CHEMICAL ECOLOGY OF *SCOLYTUS VENTRALIS* LECONTE AND *PITYOKTEINES ELEGANS* (SWAINE), TWO BARK BEETLES OF *ABIES GRANDIS* (DOUGL.) LINDL.

by

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ABSTRACT

In laboratory bioassays, Porapak Q-captured and steam-distilled volatiles from the bark of host trees, *Abies grandis* (Dougl.) Lindl., particularly from root-rot infected trees, attracted 50-70 % of male and female fir engravers, *Scolytus ventralis* LeConte. Gas chromatographic-electroantennographic detection (GC-EAD) analyses of Porapak Q-captured bark volatiles revealed 19 EAD-active compounds of which 13 (mostly monoterpenes) were identified by GC-mass spectrometry (GC-MS). Multiple-funnel traps baited with blends of these 13 volatiles released at 280 and 340 mg per 24 h, respectively, attracted 66 and 93 % of the total *S. ventralis* captured. The clerid predator, *Thanasimus undulatus* Say, also responded strongly to the kairomonal volatiles. Additional experiments (laboratory bioassays, GC and GC-EAD analyses of volatiles from infested logs and trees undergoing mass attack, female accessory gland extracts, volatiles from emerged, attacking and juvenile hormone-treated beetles, and videotape analysis of the behavior of attacking beetles failed to disclose evidence for aggregation pheromones. I conclude that the attack dynamics of *S. ventralis* can be explained solely by its primary attraction response to host volatiles. In laboratory bioassays, *Pityokteines elegans* (Swaine) were attracted to volatiles captured from bolts of grand fir colonized by *P. elegans* males. Male-specific volatiles detected by GC-EAD analysis and by GC-MS employing a chiral column were: (*S*)-(-) ipsenol, (+)- and (-)-ipsdienol and ipsenone. Combinations of (-)-ipsenol and (±)-ipsdienol (1:1) strongly attracted both sexes to multiple-funnel traps. No beetles were attracted to any of these compounds alone, and both enantiomers of ipsdienol were required with (-)-ipsenol to induce attraction. Specificity of semiochemical-based communication between sympatric species *P. elegans* and *Pityokteines minutus* Swaine appears to be based on host preference and on the composition and chirality of the pheromone blend. Biological observations showed that *S. ventralis* and *P. elegans* have different but overlapping ecological niches. The possibility of interspecific interference competition was ruled out by evidence that response to attractive semiochemicals and attack on new hosts was not adversely affected by exposure to

semiochemicals used by the other species. Therefore, exploitation competition must be the sole determinant of the negative interspecific relationships observed in hosts attacked by both species.

DEDICATION

To Laura, my companion, for her love and endless support in my life.

To Carlos and Gabriela who made my life complete.

To my parents for the opportunities they gave me.

To friendship for its happiness and joy of life.

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1 INTRODUCTION

1.1 Biology and ecology *Scolytus ventralis*

The fir engraver, *Scolytus ventralis* LeConte (Coleoptera: Scolytidae), is a major cause of chronic death and acute mortality of true firs, especially white fir, *A. concolor* (Gord. & Glend.) Hildebr. and grand fir, *A. grandis* (Dougl.) Lindl., in North America. Outbreaks of this bark beetle have occurred at irregular intervals and have been reported at least once a decade over the last 60 years (Ferrell, 1986; Wood and van Sickle, 1991). In the last decade, fir engravers have killed hundreds of thousands of grand and white fir in western North America (Wood and van Sickle, 1991; Campbell and Liegel, 1996; unpublished records from USDA, Forest Service, Northern Region). The fir engraver also infests red fir, *Abies magnifica* A. Murr. Subalpine fir, *Abies lasiocarpa* (Hook.) Nutt., Douglas-fir, *Pseudotsugae menziesii* (Mirb.) Franco, Engelmann spruce, *Picea engelmannii* Engelm., mountain hemlock, *Tsuga mertensiana* (Bong.) Sarg., and western larch, *Larix occidentalis* Nutt., have been reported as occasional hosts (Struble, 1957; Chamberlin, 1958).

Adult fir engravers emerge from infested trees and fly in search of new hosts from June to September with a flight peak in the middle of June. Flight and attack generally occur from 1400-1700 h at temperatures of 24 °C or higher. Females are the pioneer sex, and after mating a single female constructs two horizontal egg galleries extending laterally from the nuptial chamber. Eggs are laid in niches cut in the gallery wall. Larvae feed by mining the inner bark, and the galleries generally extend at right angles from the parental gallery. Both the parental galleries and the larval mines etch the sapwood, hence the name fir engraver. Six-larval instars occur in a period of about two months at room temperature. Complete development from egg to adult usually takes one year in Northern Idaho. In high altitudes, the fir engraver requires two years to complete a generation (Struble, 1957; Stark and Borden, 1965; Berryman, 1968a, 1968b, 1968c, 1969, 1972, 1973; Ashraf and Berryman, 1969; Berryman and Ferrell, 1988).

At the beginning of the flight period, fir engraver females attack living hosts in a more or less random pattern and apparently cannot distinguish between trees that will be a suitable substrate for brood development and those that will resist attack (Berryman and Ashraf, 1970). On resistant trees, however, attacking females are soon repelled by resin and aggregation then ceases. The degree of aggregation on an individual tree seems to vary with the time that females are able to remain in the tree and is reflected by the length of their egg galleries (Berryman, 1968a; Berryman and Ferrell, 1988). Grand fir logs bearing some *S. ventralis* attacks attract a larger portion of the flying population than nearby unattacked logs, and aggregation occurs earlier and reaches higher densities (Ashraf and Berryman, 1969), suggesting the existence of an aggregation pheromone. Attraction persists for at least two weeks, and termination usually coincides with the decline in the number of beetles flying in the area (Ferrell, 1971). The declining attraction to infested trees progresses steadily with no apparently abrupt "shut-off" mechanism as in other bark beetles (Raffa and Berryman, 1987).

The fir engraver exploits weakened, dying, or recently killed fir trees, which form a temporary and scarce resource in the sense that food available at one time does not necessarily remain available in the future (Berryman and Ferrell, 1988). Its attack habits are exceptional among tree-killing scolytids (Wood D. L., 1982), because of the capability to build successful individual maternal galleries without causing the death of the host (Struble, 1957; Ferrell, 1973 and 1974).

Based on detailed life-table studies over a five-year period, Berryman and Ferrell (1988) proposed the following hypothesis for the numerical behavior of *S. ventralis* populations. The primary determinant of fir engraver abundance is the quantity of food, which is set by (1) the size of susceptible trees, and (2) the density of susceptible trees per unit area of forest as affected by tree characteristics (genetics, age) and environmental factors influencing tree vigor (e.g., diseases, insects, precipitation). The fundamental mechanism regulating the size of the fir engraver population is intraspecific competition operating as a negative feedback process. At suboutbreak levels, competition for limited food acts as a "perfect" or instantaneous density-dependent mortality factor. When food limitations are relaxed the population becomes dispersed,

competition is alleviated, and the population grows. As the outbreak progresses and the food supply is utilized, or the trees recover their resistance, intraspecific competition intensifies and the population declines to a level at which a steady relationship exists between the competitive use of the resource and low-level replacement of susceptible hosts (Berryman and Ferrel, 1988).

A number of secondary bark beetles are commonly observed feeding in trees colonized by the fir engraver. Of these, several species feed in the phloem of the bole and compete with the fir engraver broods for this limited food supply. High *S. ventralis* brood mortality has been observed under bark containing the galleries of *Pityophthorus pseudotsugae* Swaine and *Pityokteines elegans* Swaine, and when > 50 % of the phloem is used by the other insects (Stark and Borden, 1965; Berryman, 1968, 1973). Other bark beetles such as *Pseudohylesinus grandis* Swaine, *P. granulatus* LeConte, *Scolytus opacus* Blackman, *S. praeceps* LeConte, *S. subscaber* LeConte and *Crypturgus borealis* LeConte are common associates of the fir engraver in white and grand fir (Struble, 1957; Ashraf and Berryman, 1969).

At least 10 species of insect parasitoids have been reared from logs infested by the fir engraver. Of these, seven wasps in the families Braconidae, Pteromalidae, Torymidae, and Ichneumonidae attack the larval stages and one phorid fly (Diptera: Phoridae) attacks the adult beetle (Ashraf and Berryman, 1969). The parasitic nematode, *Sulphuretylenchus elongatus* (Massey) Nickle, infects 45-93 % of emerging adults and is known to impair flight, gallery construction, and oviposition potential in infected beetles (Ashraf, 1968; Ashraf and Berryman, 1970).

Enoclerus lecontei (Wolcott), *E. sphegeus* F. and *Thanasimus undatulus* Say (Coleoptera: Cleridae), *Temnochila virescens* var. *chlorodia* (Mann.) (Coleoptera: Trogositidae) and *Medetera aldrichiii* Wheeler (Dipetra: Dolichopodidae) have all been reported as predators of the fir engraver (Struble, 1957; Ashraf and Berryman, 1969). Numerous predators have been attracted to the pheromones of bark beetles (Borden, 1982; Miller et al., 1991), but semiochemical-mediated association of predators with *S. ventralis* has not been investigated. *Thanasimus undatulus* has been attracted in large numbers to frontalin-baited spruce trees (Dyer, 1975) or traps (Kline et al. ,1974). *Enoclerus lecontei* was trapped in California to mixture of

ipsenol, ipsdienol and *cis*-verbenol (Wood et al. 1968), and *E. sphegeus* was attracted to *exo*-brevicomin in subalpine fir and Engelmann spruce forests in British Columbia (Borden et al., 1987).

The fir engraver has a strict mutualistic association with the phytopathogenic fungus, *Trichosporium symbionticum* Wright, that is apparently essential for successful reproduction (Wright 1935; Livingston 1971; Wong and Berryman 1977). This fungus is transported in mycangial pits on the head of both male and female beetles. *Trichosporium symbionticum* is always observed growing in advance of developing larvae and is probably involved in the death and dying of phloem tissue prior to larval feeding. The action of the fungus on host tissue causes a rapid embolism and disruption of water and nutrient transport in the outer xylem and phloem (Berryman and Ferrell, 1988).

1.2 Biology and ecology of *Pityokteines elegans*

The bark beetle, *Pityokteines elegans* (Swaine) (Coleoptera: Scolytidae), colonizes grand fir, red fir, white fir, and possibly Douglas-fir (Bright and Stark, 1973; Bright, 1976; S. L. Wood, 1982). The biology of *P. elegans* is unknown, but it is suspected to be similar to that of related species such as *P. sparsus* LeConte in balsam fir, *A. balsamea* (L.) Mill. and *P. minutus* Swaine in subalpine fir (Bright, 1976; S. L. Wood, 1982).

All *Pityokteines* spp. are polygamous (S. L. Wood, 1982). Males are the pioneer sex (Chararas, 1962; Dajoz, 1980; D. L. Wood, 1982) and both sexes build a star-shaped gallery with four to six or more egg galleries radiating from the nuptial chamber (Bright, 1976; S. L. Wood, 1982). The insects live in the phloem tissue of cut, fallen, or dying trees (Belyea, 1952; Chararas, 1962; Bright, 1976; Hosking and Knight, 1976; S. L. Wood, 1982). *Pityokteines elegans*, *P. sparsus*, *P. minutus* and *P. lasiocarpi* (Swaine) are reportedly never found in sound true firs or in the absence of other bark beetles, suggesting that they are secondary colonizers (Belyea, 1952; Hertert et al., 1975; Hosking and Knight, 1976; Basham, 1986; Furniss and Carolin, 1992; R. L. Livingston, pers. comm., Insects and Diseases Section, Idaho Department of Lands, Coeur

d'Alene, Idaho). *Pityokteines elegans* usually inhabits trees weakened by root diseases and other insects, notably the fir engraver, *Scolytus ventralis* LeConte (Hertert et al., 1975; S. L. Wood, 1982).

1.3 Biology and ecology of *Abies grandis*

Grand fir is a major timber resource with more than 38 bill. m^3 growing in British Columbia (Cochran, 1979; Franklin, 1981). In dry climates, fire control programs encourage the growth of true firs and other shade tolerant species; thus the prevalence of grand fir may increase in the future (Hall, 1981; Franklin, 1981). In addition, silviculture is becoming more sophisticated, the use of species mixtures is increasing, and firs will be frequent candidates for silvicultural management (Franklin, 1981).

Grand fir is a stately tree with a wide crown. Tree heights at maturity generally range from 43 - 61 m along the coast and from 10 - 50 m inland (Foiles et al., 1990; Hall,1984). The species is not long-lived but frequently reaches an age of 250 years and occasionally exceeds 300 years (Amo and Hammerly, 1977; Foiles et al., 1990). The trees are densely branched to the ground with sweeping flat branches. The long, blunt-tipped needles are dark green on the top and whitish underneath. They are arranged in two rows on opposite sides of the twigs and spread out flat on the lower boughs. Near the top of the tree, the needles curve upward (Amo and Hammerly, 1977).The bark is grayish to light brown, smooth or shallowly ridged and with flakes on mature trees (Hitchcock et al., 1959). Cone and seed production begin at about 20 years of age and increase with age and vigor. Pollen and ovulate cones begin development during the summer and go through a winter dormancy before pollination, fertilization, and seed production the second spring and summer (Singh and Owens, 1982).

Grand fir grows well on stream bottoms, valleys and on mountain slopes up to about 1675 m. It can be found up to 1830 m in Northern Idaho (Amo and Hammerly, 1977; Foiles et al., 1990). On the Pacific Coast, its range extends from southern British Columbia south to Sonoma County in Northwest California. It is found in the Coast Mountains, in the adjacent lowlands and

valleys, and in the Cascade Mountains as far as central Oregon. In the Continental Interior, its range includes the Okanagan and Kootenay Lakes areas of British Columbia and extends south in eastern Washington, northern and Central Idaho, Montana west of the Continental Divide, and the Blue and Ochoco Mountains in Oregon (Foiles et al., 1990; Van Pelt, 1991).

In Northern Idaho and western Montana, grand fir is the climax dominant on sites that are cooler and moister than sites with Douglas-fir or subalpine fir as the climax species (Pfister et al., 1977; Cooper et al., 1987). Floristically, climax grand fir stands are extremely diverse (Cooper et al., 1987; Foiles et al., 1990), and species such as ponderosa pine, *Pinus ponderosa* Dougl., western larch, *Larix occidentalis* Nutt., Douglas-fir, western white pine, *Pinus monticola* Dougl., and lodgepole pine, *P. contorta* Dougl. are dominant seral species (Dittberner and Olson, 1983; Hall, 1982). In northern Idaho and eastern Washington, dense seral shrub communities such as snowbrush ceanothus, *Ceanothus velutinus* Dougl., Scouler's willow, *Salix scoulariana* Barratt, Saskatoon serviceberry, *Amelanchier alnifolia* Nutt., and elderberry, *Sambucus* spp. may dominate in grand fir habitat type for many years (Daubenmier and Daubenmeir, 1968; Franklin and Dymess, 1973).

It has been shown that in such conifer genera as *Abies, Tsuga, Cedrus* and *Pseudolarix*, vertical resin ducts are absent from the secondary xylem, except at injuries where they occur in tangential series (Bannan, 1936). In the Abieteae the ducts are sac-like and are either isolated or branched and fused with others in the series to form an anastomosing network of cavities (Bannan, 1936). The formation of a secondary periderm beneath the blisters (sac-like ducts) isolates them from the cortex and later they are sloughed off with rhytidome formation (Littlefield, 1973).

Like most coniferous trees, true firs have two defenses against bark beetles and their associated fungi (Berryman, 1972). The first line of defense, the static or preformed resistance mechanism, is weak in true firs, consisting of thin resin ducts and blisters in the cortex of the bark. The second line of defense is a dynamic induced response, sometimes referred to as a hypersensitive reaction, that occurs after the beetle and fungus have invaded the tree (Berryman, 1969,1972). Although lesions induced by mechanical injury and those induced by a beetle-fungus

complex are morphological similar, the presence of the fungus results in a much more extensive reaction, involving the production of different defensive chemicals (Russell and Berryman, 1976), the resinosis and death of phloem tissue, and the formation of traumatic resin cavities in the outer sapwood at the edge of the lesion area (Berryman and Ferrell, 1988).

Phloem resinosis occurs in advance of fungal spread and is not caused directly by actual hyphal penetration (Wong and Berryman, 1977). The effects of the hypersensitive reaction on the insect are twofold. First, the metabolites of active cellular degeneration appear to be produced under pressure and therefore to flow into the insect's tunnel (Berryman, 1969). The monoterpenes found in the reaction area are highly repellent to the beetle, so attack is usually abandoned when this occurs (Berryman and Ashraf, 1970; Bordash and Berryman, 1977; Ferrell, 1978). Second, the resin-soaked tissues of the reaction zone are completely unsuitable for survival of bark beetles and larvae (Berryman and Ashraf, 1970).

1.4 Thesis Objectives

There are a number of unresolved questions regarding the biology of *S. ventralis* and *P. elegans* and the interactions between them. In particular, the role of semiochemicals in mediating mass attack by *S. ventralis* has not been definitively investigated, and no chemical ecological studies have been done on *P. elegans*. In addition, although *P. elegans* is hypothesized to be a significant competitor of *S. ventralis* little has been done on the interactions between these two species. Therefore my objectives were: 1) to isolate and identify the semiochemicals that mediate host selection and mass attack by *S. ventralis*; 2) to elucidate the biology and semiochemical-based communication in *P. elegans*; and 3) to describe any semiochemical-based and ecological interactions between *S. ventralis and P. elegans*.

2. PRIMARY ATTRACTION IN *S. ventralis*

2.1 Introduction

Bark beetles must locate and detect not only the right host species, but also the most susceptible trees within the host population (Raffa and Berryman, 1987). There is conflicting evidence as to whether bark beetles land on potential hosts at random, making a decision on host suitability at close range, or whether they orient toward host volatiles (primary attraction). For most species, it is widely accepted that after pioneer beetles have initiated attack the majority of the population orients to the host in response to secondary attractants, usually a blend of pheromones released by conspecifics and kairomones released by the tree (Wood, D. L., 1982; Birch, 1984; Borden, 1985).

