman A.A. and Safranyik, L. Proc. 2nd IUFRO Conf., Washington. Washington State University Co-op extens. Serv. pp 213-233.

70. Raffa, K.F.; Berryman, A.A. 1982. Accumulation of monoterpenes and associated volatiles following inoculation of grand fir with a fungus transmitted by the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. 114, 797-810.

71. Raffa, K.F.; Berryman, A.A. 1983a. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). Ecol. Monogr. 53, 27-49.

72. Raffa, K.F.; Berryman, A.A. 1983b. Physiological aspects of lodgepole pine wound response to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. Entomol. 115, 723-734.

73. Raffa, K.F.; Berryman, A.A.; Simaska, J.; Teal, W.; Wong, B.L. 1985. Effects of grand fir monoterpenes on the fir engraver, *Scolytus ventralis* (Coletoptera: Scolytidae) and its symbiotic fungus. Environ. Entomol. 14, 552-556.

74. Reed, A.N.; Hanover, J.W.; Furniss, M.M. 1986. Douglas-fir and western larch: chemical and physical properties in relation to Douglas-fir bark beetle attack. Tree Physiol. 1, 277-287.

75. Reid, R.W. 1963. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the East Kootenay region of British Columbia. III. Interaction between the beetle and its host, with emphasis on brood mortality and survival. Can. Entomol. 95, 225-238.

76. Reid. R.W.; Whitney, H.S.; Watson, J.A. 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Bot. 45, 1115-1126.

77. Ross, D.A. 1967. Wood- and bark-feeding Coleoptera of felled western larch in British Columbia. J. Entomol. Soc. Brit. Columbia. 64, 23-24.

78. Ross, D.W.; Daterman, G.E. 1994. Reduction of Douglas-fir beetle infestation of high-risk stands by antiaggregation pheromones. Can. J. For. Res. 24, 2184-2190.

79. Ross, D.W.; Daterman, G.E. 1995a. Efficacy of an antiaggregation pheromone for reducing Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae) infestation of high-risk stands. Can. Entomol. 127, 805-811.

80. Ross, D.W.; Daterman, G.E. 1995b. Response of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) to traps with different semiochemicals. J. Econ. Entomol. 88, 106-111.

81. Ross, D.W.; Daterman, G.E. 1997. Using pheromone-baited traps to control the amount and distribution of tree mortality during outbreaks of the Douglas-fir beetle. For. Sci. 43, 65-70.

82. Ross, D.W.; Gibson, K.E.; Daterman, G.E. 2001. Using MCH to protect trees and stands from Douglas-fir beetle infestation. USDA Forest Health Tech Enterprise Team Tech. Transfer 2001-09.

83. Rudinsky, J.A. 1963. Response of *Dendroctonus pseudotsugae* Hopkins to volatile attractants. Contr. Boyce Thompson Inst. 22, 23-38.

84. Rudinsky, J.A. 1966a. Host selection and invasion by the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, in coastal Douglas-fir forests. Can. Entomol. 98, 98-111.

85. Rudinsky, J.A. 1966b. Scolytid beetles associated with Douglas-fir response to terpenes. Science. 152, 218-219.

86. Rudinsky, J.A.; Vité, J.P. 1956. Effects of temperature upon the activity and behavior of the Douglas-fir beetle. For. Sci. 2, 258-267.

87. Ryker, L.C. 1984. Acoustic and chemical signals in the life cycle of a beetle. Sci. Am. 250, 112-123.

88. Schlyter, F.; Byers, J.A.; Lofqvist, J.A. 1987. Attraction to pheromone sources of different quantity, quality and spacing: Density regulation mechanisms in the bark beetle *Ips typographus*. J. Chem. Ecol. 13, 1503-1523.

89. Schmitz, R.F.; Gibson, K.E. 1996. Douglas-fir beetle. USDA For. Serv. Forest Insects and Dis. Leaflet 5.

90. Shrimpton, D.M.; Whitney, H.S. 1968. Inhibition of growth of blue stain fungi by wood extractives. Can. J. Bot. 46, 757-761.

91. Smith, R.H. 1963. Toxicity of resin vapors to three species of *Dendroctonus* bark beetles. J. Econ. Entomol. 56, 827-831.

92. Smith, R.H. 1964. Variation in the monterpenes of *Pinus ponderosa* Laws. Science. 143, 1337-1338.

93. Smith, R.H. 1965. Effect of monoterpene vapors on the western pine beetle. J. Econ. Entomol. 58, 509-510.

94. Smith, R.H. 1966a. Resin quality as a factor in the resistance of pines to bark beetles, in: Breeding pest-resistant trees. Eds. H.D. Gerhold; R.C. McDermott; E.J Schriner; and J.A Winieski. Pergamon. Oxford. pp 189-196.