Volatiles released by the host are attractive to a number of subcortical scolytids, including species in the genera *Dendroctonus, Hylastes, Hylurgops, Hylurgopinus, Ips, Pityogenes, Pseudhohylesinus, Scolytus, Tomicus and Trypodendron* (Goeden and Norris, 1964; Rudinsky, 1966a, b; Meyer and Norris, 1967; Moeck, 1970; Moeck et al., 1981; Byers et al., 1985 and 1990; Miller et al., 1986; Lanne et al., 1987; Swedenborg et al., 1988; Voltz, 1988; Byers, 1989; Moeck and Simmons, 1991; Lindelöw et al., 1992; Hobson et al., 1993; Tunset et al., 1993). Oleoresins are the main sources of these attractive compounds, and their terpenoid constituents, primarily monoterpenes and sesquiterpenes, have proved to be active as kairomones. Monoterpenes such as α-pinene, myrcene, terpinolene, β-pinene, β-phellandrene, and 3-carene, as well as sesquiterpenes like α-atlantone, α-cubebene and cadinene (Table 1) appear in the literature as attractants for bark beetles (Chararas, 1980; Byers et al., 1985; Millar et al., 1986; Philips et al., 1988; Schroeder, 1988, Siegfried, 1987; Chénier and Philogéne, 1989; Schroeder and Lindelöw, 1989; Miller and Borden, 1990b; Phillips, 1990; Byers, 1992; Hobson et al., 1993). Synergistic effects on attraction often occur when terpenes are combined with the host kairomone ethanol or with insect-produced pheromones (Borden, 1985).

Some field observations appear to indicate that *S. ventralis* selects its host through a process of random landing on both resistant and susceptible trees (Struble 1957; Ashraf and

Table 1.Terpenoids (monoterpenes and sesquiterpenes) and the behavior they elicit in several scolytid species. Note that some insect behaviours change when the terpenoids are combined. Symbols are as follows: 1, attraction alone; 2, attraction alone or in combination; 3, attractive when combined; 4, decreases pheromone activity; 5, synergistic with pheromone.

INSECT SPECIES	α– pinene	camphene	β– pinene	myrcene	3- carene	limonene	β– phellandrene	terpinolene	α– cubebene	REFERENCES
Ips pini			4				1			Miller and Borden, 1990b
I. latidens	4	4		4	4		4	4		Miller and Borden, 1990a
I. grandicollis	1	1		1		1				Werner, 1972
Dendroctonus valens	2		2	1	2					Hobson et al., 1993
D. brevicomis				5						Bedard et al., 1969
D. frontalis	5									Borden, 1985
D. ponderosae	5	5		5	5		1	5		Borden, 1985
D. rufipennis		5								Borden, 1985
D. pseudotsugae	3	3								Furniss and Schmitz, 1971
Scolytus multistriatus									5	Lanier et al., 1976
S. scolytus									5	Blight et al., 1978
Tomicus piniperda	2				2			2		Byers et al., 1985
Gnathotrichus spp	5	5	5							Byers et al., 1990

Berryman, 1969, Berryman and Ashraf, 1970). However, a high correlation between root rot infections and successful fir engraver attacks has been repeatedly reported (Cobb et al., 1973; Hertert et al., 1975; Ferrell and Smith, 1976; Wright et al., 1984), which suggests that the insect can detect root rot-infected trees, possibly in response to an altered semiochemical profile. There is a growing body of evidence that indicates that diseased and healthy conifers can be detected by their volatile profiles. White fir trees that survived fir engraver attacks in Lake Tahoe, California, had a different monoterpene composition than trees that were killed (G. T. Ferrell, USDA Forest Service, Redding, California, pers. comm.). Concentrations of five monoterpenes, tricyclene, α-pinene, camphene, γ-terpinene, and bornyl acetate were significantly higher in lodgepole pine, *Pinus contorta*, infected by one or more diseases (dwarf mistletoe, comandra blister rust, and Armillaria root rot) than in healthy ones (Nebeker et al., 1995). Similarly, spruces, *Picea excelsa* Lk., infected with Armillaria root rot, contained increased amounts of oils (Madziara-Borusiewicz and Strzelecka, 1977). Moreover, needles of experimentally drought-stressed Norway spruces, *Picea abies* (L.), had a higher total monoterpene content and greater amounts of tricyclene, α-pinene, and camphene than control trees (Kainulainen et al., 1993).

In the last three decades several attempts have been made to find evidence for primary and secondary attraction of the fir engraver. Vité and Pitman (1967) reported that *S. ventralis* and *S. unispinosus* LeConte respond to host odors in field trials and suggested that an insect-produced attractant was not indicated. Ferrell's (1969, 1971) field experiments showed that the fir engraver can land on different species but will land preferentially on its host, white fir. Fir engravers were trapped twice as frequently on girdled trees as on ungirdled controls. Similar results were obtained comparing the responses to severed and uninjured standing trees. However, these experiments could not attribute orientation to either primary or secondary attraction, because test trees were not protected from insect attacks and thus secondary attraction was not prevented. Results from laboratory bioassays showed that both male and female *S. ventralis* were highly attracted to aged host phloem and less so to frass produced by virgin females (Ferrell, 1969).

Fir engravers exposed to constitutive grand fir oleoresin or to its monoterpene vapors (individually presented) died at significant rates within 4-12 h after exposure (Ferrell, 1969; Raffa et al., 1985). The monoterpenes tricyclene, α-pinene, β-pinene, camphene, myrcene, sabinene, limonene, β-phellandrene, bornyl acetate and terpinolene were present in the constitutive resin. The composition of traumatic resin induced by wounding was similar, except for the addition of Δ^3-carene, the absence of bornyl acetate and a significant increase in the quantities of β-pinene and myrcene (Russell and Berryman, 1976; Raffa and Berryman, 1987, Lewinsohn et al., 1990). Each of these compounds was repellent in a dose-dependent manner to walking beetles in laboratory olfactometer bioassays (Bordash and Berryman, 1977). Myrcene, in particular, has been reported to have different biological effects on bark beetles and their symbiotic fungi, e.g. inhibition of the growth of *Ceratocystis minor* (Hedgecock) Hunt (Cobb et al., 1968), attraction of *Pseudohylesinus grandis* Swaine, an associate of the fir engraver (Rudinsky, 1966b), and a synergistic effect on attraction to the pheromone of the western pine beetle, *Dendroctonus brevicomis* LeConte (Bedard et al., 1969). Growth of *T. symbionticum* was inhibited by camphene, β-pinene, myrcene, Δ^3-carene and limonene (Wong and Berryman, 1977; Raffa et al., 1993).

Possible evidence for secondary attraction in *S. ventralis* was found by Ashraf and Berryman (1969). In field experiments, they observed that grand fir logs previously attacked by the fir engraver attracted many more flying conspecific beetles than uninfested control logs. Ethanolic extracts of *S. ventralis* frass were strongly attractive to beetles in the field. However, there were only two replicates, the frass-producing sex was not reported, and the experiment did not include a control for ethanol, a known semiochemical for other bark beetles (Pitman *et al.*, 1975; Moeck, 1970). However, Ferrell (1969) found that *S. ventralis* were not arrested by ethanol in laboratory bioassays. As the season progressed, attacks by *S. ventralis* became increasingly aggregated, but because attack density is directly related to gallery elongation (Ashraf and Berryman, 1969), an attractant could either be released by the beetle or by the host tree.

In a 1968 study by G. T. Ferrell and J. H. Borden (unpublished) laboratory bioassays revealed that virgin female frass and fresh grand fir phloem sawdust arrested equal numbers of *S. ventralis* at high doses, but when the quantities tested were progressively decreased, positive response to the frass disappeared before the response to sawdust. Frass produced by virgin males or females was equally attractive. Grand fir phloem disks containing a mining female remained highly attractive for hours, while disks lacking a beetle rapidly lost potency. Fecal pellets separated from virgin female frass proved no more attractive on an equal weight basis than whole frass. The above results support the hypothesis of primary attraction for *S. ventralis*, but do not rule out the possibility of a pheromone.

Secondary attraction does occur in the genus *Scolytus*. In particular, the smaller European elm bark beetle, *S. multistriatus* Marsham, produces and responds to the pheromones 4-methyl-3-heptanol and multistriatin (2,4-dimethyl-5-ethyl-6,8-dioxabicyclo[3.2.1]octane) in combination with the sesquiterpene α-cubebene; the large elm bark beetle, *S. scolytus* F. utilizes only 4-methyl-3-heptanol and α-cubebene (Lanier et al., 1977; Blight et al., 1978). Field tests with these three components have also caught *S. pygmaeus* F. and *S. laevis* Chapuis (Minks and Van Deventer, 1978; Bejer, 1979), suggesting that the same compounds are involved in secondary attraction for these beetles. For *S. quadrispinosus* Say (Goeden and Norris, 1964), *S. numidicus* Brisout (Chararas, 1980), and *S. rugulosus* Ratzeburg (Kovach and Gorsuch, 1985) there is evidence only for primary attraction.

In this chapter, I report the results of laboratory and field experiments supporting the hypothesis that primary attraction occurs in *S. ventralis* and elucidating the kairomones involved. I argue against the hypothesis that *S. ventralis* requires secondary attraction for successful host selection.

2.2 Methods and Materials[1]

2.2.1 Collection of Insects and Host Material

Bolts of grand fir from healthy and root rot-infected trees as well as from trees infested with *S. ventralis*, were collected in August and September 1993 - 1995, from felled trees near Coeur d'Alene, Idaho. Root rot-infected trees were characterized by the thinning of their crowns and by finding decay on the dissected root system (Hagle et al., 1987). All logs were kept at 2 °C until used. Infested logs were transferred in mesh screen cages held at 24-30 °C; water was sprayed on them every 5 - 6 days to prevent desiccation. Emerged beetles were collected daily, and sexed by comparing morphological characteristics of the abdominal stemites (Blackman, 1934; Edson, 1967; Bright, 1976; S. L. Wood, 1982) and the frons (Edson, 1967; Ashraf and Berryman, 1969)(see also appendix 1).

2.2.2 Collection and Analysis of Beetle and Host Volatiles

Volatiles from logs were obtained by drilling "entrance holes" (1.5 mm diam.), approximately 3 cm apart, in the bark of fresh grand fir bolts ca. 21 cm long and 12 cm diam. These bolts were set inside separate glass aeration chambers (28 cm long and 15 cm diam.). Either 130 males or 130 females were then allowed to bore into the bark, or the log remained without beetles as an uninfested control. Air was drawn through the chamber at 1.7 L per min, and then through a glass trap (14 mm O.D., 20 cm long) containing Porapak-Q (Byrne et al., 1975). Volatiles were eluted from the trap with 150 ml of distilled pentane, and the eluent concentrated to 5 ml by distilling off the pentane through a 30 cm Dufton column.

Differential diagnosis (Vité and Renwick, 1970) of male- and female-produced volatiles was used to search for sex-specific compounds. GC analyses employed Hewlett Packard 5830A, 5880A, and 5890A instruments equipped with capillary inlet systems, and FID. Capillary columns (30 m x 0.25 or 0.32 mm I.D.) coated with SP-1000 (Supelco, Bellefonte, Pennsylvania) or DB-1

[1] Volatile capture, extraction and macro-fractionation in this and the following chapters were performed by D. H. D. Pierce, Jr., Department of Chemistry, Simon Fraser University. Coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis, micro-fractionation and GC-mass spectrometry were done by Ms. Regina Gries, Department of Biological Sciences, Simon Fraser University.

(J & W Scientific Inc., Folsom, California) were used. A Varian Satum ion trap GC-MS fitted with a DB-23 column was employed for coupled GC-mass spectrometry (MS). Helium was the carrier gas for the GC and GC-MS.

2.2.3 Isolation of Bark Oil

Bark tissue (cortex plus phloem) was peeled from fresh logs of either healthy or root rot-infected grand fir and cut into small chips (ca. 1 cm^2). Bark oil was obtained by steam distillation procedures, yielding two products (BO1 and BO2). In the first procedure, a concurrent steam distillation-continuous extraction still head (Flath and Forrey, 1977) was employed for the isolation of volatile oil from bark chips. The steam distillation was conducted for 4 h after boil-up and pentane was used as the extraction solvent. After evaporation of most of the pentane under a stream of nitrogen, residual solvent was removed by brief vacuum pumping. The second procedure was conducted under the supervision of Dr. D. Jones, Pacific Cedar Industries, Langley, B.C. It employed a steel retort with a water condenser. The apparatus had a drum with a ca. 60 kg capacity, and two batches of fresh grand fir peeled bark were processed under ca. 2 psi of pressure at 110 - 120 °C for 1 h, each. The bulk of water of the condensate was removed by siphon. The remaining oil and water were transferred to separatory funnel and the lower aqueous layer removed. The oil was dried (Na_2SO_4) and decanted from the drying agent. The yield of crude oil was ca. 0.1 %. GC analysis of BO1 and BO2 showed that both bark oils were qualitatively and quantitatively similar. Both were used without further purification in field experiments.

2.2.4 Fractionation of Bark Oil

A Varian 1200 gas chomatograph (GC) equipped with 10:1 effluent splitter and thermal gradient collector (Brownlee and Silverstein, 1968) was employed for micropreparative fractionation of *A. grandis* bark oil. The column was a stainless steel tube (3.05 m x 3.18 mm

O.D.) packed with 10 % SP-1000 on Supelcoport (100/120 mesh) (Supelco, Inc. Bellefonte, PA). The temperature program was 70 °C for 2 min, then 4 °C/min to 180 °C holding for 20 min. The injection port and flame-ionization detector (FID) temperatures were 260 °C and 270 °C, respectively, and helium was the carrier gas. Typically, 1.5 µl aliquots of oil were used per run, and fractions were rinsed from the collection tubes with pentane into 1 ml volumetric tubes which were made up to volume. A Hewlett Packard 5830 GC fitted with a glass column (30 m x 0.50 mm I.D.) coated with SP-1000, and flame-ionization detector (FID), was employed for determination of components in the fractions by the external standard method. The temperatures and carrier gas were as above. The FID was calibrated by analyzing a solution containing a known concentration of bark oil. Fraction 1 contained monoterpenes (up to a retention time slightly beyond that of β-phellandrene), and Fraction 2 contained all the rest, mostly sesquiterpenes. A subsequent three-part fractionation of each of the two fractions was done under the same conditions. Table 2 shows the major chemical components of each subfraction.

2.2.5 GC-EAD Analysis

Extracts and oils obtained by steam distillation and by laboratory and field aerations were subjected to coupled gas chomatographic-electroantennographic detection (GC-EAD) analyses (Arn et al., 1975) adapted for intact bark beetles (Gries, 1995) or excised bark beetle antennae. Analyses by GC employed a Hewlett Packard 5890 A instrument equipped with a DB-23-coated fused silica column (30 m x 0.32 mm ID; J & W Scientific, Folson, CA). Responses of excised antennae were amplified utilizing a custom-built amplifier with a passive low pass filter and a cutoff frequency of 10 kHz. Compound identities were confirmed by comparison of their mass spectra and retention times with those of authentic samples.

Analysis of the chirality of α- and β-pinene, camphene, and limonene was performed as follows: ca. 10 µg of grand fir steam-distilled oil were injected twice under split conditions into a Varian 3400 GC remodified according to Brownlee and Silverstein (1968) to a preparative GC.

Table 2. Terpenoids present in fractions of steam-distilled bark oil (BO1) tested in laboratory bioassays for attraction of female *S. ventralis*.

	Occurrence (+) by fraction					
Compound	$F1_1$	$F1_2$	$F1_3$	$F2_1$	$F2_2$	$F2_3$
α-pinene	+					
camphene	+					
β-pinene		+				
myrcene		+				
β-phellandrene			+			
limonene			+			
α-terpinolene			+			
bornyl acetate				+	tr	
longifolene				+		
borneol					+	
(*E*)-nerolidol						+
methyl isoeugenol						+

tr = trace

The GC was equipped with a DB-23 column (30 m x 0.32 mm ID; J & W Scientific, Folsom CA; GC temperature program: 40 °C hold for 5 min, then program 5 °C/min up to 200 °C. The injector was set to 240 °C and the auxiliary heater for the preparative unit at 250 °C. At these conditions α-pinene eluted at 3.6, camphene at 4.7, β-pinene at 5.7 and limonene at 7.8 min, and these were condensed in glass tubes (25 cm long and 1 mm ID) which were then rinsed with 25 µl of hexane into a 1.5 ml vial. The 2 collections were combined to give a total of 50 µl and the samples of camphene and limonene were concentrated to ca. 10 µl. Each singly-collected monoterpene was then analyzed on a Varian 3400 GC equipped with a Cyclodex-B column (30m x 0.25 mm ID, J & W Scientific; GC conditions: split injection, 80 °C isothermal, injector and detector at 200 °C). The four chiral monoterpenes coincided with authentic standards of (+)-α-pinene and (-)-α-pinene, (-)-camphene, (-)-β-pinene, and (-)-limonene.

2.2.6 Preparation of Test Stimuli for Laboratory and Field Bioassays

Bark and sapwood sawdust from fresh logs of grand fir were obtained by drilling with a 1.5 mm diam. bit. Frass produced by the insects was obtained by confining male or female beetles in gelatin capsules attached to a fresh bolt of grand fir. Frass deposited into the capsules was collected on days 2-8 after the insects began to bore into the bark. Frass was stored in air-tight vials and kept at -15 °C until used.

All chemicals used in preparation of test stimuli, their purity, and their sources are listed in Table 3. Three made with commercial compounds (SB1, SB2 and SB3) (Table 4) were made up. SB1 and SB2 contained 13 components each, while SB3 had only 12. These synthetic blends mimicked as closely as possible the relative amounts of antennally-active volatiles in the bark oil. Blend SB1 was prepared using β-phellandrene that which had limonene as an impurity in a 2:1 ratio (limonene:β-phellandrene), much higher than the 1:10 ratio in the grand fir bark oil. Synthetic blend SB2 was prepared with synthetic β-phellandrene of greater purity (53 %) and without

Table 3. Chemical purity and sources of the compounds used in this study.

Chemical	Purity	Source
(±)-α-pinene	98 %	Sigma Chemical Co.
camphene	81 %	Matheson, Coleman & Bell
(-)-β-pinene	99%	Aldrich Chemical Co.
(+)-β-pinene	98 %	Aldrich Chemical Co.
myrcene	90 %	Aldrich Chemical Co.
(*S*)-(-)-limonene	96 %	Aldrich Chemical Co.
(*R*)-(+)-limonene	97 %	Aldrich Chemical Co.
β-phellandrene (synthetic)	53 %	Synthesized
β-phellandrene (comm.)	30 %	Glidco Organics
α-terpinolene	29 %	Givaudan Lab.
para-cymene	99 %	Aldrich Chemical Co.
(-)-α-cubebene	98 %	Fluka Chemical Corp.
(+)-longifolene	90 %	Sigma Chemical Co.
(*E*)-pinocarveol	90.05 %	Phero Tech Inc.
bornyl acetate	98 %	Matheson, Coleman & Bell
α-terpineol	95 %	Aesar
(-)-borneol	99 %	Aldrich Chemical Co.
cadinene	72 %	Phero Tech Inc.
verbenone	93 %	Phero Tech Inc.
(*E*)-nerolidol	95 %	Aldrich Chemical Co.
nerolidol	98 %	Aldrich Chemical Co.
methyl isoeugenol	99 %	Aldrich Chemical Co.

Table 4. Chemical components and their percentages in the composition of synthetic blends SB1 SB2 and SB3. Because commercial β-phellandrene contained limonene as an impurity, limonene was not added to SB1. β-Pinene was deployed in a 1:50 ratio of (-)- and (+)-enantiomers, and nerolidol was deployed in a 2:1 ratio of (*E*)- and (*Z*)- isomers. α-Cubebene and methyl isoeugenol were present in the bark oil at 3.53 and 0.49 % respectively. However, due to limited supply they were deployed in the low percentages that appear in this table. α-Cubebene was erroneously included in the synthetic blends SB1 and SB2 because of its misidentification as an antennally-active compound instead of a nearly coeluting unidentified compound. Therefore it was removed from SB3.

	Percent composition in blend		
Chemical	SB1	SB2	SB3
(±)-α-pinene	13.6	13.6	13.6
camphene	3.2	3.2	3.2
(-)-β-pinene	43.7	43.7	43.7
myrcene	1.5	1.5	1.5
(±)-limonene	18.8	1.3	1.3
β-phellandrene (synthetic)	—	18.8	—
β-phellandrene (comm.)	9.4	—	9.4
α-terpinolene	0.8	0.8	0.8
α-cubebene	0.03	0.03	—
(+)-longifolene	0.5	0.5	0.5
bornyl acetate	7.3	7.3	7.3
(-)-borneol	2.5	2.5	2.5
nerolidol	2.6	2.6	2.6
methyl isoeugenol	0.2	0.2	0.2

limonene. Synthetic β-phellandrene was prepared by Dr. G. G. S. King, Department of Chemistry, Simon Fraser University, as follows. A solution of dimsyl anion was prepared by adding, after washing, 29.6 g (0.74 mole), 60% sodium hydride dispersion to 400 ml DMSO. The mixture was slowly warmed, stirring 3 h until H_2 evolution had ceased. To this was added methyltriphenylphosphonium bromide, 289 g (0.71 mole), resulting in a yellow mixture, difficult to stir until more DMSO was added. A solution of 100 g (0.68 mole) 4-isopropyl-2-cyclohexenone (Aldrich Chemical Co.) in 100 ml dimethyl sulfoxide (DMSO) was added via a dropping funnel and the reaction was stirred overnight. The reddish mixture was quenched with 50% aqueous methanol, and extracted with hexane. The combined hexane extracts were filtered, washed with more 50% methanol and then with saturated salt solution, and dried over sodium sulfate. After removal of solvent the crude product was distilled (120 °C @ 20 torr.) to yield 22.7 g of β-phellandrene. The product was identical to authentic β-phellandrene by GC and GC-MS analysis.

2.2.7 Laboratory Experiments

The bioactivity of captured volatiles was tested in laboratory experiments, employing an arena olfactometer in which beetles made a choice between responding to a photic and an olfactory stimulus (Moeck, 1970). A light source (microscope lamp, low power) was located 49 cm from the insect release point (which received a light intensity of 76.6 lux); the air carrying test stimuli was delivered at a right angle to the light beam 6.5 cm from the release point. The arena surface was a filter paper strip (Whatman chomatographic 3 MM) 30 cm long and 15 cm wide; it was replaced every time a different sex or stimulus was tested. Prior to bioassays, beetles were held in groups of five (sexes kept separately) in petri dishes with moistened paper at 21 °C and 69 lux for 2 h. Bulk stimulus (frass or sawdust) was deposited into weighting boats and placed directly below the air outlet even with the arena surface. Extracted or captured volatiles were released from a glass tube (9 mm I.D.) lined with filter-paper (10 cm diam.) impregnated with volatile extract in pentane. Medical air was passed continuously through the tube at 1200 ml per

min. A positive response was recorded if a beetle walked to and stayed or milled around inside a rectangular area (3 cm x 1.5 cm) transverse to the runway just in front of the air outlet.

Attractiveness of stimuli was tested in nine bioassay experiments. Experiments 1 - 5 and 7, used male and female beetles; Experiments 6, 8 and 9 employed only females, the most responsive sex. Experiment 1 tested volatiles emanating from 0.250 g of freshly ground grand fir sapwood, bark, or frass produced by males or females; medical air was the control stimulus. Experiment 2 tested Porapak Q-trapped volatiles from female-infested grand fir logs, at doses of 0.03, 0.3, 3, 30, and 90 beetle-hours (bh) (one bh = the volatiles released by one female in 1 h). Porapak Q trapped-volatiles from an uninfested grand fir log were used as the control stimulus. Experiment 3 tested Porapak Q-trapped volatiles from male-infested grand fir logs at doses of 0.3, and 3 bh. Trapped volatiles from an uninfested grand fir log were used as the control stimulus. Experiment 4 tested steam-distilled bark oil from a healthy tree at doses of 0.009, 0.097, 0.975, 9.75 and 97.5 mg-equivalents, with pentane as a control stimulus (one mg-equiv. = the amount of oil distilled from 1 mg of starting material). Experiment 5 tested Porapak Q-trapped volatiles from an uninfested grand fir log at doses of 0.001, 0.01, 0.1, 1, 10 mg equiv. with pentane as a control stimulus. Experiment 6 compared female responses towards steam-distilled bark oil from healthy and root rot-infected grand fir at doses of 0.0006, 0.006, 0.006, 0.06, 0.6, 6, and 60 mg-equiv., with pentane as a control stimulus. Experiment 7 tested two fractions (Fractions 1 and 2) of steam-distilled bark oil from root rot-infected grand fir, at a 1 μg dose, with pentane as a control stimulus. Experiment 8 compared the activity of the two fractions tested in Experiment 7 with the activity of two tentative synthetic fractions[2] without complete confirmation of the bioactivity of all components. Experiment 9 compared the responses of females to six subfractions of BO1 by presenting Fraction 1 (from Experiment 7 and 8) alone or with all possible binary combinations and the ternary combination of the three subfractions of Fraction 2, and then

[2]Composition (μg) of synthetic Fraction 1: α-pinene (6.9), camphene (1.2), β-pinene (21.4), myrcene (0.2), limonene (0.5), and commercial β-phellandrene (3). Composition of synthetic Fraction 2: α-terpinolene (0.3), *para*-cymene (0.001), longifolene (0.02), (*E*)-pinocarveol (0.04), bornyl acetate (1), α-terpineol (0.3), borneol (0.4), cadinene (0.8), verbenone (0.06), (*E*)-nerolidol (0.04), (*Z*)-methyl *iso*-eugenol (0.04) and (*E*)-methyl *iso*-eugenol (0.08).

presenting Fraction 2 alone or with binary combinations and the ternary combination of the three subfractions of Fraction 1.

2.2.8 Field Experiments

Blends prepared with commercially obtained compounds that were antennally-active, attractive in the laboratory and available in sufficient quantity were field tested in a mature *Abies grandis/Acer rubrum* forest with well represented Douglas-fir (Steel and Gier-Hayes, 1992), located 10 km north of Coeur d'Alene, Idaho. Twelve-unit, multiple-funnel traps (Lindgren, 1983) (Phero Tech, Inc.) baited with candidate kairomonal blends were deployed in 10 randomized complete blocks, with ≥15 m between traps and 15 m between trap lines. Captured beetles were bagged and frozen until they could be counted and sexed. Experiments 10 and 11, respectively, tested attraction to two different synthetic blends (SB1 and SB2) of 13 components each (Table 4), as well as steam distilled-bark oil, and an unbaited control. The synthetic blends used in these experiments differ from those employed in Experiment 8, only by the presence of α-cubebene and the lack of *para*-cymene, (*E*)-pinocarveol and verbenone. Volatile stimuli were deployed in open 1.5 ml plastic

centrifuge Eppendorf tubes. Release rates of SB1 and SB2 and the bark oil determined under laboratory conditions at 32 °C using 10 replicates for each stimulus were 340 mg, 280 mg and 5 mg per 24 h, respectively. Experiment 12 compared the activity of SB3 and BO2 released at 762 and 389 mg, respectively (determined as above), to test the hypothesis that at a high release rate bark oil would be equivalent in attractiveness to that of the synthetic blends in Experiments 10 and 11.

2.2.9 Statistical Analysis

Percentages of male and female beetles responding in laboratory bioassays were transformed by $\arcsin\sqrt{x}$ to normalize the data and stabilize the variances between replicates

(Zar, 1984), except for Experiment 6, and were analyzed by ANOVA followed by the Ryan-Einot-Gabriel-Welsh (REGW) Multiple Range test (Day and Quinn, 1989). For Experiment 6 responses to the volatiles from healthy or root rot-infected trees at each dose were compared by *t*-tests. The REGW test was also used for data from field experiments, but with a $\log_{10}(x+1)$ transformation (Zar, 1984). Responses to combinations of fractions and subfractions in Experiment 9 were compared to those to the pentane control stimulus by using Dunnett's test (Zar, 1984). All analyses employed SAS computer software (SAS Institute, 1990) with $\alpha = 0.05$.

2.3 Results

Analyses by GC of volatiles produced by male or female *S. ventralis* boring into grand fir logs gave virtually identical chromatograms. There were no conspicuous sex-specific peaks.

In all laboratory bioassays, females were more responsive and less variable in their responses than males. All stimuli in Experiment 1 (Figure 1) were significantly more attractive to females than the air control; host bark, sapwood sawdust, and male or female frass were equally attractive. Male responses to sawdust and female frass (but not male frass) were significantly different from those to the control stimulus (Figure 1). Captured volatiles from female-infested logs were most attractive at doses of 3 and 30 bh per μl (females) and 3 bh per μl (males); responses declined at higher and lower doses (Figure 1, Experiment 2). In no case was attraction to the volatiles from female-infested logs significantly different from that to the volatiles from uninfested control logs. In Experiment 3, volatiles from male-infested logs were less attractive to females than were volatiles from uninfested logs, but for males there was no difference in responses to treatments (Figure 1).

In Experiment 4 (Figure 2) responses by females to steam-distilled bark oil were significantly higher than those to pentane at doses of 0.975 and 9.75 mg equiv.; males responded significantly to stimuli at these doses and also at a dose of 0.097 mg equiv. Experiment 5 (Figure 2) showed a similar trend for captured volatiles; females responded significantly at doses of 0.1, 1.0 and 10.0 mg equiv., while males responded only at the two

Figure 1. Results of laboratory bioassay Experiments 1-3 showing the percent responses of walking *S. ventralis* tested in 10 groups of 5 insects (males or females) to each stimulus as follows: sawdust or frass presented in 250 mg doses (Experiment 1), Porapak Q-trapped volatiles from logs infested with female *S. ventralis* (Experiment 2), or with males (Experiment 3). Control stimuli were medical air (Experiment 1) or volatiles from an uninfested grand fir log (Experiments 2, 3). Bars for each sex with the same letter are not significantly different, REGW test, $P < 0.05$.

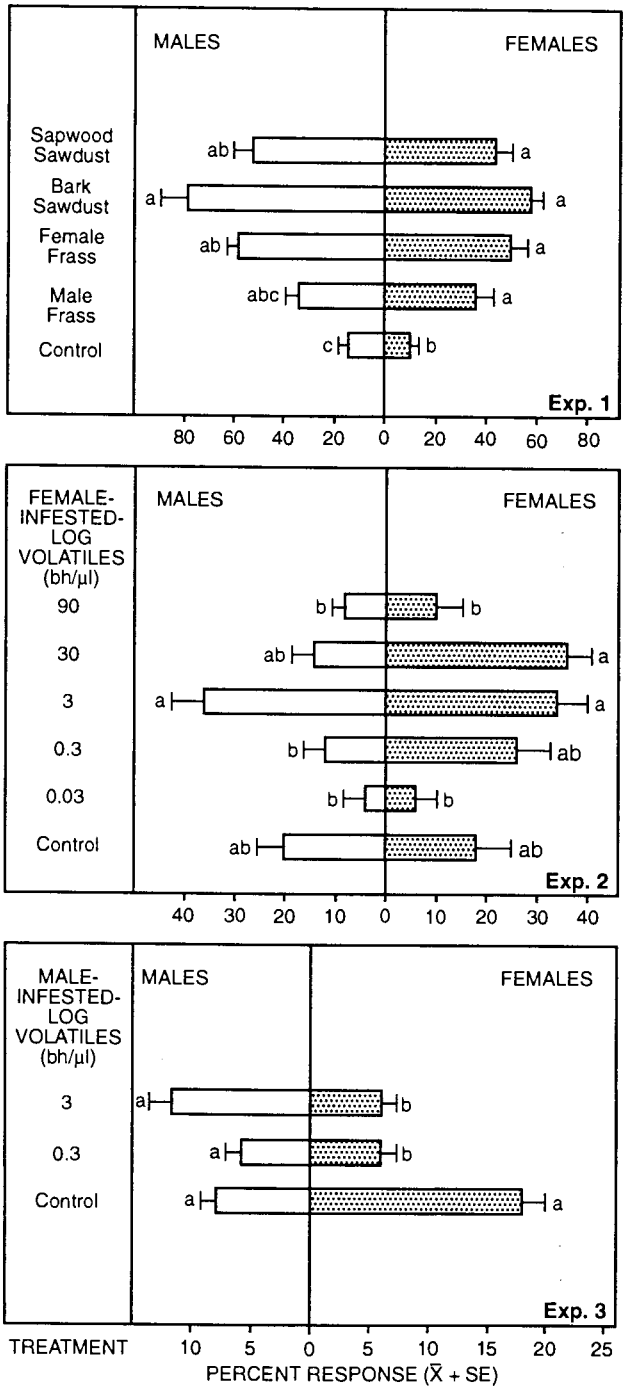
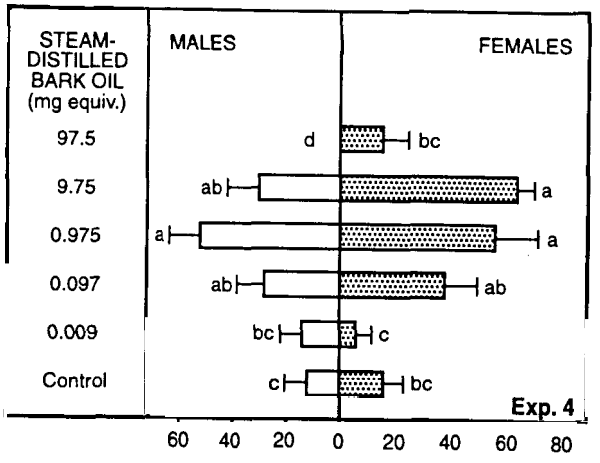
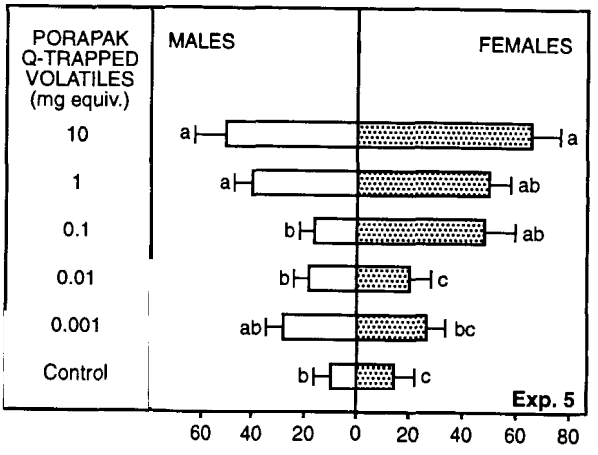
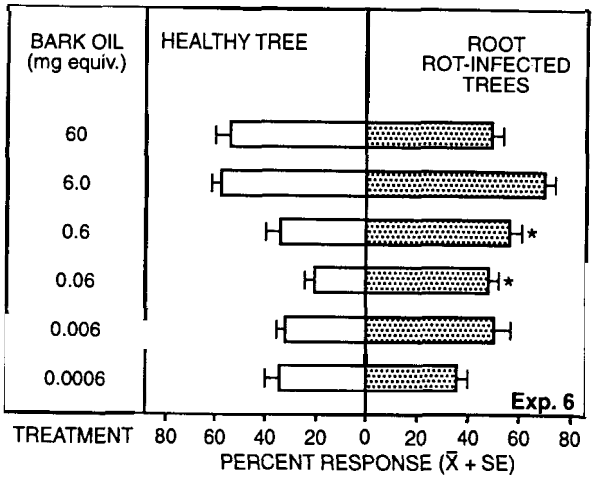

MALES
FEMALES
Sapwood Sawdust
Bark Sawdust
Female Frass
Male Frass
Control
ab
a
a
a
ab
a
abc
a
c
b
Exp. 1
80 60 40 20 0 20 40 60 80
FEMALE-INFESTED-LOG VOLATILES (bh/µl)
90
30
3
0.3
0.03
Control
b
b
ab
a
a
a
b
ab
b
b
ab
ab
Exp. 2
40 30 20 10 0 10 20 30 40
MALE-INFESTED-LOG VOLATILES (bh/µl)
3
0.3
Control
a
b
a
b
a
a
Exp. 3
TREATMENT
10 5 0 5 10 15 20 25
PERCENT RESPONSE (X̄ + SE)

Figure 2. Results of laboratory bioassay Experiments 4-6 showing the percent responses of walking *S. ventralis* tested in 10 groups of 5 insects (males or females) to each stimulus as follows: steam-distilled-bark oil (Experiment 4), Porapak Q-captured volatiles from grand fir bark chips (Experiment 5); and steam-distilled bark oil from root rot-infected or healthy grand firs (Experiment 6, only females tested). Pentane (1μl) was the control stimulus for Experiments 4 and 5. For Experiments 4-5, bars for each sex with the same letter are not significantly different, REGW Test, $P < 0.05$. Bars with asterisks in Experiment 6 indicate significant differences in paired responses within a dose, *t*-test. $P < 0.05$.