95. Smith, R.H. 1966b. The monoterpene composition of *Pinus ponderosae* xylem resin and *Dendroctonus brevicomis* pitch tubes. For. Sci. 12, 63-68.

96. Smith, R.H. 1969. Local and regional variation in the monoterpenes of ponderosa pine xylem resin. USDA For. Ser. Res. Pap. PSW-56.

97. Smith, R.H. 1972. Xylem resin in the resistance of the *Pinacea* to bark beetles. USDA For. Ser. Gen. Tech. Rep. PSW-1.

98. Smith, R.H. 1977. Monoterpenes of ponderosa pine xylem in western United States. USDA Tech. Bull. No. 1532.

99. Solheim, H. 1988. Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway spruce. Medd Nor. Inst. Skogforsk. 40, 1-11.

100. Solheim, H. 1993. Ecological aspects of fungi associated with the spruce beetle *Ips typographus* in Norway, in: *Ceratocystis* and *Ophiostoma* taxonomy, ecology, and pathogenicity. Ed. M.J. Wingfield; K.A. Seifert; and J.F. Webber. American Phytopathological Soc. Press St. Paul, Minnesota pp 235-242.

101. Sturgeon, K.B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. Evolution. 33, 803-814.

102. Tunset, K.; Nilssen, A.C.; Anderson, J. 1993. Primary attraction in host recognition of coniferous bark beetles and bark weevils (Col.: Scolytidae and Curculionidae). Z. ang. Ent. 115, 155-169.

103. Vité , J.P.; Gara R.I. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). Contr. Boyce Thompson Inst. 21, 251-273.

104. Vité , J.P.; Pitman, G.B. 1968. Bark beetle aggregation: Effect of feeding on the release of pheromones in *Dendroctonus* and *Ips*. Nature 218, 169-170.

105. Vité , J.P.; Pitman, G.B. 1969. Insect and host odors in the aggregation of the western pine beetle. Can. Entomol. 101, 113-117.

106. Vité , J.P.; Rudinsly, J.A. 1962. Investigation on the resistance of conifers to bark beetle infestation. Proc. Intern. Congr. Entomol. 11th. Vienna, 1960. 2, 219-225.

107. Vité , J.P.; Wood, D.L. 1961. A study on the applicability of the measurement of oleoresin exudation pressure in determining susceptibility of second growth ponderosa pine to bark beetle infestations. Contr. Boyce Thompson Inst. 21, 67-78.

108. Werner, R.A. 1972a. Aggregation behavior of the beetle *Ips grandicollis*, in response to combinations of host-produced attractants. J. Insect. Physiol. 18, 423-437.

109. Werner, R.A. 1972b. Aggregation behavior of the beetle *Ips grandicollis*, in response to combinations of host and insect produced attractants. J. Insect. Physiol. 18, 1403-1412.

110. Werner, R.A. 1995. Toxicity and repellency of 4-allylanisole and monoterpenes from white spruce and tamarack to the spruce beetle and eastern larch beetle (Coleopter: Scolytidae). Environ. Entomol. 24, 372-379.

111. Whitney, H.S. 1982. Relationships between bark beetles and symbiotic organisms, in: Bark beetles in North American conifers. Ed J.B. Mitton and K.B. Sturgeon. University of Texan Press. Austin, TX: pp 183-211.

112. Wong, L.; Berryman, A.A. 1977. Host resistance to the fir engraver. 3. Lesion developments and containment of infection by resistant *Abies grandis* incolulated with *Tricosporidium symbioticum*. Can. J. Bot. 55, 2358-2365.

113. Wood, D.L. 1970. Pheromones of bark beetles, in: Control of insect behavior by natural products. Eds. D.L. Wood, R.M. Silverstein, and M. Nakajima. Academic Press, New York. pp 301-316.

114. Wood, D.L. 1972. Selection and colonization of ponderosa pine by bark beetles, in: Insect / plant relationships. Ed. H.F. van Emden, pp 101-117. Blackwell Sci. Publ. Oxford. pp215.

115. Wood, D.L. 1976. Host selection by bark beetles in the mixed-conifer forests of California, in: The host plant in relation to insect behavior and reproduction. Ed. by T. Jeremy. Plenum Press, New York. pp 301.

116. Wood, D.L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Ann. Rev. Entomol. 27, 411-446.

117. Wright, L.E.; Berryman, A.A.; Gurusiddaiah, S. 1979. Host resistance to the fir engraver beetle, *Scolytus ventralis* (Coleoptera: Scolytidae). Effect of defoliation on wound monoterpenes and inner bark carbohydrate concentrations. Can. Entomol. 111, 1255-1261.