STEAM-DISTILLED BARK OIL (mg equiv.)
MALES
FEMALES
97.5
9.75
0.975
0.097
0.009
Control
d
bc
ab
a
a
a
ab
ab
bc
c
c
bc
Exp. 4
60 40 20 0 20 40 60 80


PORAPAK Q-TRAPPED VOLATILES (mg equiv.)
MALES
FEMALES
10
1
0.1
0.01
0.001
Control
a
a
a
ab
b
ab
b
c
ab
bc
b
c
Exp. 5
60 40 20 0 20 40 60 80


BARK OIL (mg equiv.)
HEALTHY TREE
ROOT ROT-INFECTED TREES
60
6.0
0.6
0.06
0.006
0.0006
*
*
Exp. 6
TREATMENT
80 60 40 20 0 20 40 60 80
PERCENT RESPONSE (X̄ + SE)

highest doses. In Experiment 6 (Figure 2) steam-distilled bark volatiles from root rot-infected trees were slightly more attractive to walking beetles at most doses than volatiles from uninfected trees, and significantly at two doses. At doses > 0.0006 mg-equiv. and < 60 mg-equiv., 61 % of all responses were to the volatiles from infected trees.

In Experiment 7, neither fraction of bark oil alone was more attractive to walking beetles than the pentane control stimulus, but there was a very clear synergistic effect for females by combining the two fractions (Figure 3). This effect was reproduced in Experiment 8 by combining the defined synthetic fractions (Figure 3). The results of Experiment 9 (Figure 4) suggest that some bioactivity was lost in fractionation. However, it appears that all or most components of bark oil are needed to achieve attraction similar to that elicited by the unfractionated oil. The lowest responses were obtained after omission of subfractions 1 and 2 of Fraction 1, containing α-pinene and camphene or β-pinene and myrcene, respectively (Table 2), or subfractions 1 and 2 of Fraction 2, containing bornyl acetate and longifolene or borneol with trace amounts of bornyl acetate, respectively. Omission of subfraction 3 of Fraction 1, containing β-phellandrene, limonene and terpinolene, appeared to have the least effect on response; this was the only treatment that did not lower the response by females to a level not significantly different from that to the solvent control.

In GC-EAD recordings of *A. grandis* volatiles female *S. ventralis* antennae responded to many compounds, including (±)-α-pinene, (-)-camphene, (-)-β-pinene, myrcene, (-)-limonene, β-phellandrene, α-terpinolene, longifolene, bornyl acetate, borneol, (*E*)-nerolidol, and methyl isoeugenol (Figure 5). When the synthetic blends SB1 and SB2 (the latter with a correct ratio of limonene:β-phellandrene) were tested in the field in Experiments 10 and 11, both *S. ventralis* and the clerid predator *T. undatulus* (Figure 6) were captured in significant numbers in traps baited with the synthetic blends. Traps baited with bark oil released at 5 mg per 24 h in Experiment 11 did not catch significant numbers of beetles. As predicted, increasing the release rate of bark oil to 384 mg per 24 h in Experiment 12 resulted in significant catches of *S. ventralis* in traps baited with BO2 (Table 5). *Pseudohylesinus granulatus* (LeConte) was also significantly

Figure 3. Results of laboratory bioassay Experiments 7-8 showing the percent responses of walking male and/or female *S. ventralis* tested in 10 groups of 5 insects (males or females) to each stimulus as follows: fractionated and unfractionated steam-distilled bark oil from root rot-infected grand fir presented in 1 μg doses (Experiment 7); and combinations of fractions of natural and synthetic bark oil from grand fir presented in 100 ng doses (Experiment 8). Within each experiment and sex of beetles, bars with the same letter are not significantly different, REGW Test, $P < 0.05$.

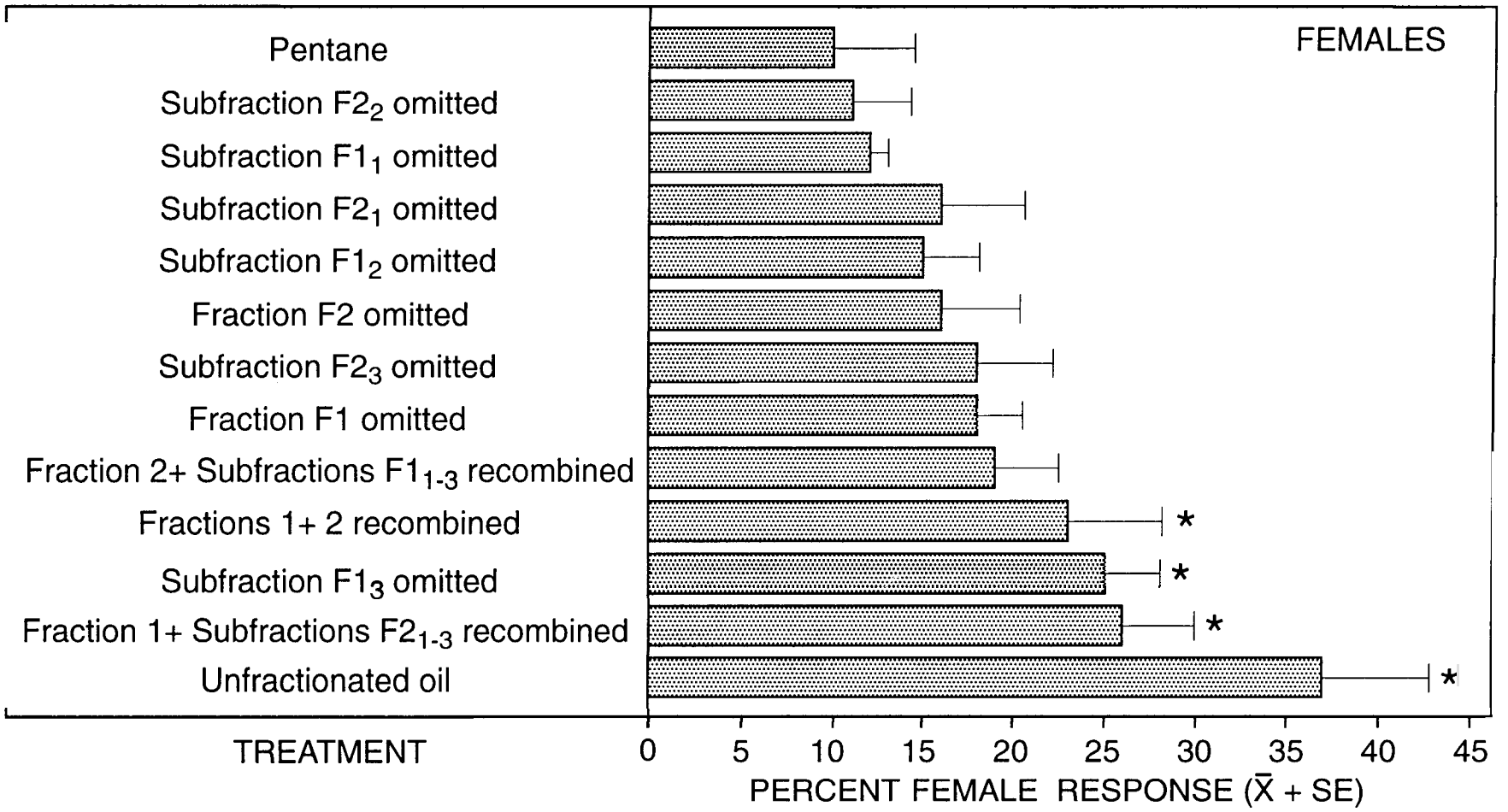
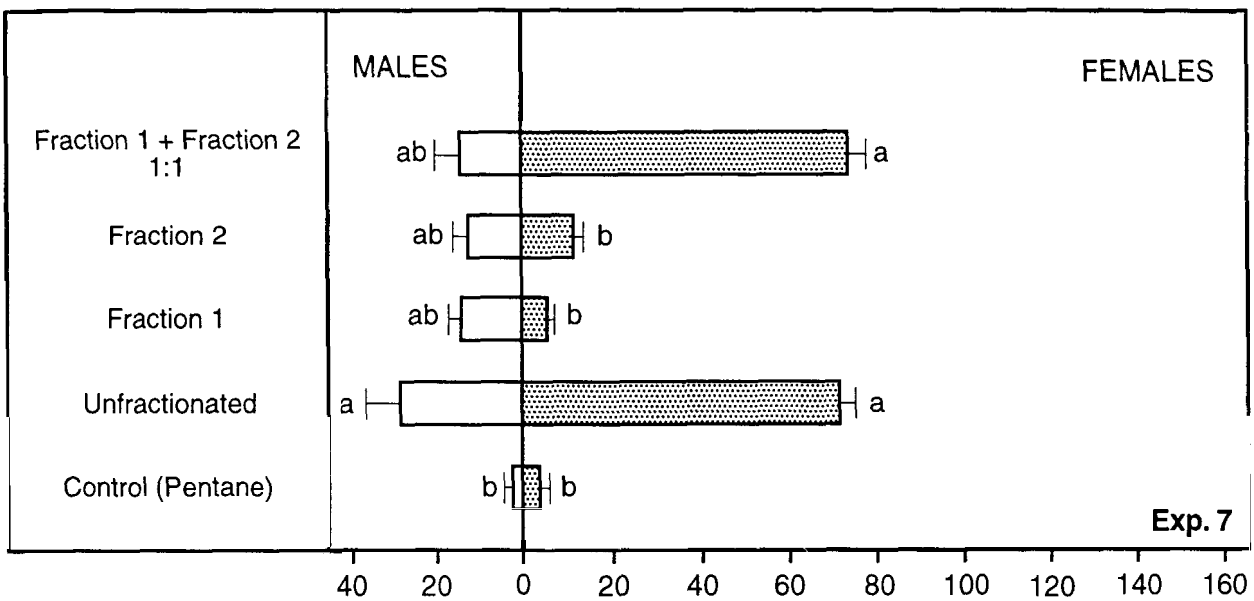

FEMALES
Pentane
Subfraction F2$_2$ omitted
Subfraction F1$_1$ omitted
Subfraction F2$_1$ omitted
Subfraction F1$_2$ omitted
Fraction F2 omitted
Subfraction F2$_3$ omitted
Fraction F1 omitted
Fraction 2+ Subfractions F1$_{1-3}$ recombined
Fractions 1+ 2 recombined
Subfraction F1$_3$ omitted
Fraction 1+ Subfractions F2$_{1-3}$ recombined
Unfractionated oil
*
*
*
*
TREATMENT
0 5 10 15 20 25 30 35 40 45
PERCENT FEMALE RESPONSE ($\bar{X}$ + SE)

Figure 4. Results of laboratory bioassays in Experiment 9 showing the percent responses of walking female *S. ventralis* tested in 10 groups of 5 insects to fractions 1 and 2 of steam-distilled bark oil from grand fir and to binary and ternary combinations of these subfractions of each. Each stimulus presented at 100 ng doses with all combinations of Fractions 1 and 2 (even with omission of single subfractions). Bars with asterisks indicate significant difference from response to pentane, Dunnett's test, $P < 0.05$.


MALES
FEMALES
Fraction 1 + Fraction 2 1:1
Fraction 2
Fraction 1
Unfractionated
Control (Pentane)
ab
a
ab
b
ab
b
a
a
b
b
Exp. 7
40
20
0
20
40
60
80
100
120
140
160

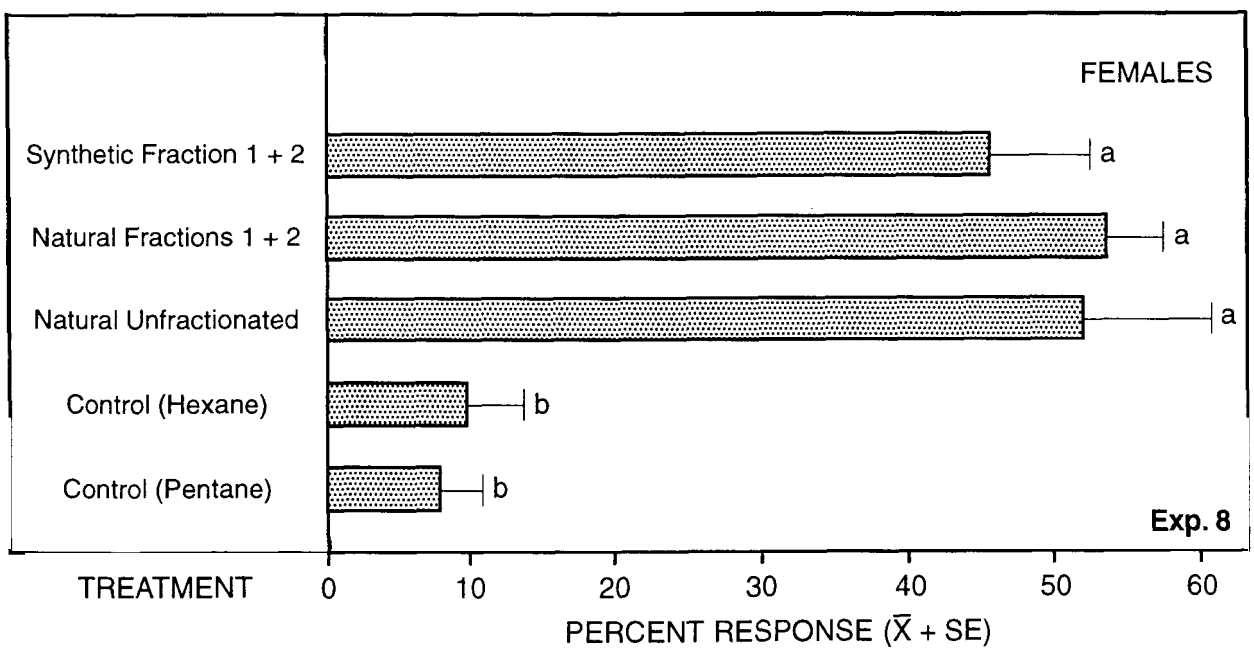

FEMALES
Synthetic Fraction 1 + 2
Natural Fractions 1 + 2
Natural Unfractionated
Control (Hexane)
Control (Pentane)
a
a
a
b
b
Exp. 8
TREATMENT
0
10
20
30
40
50
60
PERCENT RESPONSE (X̄ + SE)

Figure 5. Representative paired recordings of flame-ionization detector (FID) and electroantennographic detector (EAD) of the response of female *S. ventralis* antennae to steam-distilled grand fir bark volatiles. Chromatography: DB-23-column (30 m x 0.32 mm ID), splitless injection; injector and FID temp; 240 °C. Temperature program: 50 °C for 1 min, then 10 °C/min to 200 °C. FID 1 = Porapak Q-trapped volatiles from female-infested logs; FID 2 = Porapak Q-trapped volatiles from field aeration of a 1 m-section of a root rot-infected, uninfested standing grand fir.

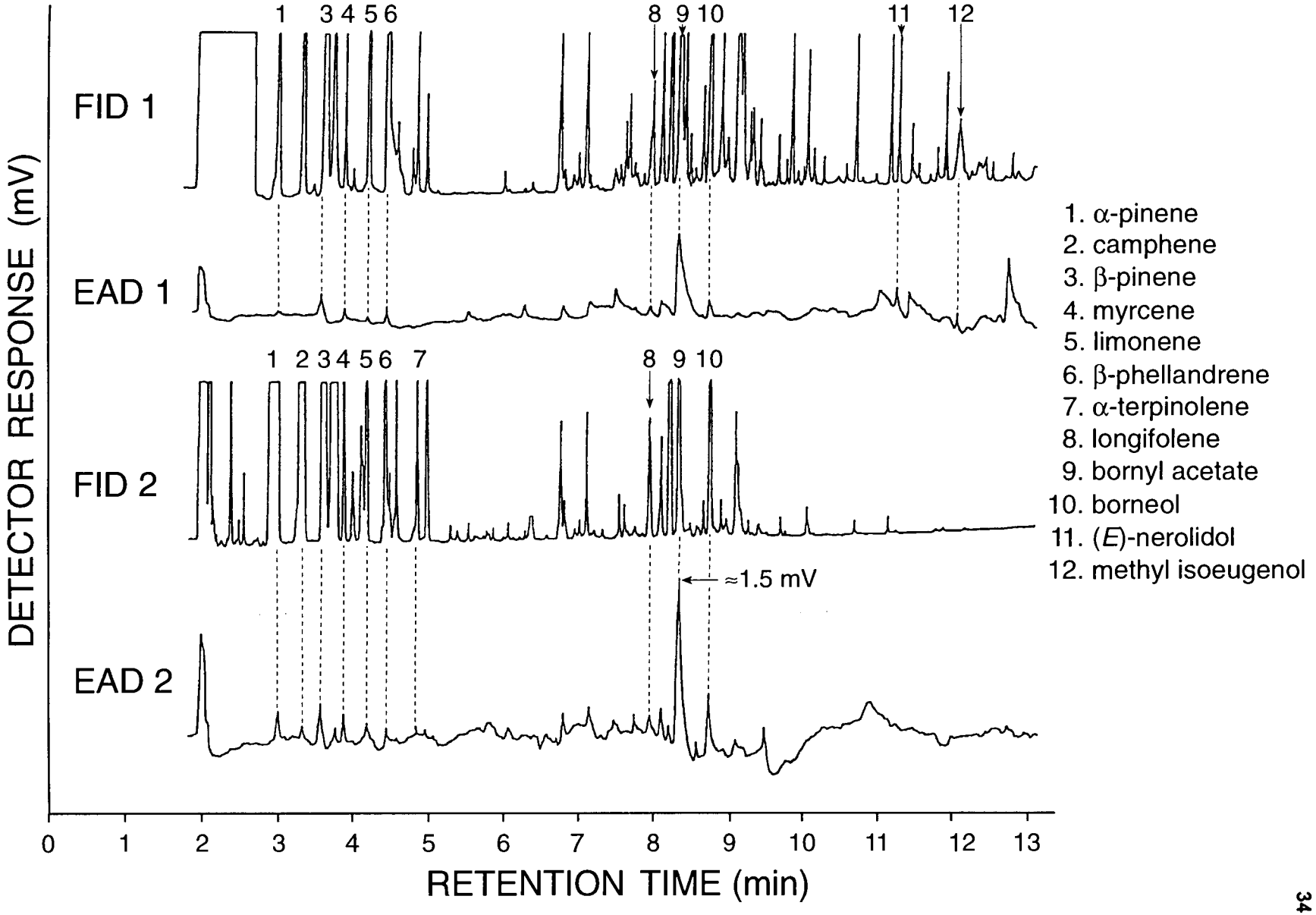

FID 1
EAD 1
FID 2
EAD 2
1 3 4 5 6
8 9 10
11
12
1 2 3 4 5 6 7
8 9 10
≈1.5 mV
DETECTOR RESPONSE (mV)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
RETENTION TIME (min)
1. α-pinene
2. camphene
3. β-pinene
4. myrcene
5. limonene
6. β-phellandrene
7. α-terpinolene
8. longifolene
9. bornyl acetate
10. borneol
11. (E)-nerolidol
12. methyl isoeugenol

Figure 6. Numbers of *S. ventralis* and *T. undatulus* captured in multiple-funnel traps in Experiment 10 (July 7-16, 1996) and Experiment 11 (July 2-9, 1996), Coeur d'Alene, Idaho; (N = 10). Release rate for SB1, SB2 and bark oil were 340 mg, 280 mg, and 50 mg per 24 h, respectively. For each experiment and insect bars with the same letter are not significantly different, REGW test, $P < 0.05$.

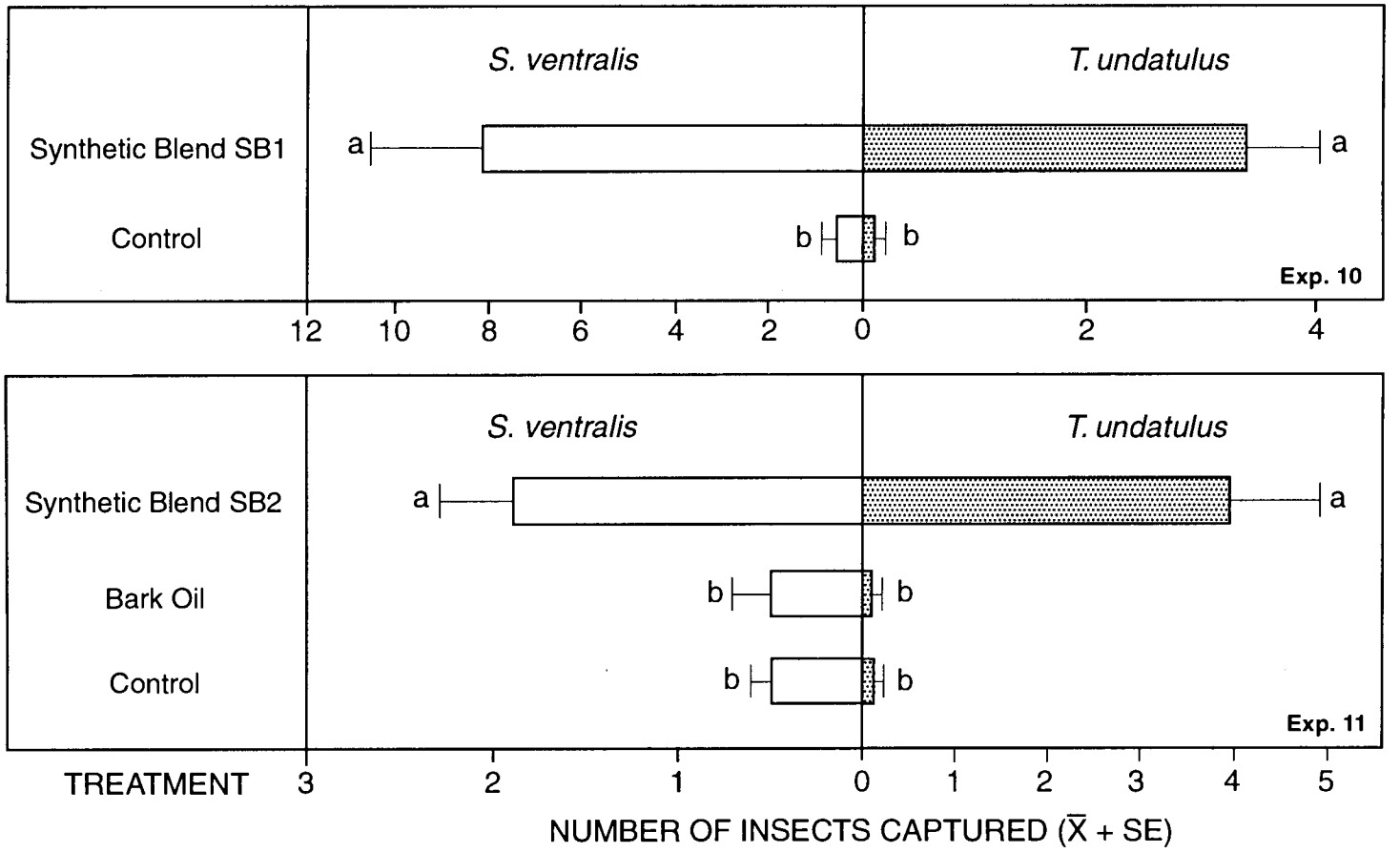

S. ventralis
T. undatulus
Synthetic Blend SB1
Control
a
a
b
b
Exp. 10
12
10
8
6
4
2
0
2
4
S. ventralis
T. undatulus
Synthetic Blend SB2
Bark Oil
Control
a
a
b
b
b
b
Exp. 11
TREATMENT
3
2
1
0
1
2
3
4
5
NUMBER OF INSECTS CAPTURED (X̄ + SE)

Table 5. Numbers of *S. ventralis* and *P. granulatus* captured in multiple funnel traps in Experiment 12 (N = 10). Bark oil (BO2) and SB3 were released at 384 and 762 mg per 24 h, respectively. Experiment was conducted 10 km North Coeur d'Alene, Idaho, June 25 - July 21, 1997.

Treatment	Release rate (mg per 24 h)	Number of beetles captured ($\bar{X} \pm SE$)[a]	
		S. ventralis	*P. granulatus*
Unbaited trap (control)		0.7 ± 0.3b	0.9 ± 0.34b
Bark oil (BO2)	389	4.7 ± 0.86a	3.7 ± 0.76a
Synthetic blend (SB3)	762	3.6 ± 2.7b	0.3 ± 0.15b

[a] Means within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh Multiple Range Test, $P > 0.05$.

attracted to BO2. However, apparently because of large variation in numbers of *S. ventralis* captured, traps baited with SB3 released at 762 mg per 24 h did not catch significantly more beetles than unbaited control traps.

2.4 Discussion

When examined in detail my results (Figures 1-6) consistently support the primary attraction aggregation hypothesis. Both sexes of *S. ventralis* displayed the same general trend of response to any material tested (Figure 1, Experiments 1-3; Figure 2, Experiments 4-5; Figure 3, Experiment 7), not differentiating male or female frass from only host materials, as well as infested or uninfested logs. Female *S. ventralis* were attracted in slightly higher numbers to a broader dose range of stimuli than males. This finding is concordant with the role of females as the pioneer sex that must perceive and select the most suitable host trees. The superior attraction of volatiles from female-infested logs over those from male-infested logs (Figure 1, Experiments 2, 3) can be accounted for by the higher rate of boring by females, which would release more host volatiles than males, which in nature hardly bore at all. The results from Experiment 3 might suggest that boring males produced a repellent pheromone that significantly reduced attraction of females compared to that from logs (Figure 1), but this hypothesis was not followed further.

The results from GC-EAD analyses (Figure 5), bioassays that indicate a requirement for a blend of host volatiles to achieve attraction (Figure 3 and 5, Experiment 7 and 9), and the finding that attraction can be reproduced by substituting synthetic blends for natural ones (Figure 3, Experiments 7-9 and 12, Table 5) are all indicative of a species highly adapted to respond to host kairomones. Further evidence of the sensitivity of *S. ventralis* to specific volatiles from its host is that it is repelled in laboratory bioassays at equivalent doses to bark oil from subalpine fir (unpublished results). Although both *A. grandis* and *A. lasiocarpa* are remarkably similar in their major volatile components, some minor components are not shared by both (Zavarin, 1968; von Rudlof, 1975).

The repellent effect of individual terpenoid components found by Bordash and Berryman (1977) was apparently overturned by offering a blend of the same materials in a (Table 3) similar to that found in the naturally-occurring bark volatiles. Most of these components are required to maintain attractiveness (Figure 4). An equivalent situation was found by Visser and Avé (1978), in which the Colorado potato beetle, *Leptinotarsa decemlineata* Say, was attracted to a specific blend of green leaf volatiles. When these compounds were tested individually or incorporated into the blend at different ratios, the attraction ceased or decreased, respectively. Similarly, Anderson et al. (1993) reported that oviposition by the cotton leafworm, *Spodoptera littoralis* (Boisd.), was strongly deterred by a mixture of six compounds from conspecific larval frass. If one of the compounds was excluded from the mixture the deterrent effect was lost.

The response of the predator *T. undatulus* to the same blend that attracts their prey (Figure 6) is analogous to similar predator-host interactions in which this same predator, responded to the pheromone frontalin produced by *Dendroctonus pseudotsugae* Hopkins (Ross and Daterman, 1995) and ipsdienol produced by *Ips* spp. (Miller et al., 1991). In each type of association the predators and their prey responded to identical stimuli, suggesting that *T. undatulus* may have distinct semiochemical-based, prey-adapted races.

To my knowledge this is the first report of any *Thanasimus* sp. that is attracted in the field to a blend of terpenoids. *Thanasimus* spp. are characteristically attracted to the pheromones of several host scolytid species (Vité and Williamson, 1970; Bakke and Kvamme, 1981; Mizell et al., 1982; Payne et al., 1984; Raffa and Klepzig, 1989; Herms et al., 1991; Miller et al., 1991). Another predator, the blackbellied clerid, *Enoclerus lecontei* (Wolcot), reported as the most abundant predator of the fir engraver (Struble, 1957; Ashraf and Berryman, 1969; Berryman and Ferrell, 1988), was not trapped in response to the synthetic blends, but at the same field site was attracted by the aggregation pheromone of *P. elegans* (see Chapter 3).

The thresholds for perception of and response to bark oil and the blend of host kairomones (Figures 2, 3) are similar to those for pheromones in the genera *Dendroctonus* and *Ips* (Borden, 1985), and much lower than for kairomones in other scolytid genera (Dickens, 1979). In particular the threshold for response near 0.097 mg equiv. of bark oil distillate (Figure 2,

Experiment 4) is equivalent to the response of male *S. multistriatus* in laboratory bioassays to 10 mg of pheromone-laden frass from virgin females (Peacock et al., 1973). The ability of the synthetic blends released at 340, 280 and 762 mg per 24 h for SB1, SB2 and SB3, respectively, to attract *S. ventralis* in the field (Figure 6, Table 5) is also remarkable, considering the competition from natural odor sources in the forest, and the fact that these are the summed release rates for 12 or 13 components (Table 4). In comparison, red turpentine beetles, *Dendroctonus valens* LeConte, were attracted in the field to specific enantiomers of α- and β-pinene released at 0.8 ml to 70 ml per 24 h (Hobson et al., 1993), the doses ranging from 2 to 200 times those at which SB1 and SB2 were released.

Although there was a significant response to the synthetic blends of host components in the field, the blends were not exactly the same as the natural mixture. For example, the optical purity of the compounds used in the blend's preparation ranged from 29 to 99 %, which could possibly have influenced the field catches.

As mentioned above numerous antennally-active compounds were found by GC-EAD analysis, but only 13 were identified and used in the synthetic blends SB1 and SB2. However, it is possible that redundancy can occur, making some of the compounds unnecessary to achieve attraction. Such a phenomenon has been shown for *Rhyzophagus grandis* Gyllenhal, a predator of the great spruce bark beetle, *Dendroctonus micans* Kugelann., and a potential biological control agent for *D. valens*. *Rhyzophagus grandis* oviposits in frass produced by larvae of these two bark beetle species. Larval frass volatiles of *D. micans* contained nine oxygenated monoterpenes, while those of *D. valens* included 12. A synthetic blend of the seven components common to both species elicited as much oviposition by *R. grandis* in laboratory bioassays as did larval frass of *D. micans* (Grégoire et al., 1991).

Although grand fir bark oil (B01) was highly attractive to *S. ventralis* in laboratory bioassays (Figures 2, 3), its failure to attract *S. ventralis* in the field when released at 50 mg per 24 h (Figure 6) suggests that the release rates of 340 and 280 mg per 24 h for SB1 and SB2, respectively, were just above the threshold for attraction. This was confirmed when BO2 was released at 389 mg per 24 h, and *S. ventralis* was significantly attracted (Table 6). The superiority

of BO2 over SB3 (Table 6) may indicate that the synthetic blends may be missing one or more bioactive components. In addition, during the time that the Experiment 12 was in progress, I observed large populations of *S. ventralis* attacking freshly-felled trees in cutting areas adjacent to the experimental site, indicating a probable response to this large alternative source of primary attractants, and leaving very few beetles to respond to baited traps.

Because of the intolerance of *S. ventralis* to resin and the inhibition of growth by *T. symbionticum* in the presence of monoterpenes (Raffa et al., 1985), there is a low likelihood that fir engravers could overcome the induced defense system of healthy trees (Raffa 1991), even by mass attack. Therefore, avoidance of stimuli associated with host resistance would be adaptive (Raffa and Berryman, 1987), as would orientation toward stimuli associated with susceptible weakened hosts. Mass attack behavior would be adaptive only to the extent that slightly resistant trees could be included as suitable hosts. A preference for weakened hosts is supported by the high correlation of root rot infections and fir engraver attacks (Cobb *et al.*, 1973; Hertert *et al.*, 1975; Ferrell and Smith, 1976; Wright *et al.*, 1984), and by my finding that fir engravers are more attracted to oil from the bark of root rot-infected trees than from the bark of healthy trees (Figure 2, Experiment 6). Other bark beetles can apparently also detect fungus-infested hosts. For example, seven times more pine engravers, *Ips pini* (Say), bored into trees infected by *Leptographium terebrantis* Barras and Perry, than into healthy ones (Raffa and Klepzig, 1996). Nebeker et al. (1995) showed that lodgepole pines infested with Armillaria root rot had a 20 times higher bornyl acetate content in the resin than healthy trees. In my experiments bornyl acetate elicited a very clear and strong EAD response in fir engraver antennae (Figure 4) and was attractive to walking *S. ventralis* when tested alone at doses ranging from 10 - 100 ng in laboratory bioassays (results not shown).

In a kairomone-driven system, it would be adaptive for both sexes to respond at high levels to a host kairomonal signal and to rely on close-range recognition factors for mate selection. Accordingly, one would not expect the sex ratio of responding beetles to be altered if the host were under attack by conspecifics. Field investigations by Ferrell (1969) support this hypothesis. The sex ratios of *S. ventralis* caught on girdled-unattacked and girdled-attacked trees

did not differ, nor did they differ from the sex ratio at emergence. Results from Experiment 1 (Figure 1) and the female : male sex ratios of 12:8, 47:35, and 20:21 in Experiments 10, 11, and 12, respectively, are consistent with Ferrell's (1969) observations.

There are other bark beetles that are attracted to host resin and/or some of its components and that also do not seem to have aggregation pheromones. The red turpentine beetle is attracted to mixtures of α-pinene, myrcene, and 3-carene (Hobson et al., 1993), and demonstrates a remarkable chiral specificity toward *S*-(-)-α-pinene (White and Hobson, 1993). The native elm bark beetle, *Hylurgopinus rufipes* (Eichhoff), is consistently reported as attracted to cut elm wood (Martin, 1936), wounded elms (Landweh et al., 1981), and to naturally and artificially moribund elms (Gardiner, 1979; Millar et al., 1986). Several sesquiterpenes seem to play a role in host-finding by *H. rufipes,* since they were attractive when deployed in traps (Millar et al., 1986). Despite extensive research no pheromone could be demonstrated in this species (Swedenborg et al., 1988).

Historically research on the chemical ecology of scolytids has disclosed occasions when species were considered to be kairomone-driven, but were later shown to employ aggregation pheromones. For example, Meyer and Norris (1967) attributed the higher attraction of *S. multistriatus* to female- than male-infested logs to the greater release of host volatiles by the actively boring females. However, Peacock et al. (1971) clearly demonstrated the presence of an aggregation pheromone produced by female *S. multistriatus* by showing much higher attraction to logs infested with 40 females than to logs infested by 400 males. More recently, the contention by Byers et al. (1985) and Vité et al. (1986) that the pine shoot beetle, *Tomicus piniperda* (L.), relies solely on primary attraction in host selection has been countered by Teale (1996) who used GC-EAD analysis as the basis for demonstrating that this species also uses an aggregation pheromone.

One might question, as I did, whether the fir engraver has a yet undiscovered aggregation pheromone. Therefore I pursued an exhaustive series of experiments using GC and GC-EAD techniques as well as other approaches to test the hypothesis of secondary attraction in *S. ventralis,* with consistently negative results (Table 6, Appendix 2). Moreover, a review of 50

Table 6. Summary of additional experiments performed to test the hypothesis of secondary attraction in *S. ventralis* (for details see Appendix 2).

Experiments	Results
Laboratory aerations	
Volatiles collected on Porapak-Q from groups of 20-500 male, female or mixed-sex *S. ventralis* held in glass tubes (Rudinsky, et al., 1973). Beetles were virgin-unfed, virgin-fed or mated-fed.	GC and GC-EAD analyzes of captured volatiles revealed no sex-specific compounds. No preferential response by beetles of either sex to volatiles from either sex or type of treatment.
Field aerations	
Adapting the methodology of Browne et al. (1979), 1 m long sections of bole of standing grand fir trees under attack by *S. ventralis*, were wrapped in a plastic sheet open at the top with the bottom attached to a Porapak Q trap under vacuum from a portable pump. Aerations continued for 50 h. Volatiles from unattacked control trees captured in identical manner.	GC analysis of the captured volatiles revealed no volatiles specific to attacked trees and no differences in ratios of components. This was confirmed by comparing the GC-EAD analysis of extracts from one of the infested trees with one uninfested tree.
Methoprene Treatments	
Using Harring's (1978) technique, 120 *S. ventralis* of each sex were treated topically with 1 and 10 µg of methoprene in 1 and 10 µl of pentane. After 24 h treated and control beetles extracted in pentane. Extracts also made of male and female fir engravers allowed to bore into phloem pieces treated with 1 and 10 µg of	No sex-specific volatiles revealed by GC-analysis of extracted beetles.

methoprene.

Gland Extracts

Exploring the hypothesis of Gore's et al. (1977) that accessory glands at the base of vaginal palpi of *S. multistriatus* were associated with pheromone production, 276 abdominal tips (containing the palpi and gland) were excised from unfed female *S. ventralis* as well as from females that had been fed for two days. Excised tips macerated in ice-cold pentane.

GC analysis of abdominal tip extracts revealed trace amounts of *exo*-brevicomin as confirmed by GC-MS. No attraction to *exo*-brevicomin in laboratory bioassays or field experiments.

Videotaping

Over 20 h of videotaping of male and female *S. ventralis* walking on surface of grand fir logs, females initiating attack, and males courting females was performed.

Several females observed rubbing tip of abdomen on bark in apparent "marking" behavior, and a few others possibly "calling" while running with protruded, swollen abdominal tips (Macías-Sámano and Borden 1993). No observed response by males to either "marking" or "calling" females, and no sex-specific volatiles revealed by GC analysis of aerated or extracted beetles (above). Gallery initiation and courtship similar to that by *S. multistriatus* (Svihra and Clark, 1980), but no behavior observed that would suggest pheromone release.

years of published information on the fir engraver failed to yield compelling evidence for a pheromone-driven system and supported the hypothesis that from an evolutionary perspective the fir engraver has become well adapted to rely on host kairomones to mediate host selection. One significant adaptation is the transverse orientation of *S. ventralis* galleries. Such galleries enhance the success of the symbiotic fungus, *T. symbionticum*, because it is inoculated into the most possible vascular tissue (Berryman, 1972; Wong and Berryman, 1977). This in tum results in a large area invaded by the fungus and a correspondingly large area in which the insect can breed. Berryman (1969), Berryman and Ashraf (1970), Russell and Berryman (1976) and Ferrell (1983) found that *S. ventralis* rarely encounter large amounts of constitutive resin, which is localized in cortical pitch blisters and the very thin channels that connect them (Bannan, 1936; Littelfield, 1973; Ferrell, 1969). Therefore, there is little selective pressure to develop a resin detoxification system (Raffa and Berryman, 1987), a process that has apparently led to the exploitation of detoxification products as pheromones by other bark beetles (Renwick, 1988). Once inside the tree, fir engravers rely on their symbiotic fungus to condition the tissue in advance of their galleries and thus to protect them from exposure to the toxic effects of wound-induced resin (Raffa, 1991).

Because exposure to resin can be avoided, fir engravers may attempt to attack the majority of trees in a stand (Berryman and Ashraf, 1970). Moreover, single galleries in living trees can be as successful or more so as those in mass-attacked trees (pers. obs.). There are several reports (Struble, 1957; Johnson and Shea, 1963; Berryman, 1969; Berryman and Ashraf, 1970; Felix et al., 1971; Ferrell, 1973) that describe the scars of old, successful and unsuccessful, isolated attacks embedded in the xylem of living firs. This characteristic of being able to kill a patch of bark and potentially reproduce in it (Berryman, 1968b; Ferrell, 1973; pers. obs.) is found in very few other bark beetles. Among them is *D. micans*, which like *S. ventralis* is not known to orient by pheromonal communication (Grégoire et al., 1985).

Scolytus ventralis flies over an eight-week period (Struble, 1957; Ashraf and Berryman, 1969). With such a large flight window, the number of insects flying and attacking at any given time will be relatively low, except at peak flights, which are strongly linked to the presence of

continuous days with temperatures > 27 °C (Ashraf and Berryman, 1969). The fir engraver thus seems to be adapted to an opportunistic existence, responding to stressed trees throughout the growing season as they become available (Raffa and Berryman, 1987). Linking the long period of flight with the facts that there is no strong mass attack behavior (Ashraf and Berryman, 1969), and that when trees are "mass-attacked" there is great variability in attack density (Berryman 1968b, c, 1973), there is presumably less advantage to synchronous flight and attack than in other species of tree-killing bark beetles (Raffa and Berryman, 1987) that are driven by pheromones. This hypothesis is supported by the fact that grand fir is incapable of induced responses to wounding under conditions of intense water stress (Lewinnshon et al., 1993).

As hypothesized for pheromone-mediated mass attack (Alcock, 1982), mass attack by *S. ventralis*, when it does occur, would simply be a consequence of each beetle attempting to maximize its fitness by responding to the volatiles emitted by a potentially suitable host. In support of this hypothesis, Berryman and Ashraf (1970) found that aggregation on a host by the fir engraver is directly associated with the degree of gallery elongation.

Based on my findings and the above discussion, I hypothesize that both sexes of *S. ventralis* are attracted to and aggregate on a tree, first because of odors emitted by the tree, and subsequently because the pioneer boring insects (females) liberate host kairomones, not insect-produced pheromones, by exposing vascular tissue to the air. These compounds would signal other insects of the presence of a suitable host. Close-range phagostimulatory signals might stimulate boring, and mating might be regulated by a combination of stridulatory signals (Ferrell, 1969; Rudinsky et al., 1978; pers. obs.) and close-range pheromonal stimulation. Such close-range signals and their regulation have been suggested by observations of apparent calling and marking behavior revealed by videotape analysis (Table 6, Appendix 2).

3. SECONDARY ATTRACTION IN *P. elegans*

3.1 Introduction

Secondary attraction has been well demonstrated in several *Pityokteines spp.* Ipsenol (2-methyl-6-methylene-7-octen-4-ol) and ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) are reported as pheromones for the sympatric species, *P. curvidens* Ger. and *P. vorontzovi* Jacob, both of which infest silver fir, *A. alba* Mill, in Europe (Harring et al., 1975, Harring and Mori, 1977; Harring, 1978). *Pityokteines vorontzovi* males produce ipsenol, ipsdienol, and *cis*-verbenol, *cis*-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol (Harring, 1978). In the field, *P. vorontzovi* males were more attracted than females to mixtures of ipsenol plus (*R*)-(-)-ipsdienol. Male *P. curvidens* produce ipsenol and *cis*-verbenol (Harring *et al.*, 1975; Harring and Mori, 1977; Harring, 1978). In field tests, both sexes were attracted to (*S*)-(-)-ipsenol; the antipode was biologically inactive and (±)-ipsenol attracted fewer beetles than (*S*)-(-)-ipsenol (Harring and Mori, 1977). Neither species responded to *cis*-verbenol. Males of *P. spinidens* Reitt. produce both ipsenol and ipsdienol, but neither was bioactive in field tests (Harring, 1978).

North American *P. minutus* produce (*S*)-(-)-ipsenol and (*S*)-(+)-ipsdienol in a 1.3:1 ratio. (Camacho-Vera, 1993). In laboratory bioassays, males were equally attracted to both components alone and combined, but females were more strongly attracted to the combination. In the field, only (-)-ipsenol was attractive.

My objectives were to: 1) identify candidate pheromones produced by *P. elegans*; 2) determine the bioactivity of these compounds in the laboratory and field; and 3) investigate which optical isomers mediate attraction of *P. elegans* in the field.

3.2 Methods and Materials

3.2.1 Collection of Insects and Host Materials

Bolts of grand fir, both uninfested and infested with *S. ventralis* and *P. elegans*, were collected in August and September 1993-1995, from felled trees near Coeur d'Alene, Idaho. They were kept at 2 °C until used. To obtain beetles, infested logs were transferred into mesh screen cages held at 24-30 °C; water was sprayed on them every five-six days to prevent desiccation. Emerged beetles were collected daily, sexed by the presence (females) or absence (males) of a setal brush on their frons (Bright, 1976), and held at 4 °C in jars with moistened paper.

3.2.2 Collection and Analysis of Volatiles

Preformed "entrance holes" (1.5 mm diam.), approximately 3 cm apart, were drilled through the bark of fresh grand fir bolts ca. 21 cm long and 12 cm diam. These bolts were set inside separate glass aeration chambers (28 cm long and 15 cm diam.); 200 males, 200 females, or 200 males plus 200 females were added, and allowed to bore into the bark. Uninfested bolts were set in similar devices and used as controls. Air was drawn through the chamber at 1.7 L per min, and then through glass trap (14 mm O.D., 20 cm long) containing Porapak-Q (Byrne et al., 1975). Volatiles were eluted from the trap with 150 ml of distilled pentane, and the eluent concentrated to 5 ml by distillation of solvent through a 30 cm Dufton column.

Differential diagnosis (Vité and Renwick, 1970) of male- and female-produced volatiles allowed identification of sex-specific compounds. GC analyses employed Hewlett Packard 5830A, 5880A, and 5890A instruments equipped with capillary inlet systems, and flame-ionization detectors. Capillary columns (30 m x 0.25 or 0.32 mm I.D.) coated with SP-1000 (Supelco, Bellefonte, Pensylvania) or DB-1 (J & W Scientific Inc., Folsom, California) were used. A Hewlett Packard 5895B fitted with the above columns was employed for coupled GC-mass spectrometry (MS). The injection port, transfer line, and ion source were 260°, 250°, and 200°C, respectively. Helium was the carrier gas for the GC and GC-MS. Compound identities were confirmed by

comparison of their mass spectra with those of authentic samples. Enantiomeric compositions were determined by GC analyses using Chirasil-Dex (8) (30 m x 0.25 mm I.D., V. Schurig, Tübingen, Germany) and Cyclodex-B columns (30 m x 0.25 mm I.D., J. & W. Scientific Inc., Folsom, California).

Captured volatiles were also subjected to coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses (Arn et al., 1975) adapted for intact bark beetles (Gries, 1995). Antennal responses were amplified utilizing a custom-built amplifier with a passive low pass filter and a cutoff frequency of 10 kHz.

3.2.3 Laboratory Experiments

Behavioral activity of captured volatiles was tested in laboratory experiments employing a single-choice, open arena olfactometer (Stock and Borden, 1983). Prior to bioassays, beetles were placed in groups of 10 (sexes kept separately) into Petri dishes with moistened paper and held at 21 °C and 69 lux for 2 h. A group of 10 beetles was released in the centre of a filter paper arena (18.5 cm diam.) and the beetles were allowed 3 min to walk upwind against a gentle air stream (700 ml/min) of medical air, released through a filter-paper lined tube (10 cm diam.) impregnated with volatile extract in pentane or a pentane control stimulus. A beetle that entered a 2.0 x 3.0 cm area at the outlet of the glass tube was classed as a responder.

(*S*)-(-)-Ipsenol (97.1 % optically pure), (±)-ipsdienol (96.9 % pure), (*S*)-(+)-ipsdienol (98.3 % optically pure) and (*R*)-(-)-ipsdienol (98.4 % optically pure) were purchased from Phero Tech Inc., Delta, B. C. Except for results of chemical analyses, enantiomers are referred to hereafter as either (-) or (+). Ipsenone was synthesized by chromic acid oxidation of racemic ipsenol (Brown et al., 1971) and was 97.2 % pure after purification by preparative thin-layer chromatography on silica gel (pentane : ether, 4:1, V/V). The NMR spectral data were in agreement with that reported by Fish et al. (1984).

Attractiveness of test stimuli to walking beetles was tested in three laboratory bioassay experiments (Experiments 13-15). Experiment 13 tested volatiles from male-, female- and male

plus female-infested bolts, using pentane or medical air as control stimuli. Experiment 14 tested synthetic (*S*)-(-)-ipsenol, (±)-ipsdienol or ipsenone, employing 1µl of pentane as a control stimulus. Experiment 15 tested (-)-ipsenol in combination with (±)-ipsdienol alone or with ipsenone. Doses were much lower than in Experiments 13 and 14 and were adjusted to approach the level of ipsenone (4 ng) in 1 bh of aeration (Experiment 13).

3.2.4 Field Experiments

Compounds that were antennally-active or attractive in the laboratory were tested in a mature *Abies grandis/Acer rubrum* forest with well represented Douglas-fir (Steel and Gier-Hayes, 1992), located 10 km north of Coeur d'Alene, Idaho. Twelve-unit, multiple-funnel traps (Lindgren, 1983) (Phero Tech, Inc.) baited with candidate pheromones were deployed in 10 randomized complete blocks, with ≥15 m between traps and 15 m between lines.

All compounds were released from bubble caps (Phero Tech, Inc.) with butanediol added as stabilizer for ipsenol and ipsdienol. Release rates of ipsenol, ipsdienol and ipsenone, determined at 22°, 22° and 27°C (Phero Tech Inc), were 0.15, 0.15 and 0.5 mg per 24 h respectively.

All field experiments tested potential synergism between candidate pheromones. Captured beetles were bagged and frozen until they could be counted and sexed. Experiment 16 tested (-)-ipsenol and (±)-ipsdienol singly and in combination. Experiment 17 tested (-)- or (±)-ipsenol plus (±)-ipsdienol. Experiment 18 tested (-)-ipsenol in combination with (+)-ipsdienol, (-)-ipsdienol, or (±)-ipsdienol. Experiment 19 tested (±)-ipsenol and (±)-ipsdienol at 2:1 or 1:1 ratios. The final experiment (Experiment 20) tested (±)-ipsenol plus (±)-ipsdienol alone and in combination with ipsenone.

3.2.5 Statistical Analysis

Numbers of captured male and female beetles were analyzed separately using SAS computer software (SAS Institute, 1990). Percentages of beetles responding in laboratory bioassays were transformed by $x'=\arcsin\sqrt{x}$ (Zar, 1984) and analyzed with the Ryan-Einot-Gabriel-Welsh Multiple Range test (Day and Quinn, 1989). The same test was used for data from field experiments, but with a $x'=\log_{10}(x+1)$ transformation (Zar, 1984).

3.3 Results

In Experiment 13, female *P. elegans* were more strongly attracted in laboratory bioassays to volatiles from male- than female-infested or uninfested bolts (Table 7). Volatiles from male-infested bolts did not exceed the attractiveness of volatiles from male plus female-infested bolts. Responses by males did not differ among treatments.

Based on differential diagnosis, ipsenone, ipsenol and ipsdienol were produced only by male beetles (Figure 7). All three compounds consistently elicited antennal responses in GC-EAD analyses (Figure 8). Analysis of male-produced volatiles on a chiral column, which separated the optical isomers of ipsenol and ipsdienol, revealed that *P. elegans* males produced (-)-ipsenol and (+)- and (-)-ipsdienol in a 67:33 ratio (Figure 9).

In laboratory bioassays none of the stimuli, (-)-ipsenol, (±)-ipsdienol or ipsenone alone, induced a response that was significantly different from that to the control stimulus (Table 7, Experiment 14). The binary blend of ipsenol plus (±)-ipsdienol and the ternary blend with ipsenone added were equally attractive to females and more attractive than the pentane control stimulus (Table 7, Experiment 15).

In the field, neither (-)-ipsenol nor (±)-ipsdienol alone attracted beetles (Figure 10, Experiment 16). In contrast, (-)- or (±)-ipsenol plus (±)-ipsdienol, attracted females and

Table 7. Responses of walking male or female *P. elegans* in laboratory bioassays Experiments 13-15 (N = 5 groups of 10 insects to each treatment).

Exp. no.	Treatment	Dose	Percent response ($\bar{X}$ ± SE)[a] Males	Females
13	Pentane control	1 µl	26 ± 4.0a	36 ± 5b
	Host volatiles	5 gram-hours	12 ± 3.7a	18 ±.5.5c
	Host + female volatiles	5 beetle-hours	24 ± 6.0a	40± 7.5b
	Host + male + female volatiles	5 beetle-hours	26 ± 10.0a	55 ± 8ab
	Host + male volatiles	5 beetle-hours	42 ± 7.3a	65 ± 5.5a
14	Pentane control	1 µl	38 ± 8.0ab	38 ± 3.7a
	ipsenone	1 µg	18 ± 5.6b	26 ± 6.5a
	(-)-ipsenol	1 µg	40± 3.1a	30 ±.4.4a
	(±)-ipsdienol	1 µg	44 ± 9.2a	22 ± 5.5a
15	Pentane control	I µl	24 ± 11.5a	10 ± 4.4b
	(-)-ipsenol + (±)-ipsdienol	1:1 ng	36 ± 9.7a	42 ± 5.8a
		1:0.5 ng	44 ± 10.2a	38 ± 8.6a
	(-)-ipsenol + (±)-ipsdienol +	1:1:1 ng	36 ± 4.0a	48 ± 7.3a
	ipsenone	1:0.5:1 ng	36 ± 4.0a	42 ± 3.7a

[a] Means within an experiment and column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh Multiple Range Test, $P > 0.05$.

Figure 7. Chromatograms of volatiles produced by male and female *P. elegans* volatiles, revealing male-specific production of ipsenone, ipsenol and ipsdienol.

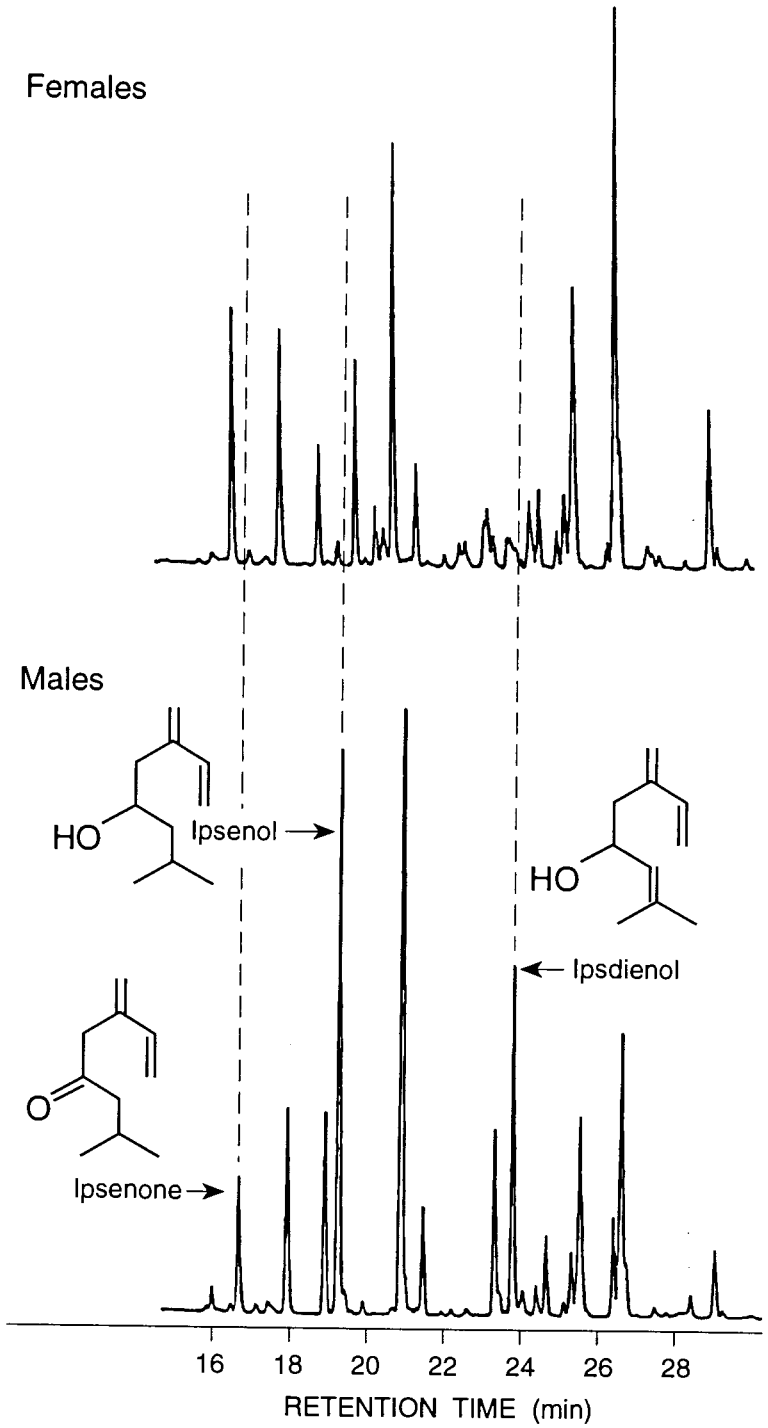

Females
Males
HO
Ipsenol
HO
Ipsdienol
O
Ipsenone
16
18
20
22
24
26
28
RETENTION TIME (min)

Figure 8. Representative recordings of flame-ionization detector (FID) and of electroantennographic detector (EAD: female *P. elegans* antenna) responses to 5 beetle-hour equivalents of volatiles released from a grand fir bolt infested with *P. elegans* males. Chromatography: DB-5 column; temperature program: 50°C (1 min), 3°C/min to 100°C then 20°C/min to 200°C.

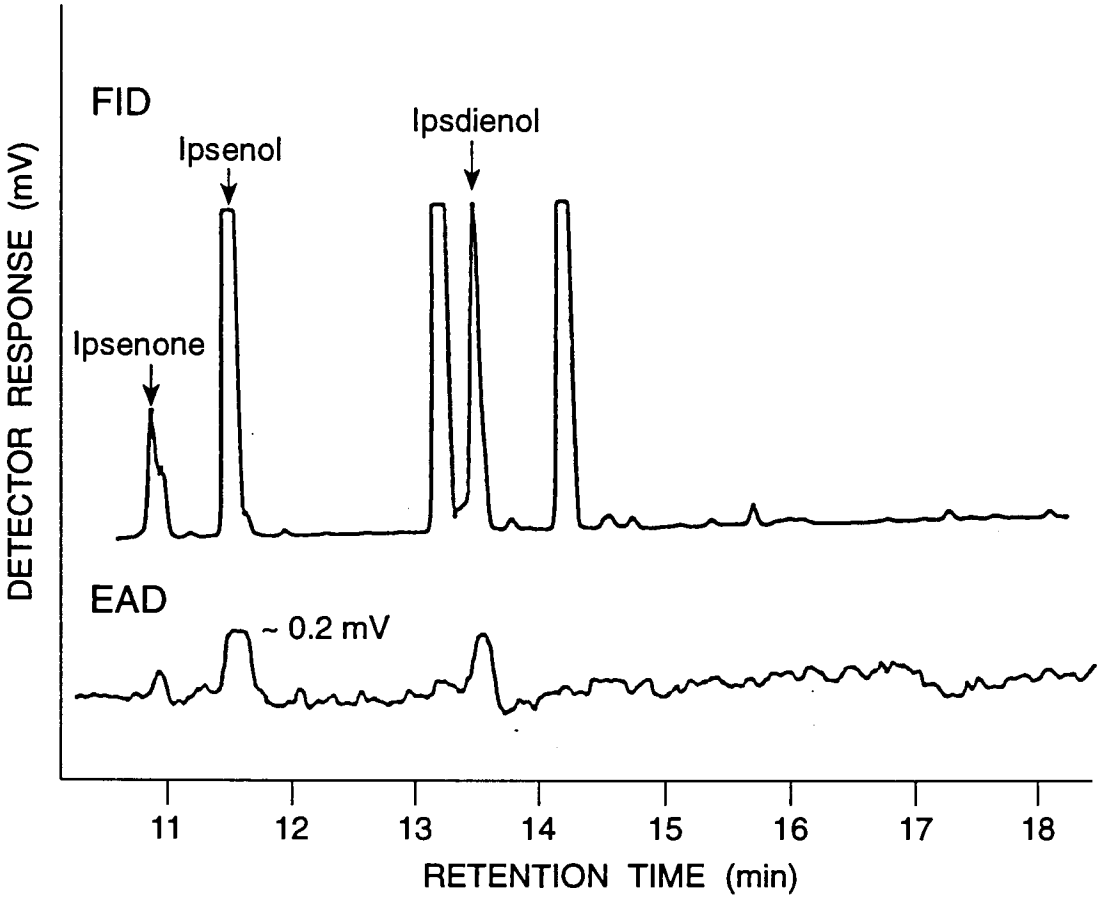

FID
Ipsenol
Ipsdienol
Ipsenone
EAD
~ 0.2 mV
DETECTOR RESPONSE (mV)
11
12
13
14
15
16
17
18
RETENTION TIME (min)

Figure 9. Selected ion *m/z* 137 (ipsenol) and *m/z* 135 (ipsdienol) chromatograms (Hewlett Packard 5985B) of enantiomeric and male *P. elegans*-produced ipsenol and ipsdienol. *M/z* 137 and *m/z* 135 were the parent ions of full scan mass spectra in chemical ionization (isobutane) mode. Chromatography: split injection; Cyclodex-B column; 110 °C isothermal; linear flow velocity of carrier gas: 35 cm/sec, injector temperature, 220 °C.

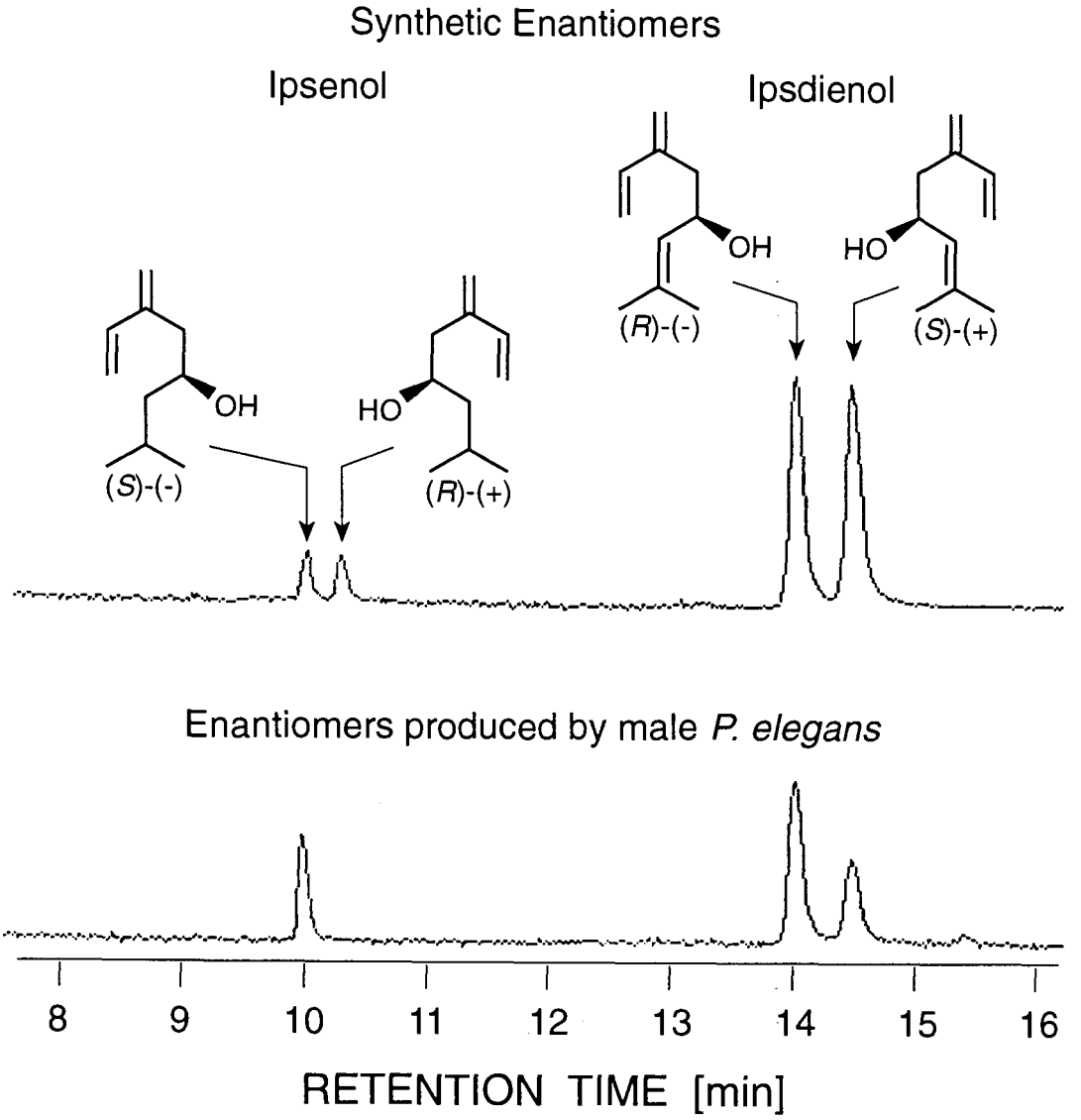
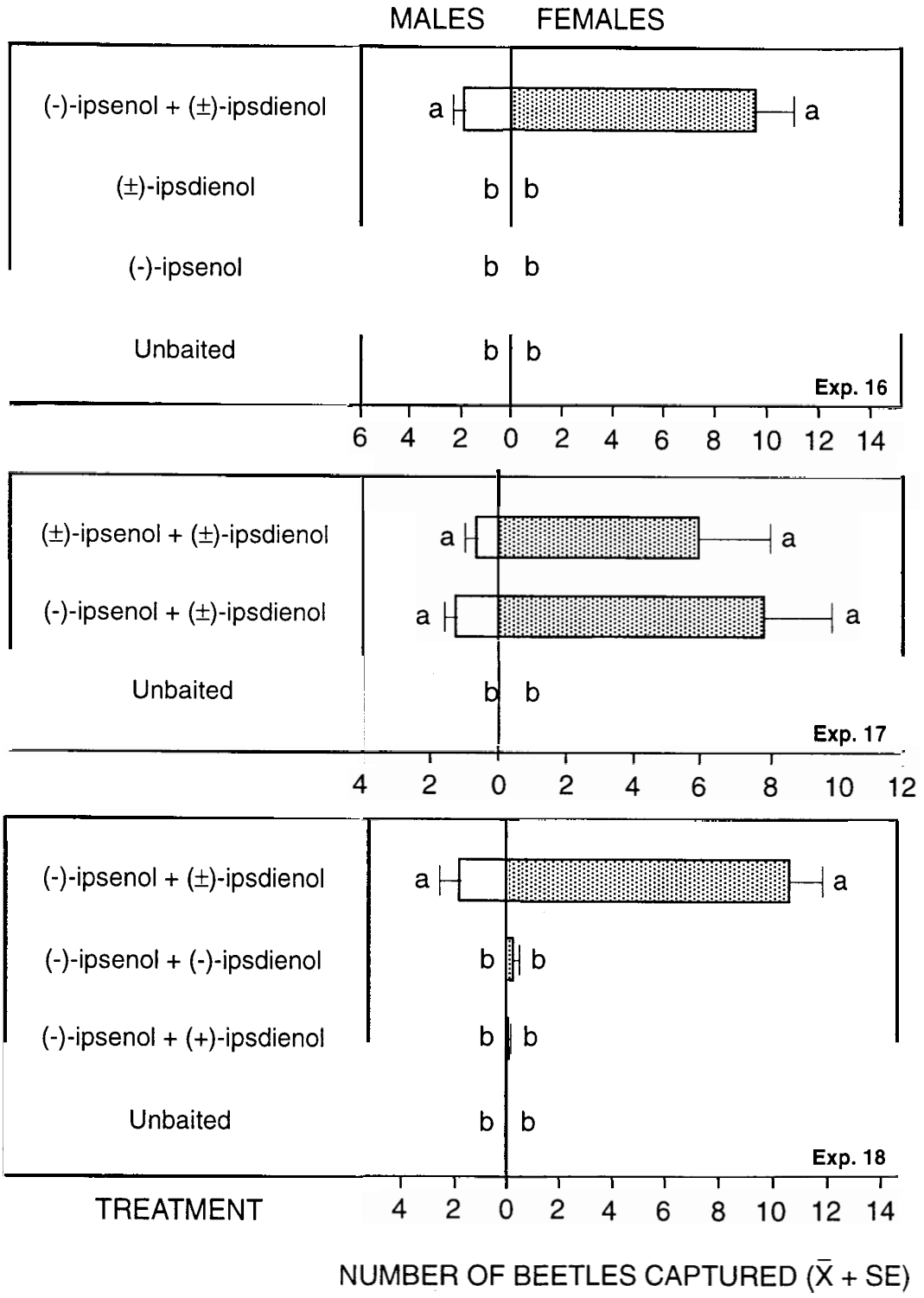

Synthetic Enantiomers
Ipsenol
Ipsdienol
OH
HO
(S)-(-)
(R)-(+)
(R)-(-)
(S)-(+)
Enantiomers produced by male P. elegans
8
9
10
11
12
13
14
15
16
RETENTION TIME [min]

Figure 10. Numbers of male and female *P. elegans* caught in multiple-funnel traps in Experiments 16 and 17 (June 20 - July 6, 1994) and Experiment 18 (July 21 - August 11, 1995) near Coeur d'Alene, Idaho; N = 10 for each experiment. Release rate for (±)-, (+)- and (-)-ipsdienol was 0.15 mg per 24 h. Release rate for (-)-ipsenol in combination with (±)-ipsdienol was 0.15 mg per 24 h, and in combination with (+)- or (-)-ipsdienol was 0.30 mg per 24 h. Release rate for (±)-ipsenol in combination with (±)-ipsdienol was 0.3 mg per 24 h. Bars for each sex within each experiment associated with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh Test, $P > 0.05$.


MALES
FEMALES
(-)-ipsenol + (±)-ipsdienol
(±)-ipsdienol
(-)-ipsenol
Unbaited
a
a
b
b
b
b
b
b
Exp. 16
6 4 2 0 2 4 6 8 10 12 14
(±)-ipsenol + (±)-ipsdienol
(-)-ipsenol + (±)-ipsdienol
Unbaited
a
a
a
a
b
b
Exp. 17
4 2 0 2 4 6 8 10 12
(-)-ipsenol + (±)-ipsdienol
(-)-ipsenol + (-)-ipsdienol
(-)-ipsenol + (+)-ipsdienol
Unbaited
a
a
b
b
b
b
b
b
Exp. 18
TREATMENT
4 2 0 2 4 6 8 10 12 14
NUMBER OF BEETLES CAPTURED (X̄ + SE)

males in low but significant numbers (Figure 10; Experiments 16-18). Both enantiomers of ipsdienol were required to induce attraction (Figure 10, Experiment 18). (±)-Ipsenol plus (±)-ipsdienol at 1:1 or 2:1 ratios were equally attractive (Table 8, Experiment 19). Addition of ipsenone to the 2:1 blend of (±)-ipsenol and (±)-ipsdienol had no effect on the number of beetles captured (Table 8, Experiment 20). Significant numbers of the clerid beetle predator *E. lecontei* responded in Experiment 18 to the two stimulus combinations involving (-)-ipsenol and (+)-ipsdienol (Figure 11). Response was not inhibited by the presence of (-)-ipsdienol.

3.4 Discussion

Enantioselective production of and response to pheromones has been demonstrated to mediate attraction of *P. elegans*. Male *P. elegans* produced (-)-ipsenol, (-)-ipsdienol and (+)-ipsdienol (Figure 9). Lack of attractiveness of these compounds singly or in binary combinations (Figure 10, Experiments 16, 18), and attraction only when all three are present (Tables 7 and 8), provides compelling evidence that each of the three chiral terpene alcohols is an essential component of the aggregation pheromone. The relatively low catches of *E. lecontei* to the temary blend (Figure 11) are probably not due to a missing component. Rather, as I later determined, the experiments were conducted between the two major seasonal flight periods of the insect. *Pityokteines elegans* produced ipsenone, and its antennae can perceive it; however, it was not behaviorally active and is of unknown function.

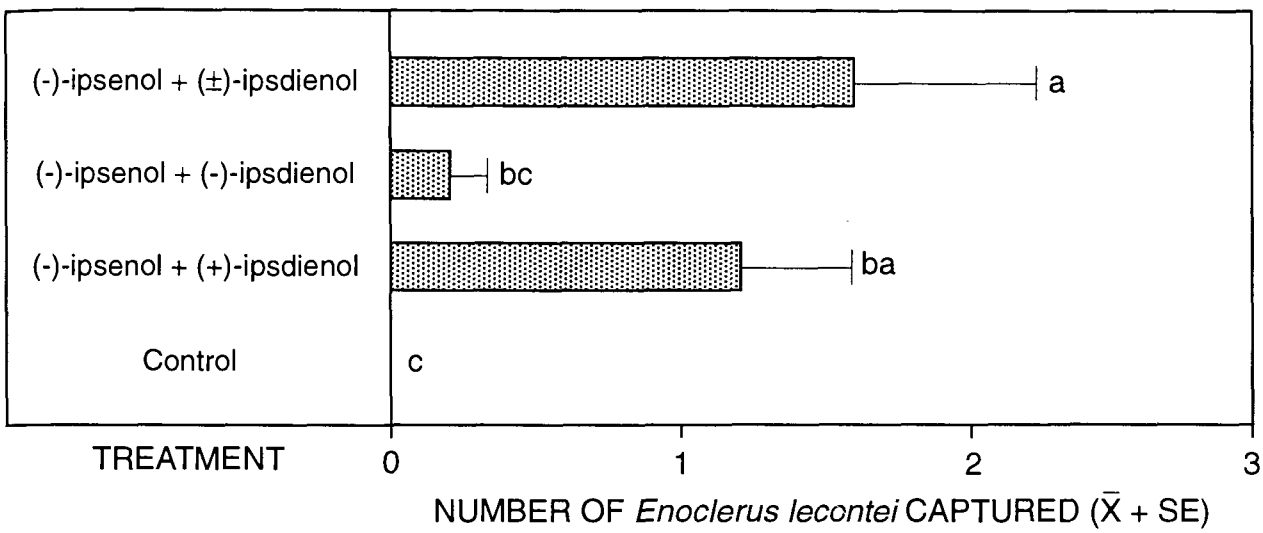
Electrophysiological studies have revealed that species such as *Ips pini* Say, *I. typographus* L., *I. paraconfusus* Lanier, *Scolytus multistriatus*, *S. scolytus and Trypodendron lineatum* (Olivier) have olfactory receptor cells specific to optical isomers of aggregation pheromones (Mustaparta et al., 1980, 1984; Angst, 1981; Wadhams et al., 1982; Tømeras et al., 1984). Based on the specificity of receptor cells in the above species and the absolute requirement of *P. elegans* for the specific optical isomers of ipsenol and ipsdienol, I hypothesize that each of the three components is perceived by specific olfactory receptor cells on *P. elegans*

Table 8. Numbers of *P. elegans* captured in multiple funnel traps in Experiments 19 and 20. Experiment 19 (N = 10) tested two different doses of (±)-ipsenol as a part of a binary pheromone blend and Experiment 20 (N = 10) tested the effect of ipsenone added to the binary blend. Experiments were conducted 10 km north of Coeur d'Alene, Idaho on the following dates: Exp. 19, June 28 - July 20; Exp. 20, June 28 - August 15, 1995.

Exp. no.	Treatment	Respective release rates (mg per 24 h)	Number of beetles captured ($\bar{X} \pm SE$)[a] Males	Females
19	Unbaited trap (control)		0.0b	0.3 ± 0.6b
	(±)-ipsenol + (±)-ipsdienol	0.15 and 0.07	1.5 ± 0.3a	8.3 ± 1.6a
	(±)-ipsenol + (±)-ipsdienol	0.3 and 0.07	1.0 ± 0.4a	8.1 ± 2.6a
20	Unbaited trap (control)		0.0 b	0.2 ± 0.1b
	(±)-ipsenol + (±)-ipsdienol	0.3 and 0.07	4.4 ± 0.7a	22.5 ± 6.0a
	(±)-ipsenol + (±)-ipsdienol + ipsenone	0.3, 0.07 and 0.26	5.2 ± 0.9a	25.9 ± 6.1a

[a] Means within an experiment and column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh Multiple Range Test, $P > 0.05$

Figure 11. Numbers of *E. lecontei* captured in multiple-funnel traps in Experiment 18. See caption of Figure 10 for experimental details. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh Test, $P > 0.05$.


(-)-ipsenol + (±)-ipsdienol
a
(-)-ipsenol + (-)-ipsdienol
bc
(-)-ipsenol + (+)-ipsdienol
ba
Control
c
TREATMENT
0
1
2
3
NUMBER OF *Enoclerus lecontei* CAPTURED ($\bar{X}$ + SE)

antennae. Enantioselective production of, and response to, pheromone has been demonstrated in many species of scolytids (Birch, 1984; Borden, 1985; Byers, 1989). To my knowledge *the P. elegans* communication system is the first example in which three specific enantiomers are required. (+)-Ipsenol is not produced by male *P. elegans* (Figure 9) and it neither enhances nor reduces attractiveness of the ternary pheromone blend (Figure 10, Experiment 17), *P. elegans* may lack receptor sites for (+)-ipsenol. The 67:33 ratio of the (*R*)-(-):(*S*)-(+)-ipsdienol produced by male *P. elegans* is identical but in reverse order to that reported for the pine engraver, *Ips pini* (Say), from California (Fish et al., 1984). However, as for *I. pini* (Miller et al., 1989), individual *male P. elegans* may produce ipsdienol of variable enantiomeric composition.

Ipsenol and ipsdienol are the only pheromones identified in the genus *Pityokteines* (Table 9), suggesting that pheromonal communication by sympatric congeners requires specificity in the enantiomeric composition of pheromones, as revealed in the genera *Ips* (Vanderwel and Ochlschlager, 1987, Seybold, 1992) and *Dryocoetes* (Camacho et al., 1993). *Pityokteines elegans* and *P. minutus* are partially sympatric in the Pacific Northwest. *Pityokteines minutus* is found primarily in subalpine fir, which is not a host for *P. elegans* (Bright and Stark, 1973; Bright, 1976; S. L. Wood, 1982). The ranges of grand fir, the principal host of *P. elegans*, and subalpine fir overlap substantially (Hitchcock et al., 1959). In addition to host preference, differential pheromone blends obviously contribute to reproductive isolation of the two sympatric congeners (Table 9). *Pityokteines elegans* would not be cross-attracted to *P. minutus* which does not produce the critical (-)-ipsdienol. In turn, *P. minutus* is attracted in the field only to (-)-ipsenol (Camacho-Vera, 1993). I suggest that (-)-ipsdienol may deter cross attraction *of P. minutus* to *P. elegans*, since this component is not present in the former species (Camacho-Vera, 1993).

One potential application for the pheromones of secondary bark beetles is to use them for induction of competitive displacement or exclusion (Payne and Richerson, 1985;

Table 9. Male-produced pheromones in *Pityokteines* spp.

Species	Pheromone produced	Attractive enantiomer (location of bioassay)	References
P. curvidens	ipsenol[a]	(-)-ipsenol (field)	Harring et al. (1975) Harring and Mori (1977) Harring (1978)
P. spinidens	ipsenol [a] ipsdienol [a]	Inconclusive results	Harring (1978)
P. vorontzovi	ipsenol [a], ipsdienol [a]	ipsenol [b] + (-)-ipsdienol (field)	Harring (1978)
P. minutus	(-)-ipsenol, (+)-ipsdienol	(-)-ipsenol (field) 1:1 blend of both enantiomers attractive to females (laboratory)	Camacho-Vera (1993)
P. elegans	(-)-ipsenol, (-)-ipsdienol (+)-ipsdienol	(-)-ipsenol, + (-)-ipsdienol and (+)-ipsdienol (laboratory and field)	Figures 8-11

[a] Chirality not yet determined.

[b] Bioactive enantiomers not yet determined.

Borden, 1992; Poland, 1997) of primary, tree-killing species.

My findings of *E. lecontei* attraction to *P. elegans* pheromone (Figure 12) confirm the fact that this predator has a wide variety of prey species which live in different tree-associated ecotypes. Since Wood et al. (1968) trapped *E. lecontei* in traps baited with ipsenol, ipsdienol, and *cis*-verbenol, Seybold et al. (1992) had similar results using lanierone and (-)-ipsdienol, and Miller and Borden (1990) used (±)-ipsdienol. Responses of *E. lecontei* to the combination of (-)-ipsenol + (-)-ipsdienol (Figure 11), seem to have an inhibitory effect if compared with the combination of (-)-ipsenol + (±)-ipsdienol, suggesting that the (-) enantiomer of ipsdienol is the factor promoting such effect. However, *E. lecontei* population examined by Seybold et al. (1992) were attracted to (-)-ipsdienol when combined with lanierone.

4. BIOLOGICAL AND SEMIOCHEMICAL INTERACTIONS BETWEEN *S. ventralis* AND *P. elegans*

4.1 Introduction

An insect guild is considered to be a group of species that exploit the same class of environmental resources in a similar manner (Arthur, 1987). The competitive exclusion principle states that competing species within a guild cannot co-exist if there is no differentiation between their niches. If differentiation of niches occurs, then the co-existence of potential competitors is feasible (Begon and Mortimer, 1986). With some exceptions, e.g., between *I. pini* and *I. paraconfusus* (Light et al., 1983), resource partitioning in bark beetles is rarely absolute (Keddy, 1989; Roughgarden, 1983). Thus varying degrees of niche differentiation must occur, and interspecific competition may also occur with its intensity varying inversely with the degree of niche differentiation.

Interspecific competition occurs when the niches of two or more species overlap and they utilize a limited common resource (Birch, 1957; Arthur, 1987). Competition can occur through direct interference (Brian, 1956), such as the display of threatening behavior, physical attack, or territoriality, and through indirect exploitation (Brian, 1956; Keddy, 1989) by reducing the available pool of resources. Recently Poland (1997) demonstrated that competition between the spruce beetle, *Dendroctonus rufipennis* Kirby, and two secondary bark beetles occurs primarily through synomonal interference by the secondary species with spruce beetle attack, and secondarily through the rapid ability of the secondary species to exploit the phloem resource. Similar competitive interactions are described by Birch and Wood (1975) and Byers and Wood (1980).

The fir engraver is widely regarded as an aggressive (primary), tree-killing bark beetle (Struble, 1957; Ashraf and Berryman, 1969). Like other primary species its galleries may intermingle in part with those of several less aggressive (secondary) species of bark beetles, particularly *P. elegans*, but also *Pityophthorus pseudotsugae*, *Pseudohylesinus grandis, P. granulatus, Scolytus opacus, S. praeceps,* and *S. subscaber* (Struble, 1957; Stark and Borden, 1965; Ashraf and Berryman, 1969). Hertert et al. (1976) always found *P. elegans* in the presence

of *S. ventralis*, and never in sound fir or in the absence of other bark beetles, which reinforces the hypothesis that it is a secondary colonizing species.

In general, the fir engraver colonizes the major part of the bole, while the other species establish themselves mostly in the lower bole or in the thin-diameter crown of the tree (Struble, 1957; Berryman, 1968a). It thus appears that pronounced, but incomplete, resource partitioning occurs within the tree among the guild of bark beetles in grand fir, as reported for other guilds of pine-infesting bark beetles (Dixon and Payne, 1979; Birch et al., 1980; Paine et al., 1981; Rankin and Borden, 1991; Poland and Borden, 1994).

At the population level, interspecific competition can reportedly modify the behavior of the fir engraver through an imperfect or delayed negative feedback process (Berryman and Ferrell, 1989). *Pityokteines elegans* and *P. pseudotsugae* are commonly cited as competitors of the fir engraver (Struble 1957, Stark and Borden, 1965, Berryman, 1973; Berryman and Ferrell, 1988). Berryman (1973) reported that attack densities of *P. elegans* in grand fir were inversely related to the overall population trend of the fir engraver and directly related to its within-tree density. An increased density of attacks by *P. elegans* during the decline of a fir engraver outbreak, was assumed to occur because of a decrease in its food supply in the form of trees killed by *S. ventralis*.

My objective was to investigate the hypothesis that *S. ventralis* and *P. elegans* interact competitively by 1) describing the characteristics of galleries in hosts attacked by both species, 2) comparing the relationships between the two species in attack distribution along the bole, in emergence patterns and gallery establishment, and 3) testing the interspecific role of semiochemicals used in host selection and mass attack by these species.

4.2 Materials and Methods

Collections of insects and host materials, as well as field observations and experiments were done in a mature *Abies grandis / Acer rubrum* forest with well represented Douglas-fir (Steel and Gier-Hayes, 1992), located 10 km North of Coeur d'Alene, Idaho.

4.2.1 Gallery Characteristics and Distribution in Hosts Attacked by Both Species

From 1993 to 1996, 88 bolts (60 cm long) from 13 standing- and 13 fallen-infested grand fir trees were carefully dissected. When co-attack by both species was observed, the characteristics of galleries etched on the exposed sapwood were examined. Additional information regarding gallery characteristics and distribution was obtained from trees used in other experiments, and from more or less monthly field observations from April to October, 1995-1997, on the successive arrival of bark beetles on grand firs that had fallen since the previous summer.

4.2.2 Emergence Pattern

Emergence was recorded separately from two groups of field-collected logs, because one collection comprised logs infested only by *S. ventralis* and *P. elegans*, and the other also included *P. pseudotsugae*. In both cases representative bolts were cut at regular intervals along the bole according to the length of the bole (Berryman, 1968c), yielding 20-30 bolts per tree. The first collection comprised 20 bolts (60 cm long) taken from five trees on 9 October, 1993, and the second 30 bolts (30 cm long), taken from a second group of five trees on 15 October, 1994. To retard desiccation the ends of the bolts were coated with paraffin and water was sprayed on them every 8-10 days. The bolts were caged and the brood beetles allowed to emerge. Emergence holes were counted from a 0.16 x 0.31 m (0.0496 m^2) sample of bark (Berryman, 1968c) removed from all 20 bolts from the first collection and from the 27 bolts of the second collection from which beetles emerged. Data were taken only from those galleries that had their nuptial chambers within the sample boundaries. Following Safranyik and Linton's (1985) technique emergence holes were separated by species using wires of different diameters: 1.9, 1.5, and 1.0 mm *for S. ventralis, P. elegans* and *P. pseudotsugae*, respectively.

4.2.3 Gallery Establishment

Following the above procedures, 10 bolts 60 cm long were cut from the same five trees that were sampled for emergence on 9 October 1993, and 28 bolts were cut from 3 trees on 16 July 1996. A 0.16 x 0.31 m (0.0496 m^2) section of bark was removed from each bolt and the numbers of successful (larval mines present) and unsuccessful galleries were counted for both *S. ventralis* and *P. elegans*.

4.2.4 Distribution of Attack Along the Bole

Fallen trees with exposed sapwood bearing evidence of attack by both *S. ventralis* and *P. elegans* were identified in the field. A string marked by meters was pinned along the entire length of the bole of 10 of these trees. For each 1.0 m long interval, the numbers of galleries of each species intersected by the string were counted, and the diameter of the bole was taken by calipers at the mid-point of each interval.

4.2.5 Semiochemical-Based Interactions

Two experiments evaluated the effect of baiting uninfested grand fir logs (Experiment 21) and of trees undergoing initial attack by *S. ventralis* (Experiment 22) with *P. elegans* pheromone, on the subsequent attack by both species. For Experiment 21, 10 green logs (130 cm long) and 15-20 cm diam., were transported to the field on 30 June 1996 and set upright in two rows with a distance of 15 m between rows and logs. At each position along the rows, one randomly selected log was baited with (±)-ipsenol and (±)-ipsdienol, released at 0.8 and 0.4 mg per 24 h, respectively, from bubble caps (Phero Tech Inc.) stapled to the log. The other log at that position served as an unbaited control. On 11 October, 1996, all logs were transported to the laboratory, where the bark was peeled off and the numbers of successful (with brood) and unsuccessful attacks for each species were counted. One pair of logs was discarded because no attack was initiated by any bark beetle, leaving four successful replicates.

In Experiment 22, 10 standing trees under initial attack by the fir engraver were baited on 14 July, 1996, with (±)-ipsenol and (±)-ipsdienol in bubble caps as above with one set stapled 2 m high on the north face of the tree and a second set at 4 m. A set of ten trees also undergoing attack by *S. ventralis*, each ≥ 8 m from one of the treated trees, were used as controls. All trees were girdled with an axe frill at ca 50 cm above ground to reduce the tree's ability to resist attack. On 11 October, all trees were felled and evaluated for attack; 9 baited trees and 9 control trees were attacked heavily enough to justify sampling. From these trees 20, bolts (40 cm long) were cut at regular intervals along the bole from the butt to the top. The bolts were pooled by tree and placed in rearing cages at 24 °C. The numbers of emerged beetles of each species were recorded.

Three field trapping experiments were conducted in 1997 to assess the effect of semiochemicals used in aggregation by each species on the response to aggregants by the other species. Experiments 23 and 24 tested the effect of SB3 (Chapter 2) and (±)-*exo*-brevicomin (Appendix 2, Section 7.4), respectively, on the catches of *P. elegans* in multiple-funnel traps baited with (±)-ipsenol and (±)-ipsdienol released from bubble caps (Phero Tech Inc.) at 0.8 mg and 0.4 mg per 24 h, respectively. The *exo*-brevicomin was dispensed from a flexlure (Phero Tech Inc.) at 0.28 mg per 24 h. These experiments had the following treatments: unbaited control, *P. elegans* pheromone [(±)-ipsenol and (±)-ipsdienol], and *P. elegans* pheromone with SB3 (Experiment 23) or (±)-*exo*-brevicomin (Experiment 24). They were laid out as randomized complete blocks, with 10 replicates each on the following dates: Experiment 23, 25 May - 24 June; and Experiment 24, 26 June - 7 July. Captured beetles were stored and their sex determined as in Chapter 2 and 3. A final Experiment (No. 25) was designed to test the effect of *P. elegans* pheromone on the response by *S. ventralis* to grand fir volatiles, and had the same treatments as Experiment 23 and ran from 25 June - 21 July. The experiment failed to catch sufficient numbers of *S. ventralis* to analyze, apparently because concurrent active logging activity of grand fir within 10 m of the experiment set up such a potent source of competing

attraction, that hundreds of *S. ventralis* could be observed crawling on the boles of each fallen tree.

4.2.6 Statistical Analysis

The relationships between *S. ventralis* and *P. elegans* (and in one instance *P. pseudotsugae*) for the numbers of emergence holes, successful and unsuccessful galleries, and parental galleries were analyzed by Spearman's rank correlations (Zar, 1984). These correlations were compiled, not to describe in detail the relationship between the parameters being compared, but solely to determine if there was a negative association between the fir engraver and the secondary species. Numbers of parental galleries (Experiment 21) and emerged insects (Experiment 22) were transformed by $\sqrt{(X + 0.5)}$ to normalize the data and stabilize the variances between replicates, and results for baited and control treatments for each species were compared using *t*-tests (Zar, 1984). The numbers of insects captured in traps (Experiments 23 and 24) were transformed by $\log_{10}(x+1)$ and analyzed by ANOVA followed by the Ryan-Einot-Gabriel-Welsh (REGW) Multiple Range test (Day and Quinn, 1989). All analyses employed SAS computer software (SAS Institute, 1990).

4.3 Results

4.3.1 Observations on Gallery Characteristics and Distribution

At stand level, I found fallen grand firs that were colonized by either or both of the two species. Among trees attacked only by *S. ventralis*, I found three trees in which the attack density was so low that each gallery (successful or unsuccessful) was isolated from each other. At the tree level, a discrete segregation of the two bark beetles occasionally occurred with *P. elegans* in the crown of the tree and with *S. ventralis* occupying the rest of the bole. At the other extreme, the galleries sometimes completely intermingled throughout the bole (Figure 12).

When the galleries of *P. elegans* and *S. ventralis* were intermingled, two types of interaction apparently occurred. In the first, *S. ventralis* had evidently attacked in the previous year and had stimulated the tree to form a reaction zone, probably bordering the area infected by *T. symbionticum*. Neither *P. elegans* nor any other bark beetle built galleries within or transecting this zone. In the second type of interaction there was complete overlap of galleries of both species, in the absence of reaction zones. Larval *S. ventralis* avoided contact of their galleries with any type of gallery including those of conspecific larvae, or areas with blemished phloem. Conversely, in addition to using phloem not touched by *S. ventralis*, *P. elegans* adults or larvae built galleries that freely crossed those of *S. ventralis*.

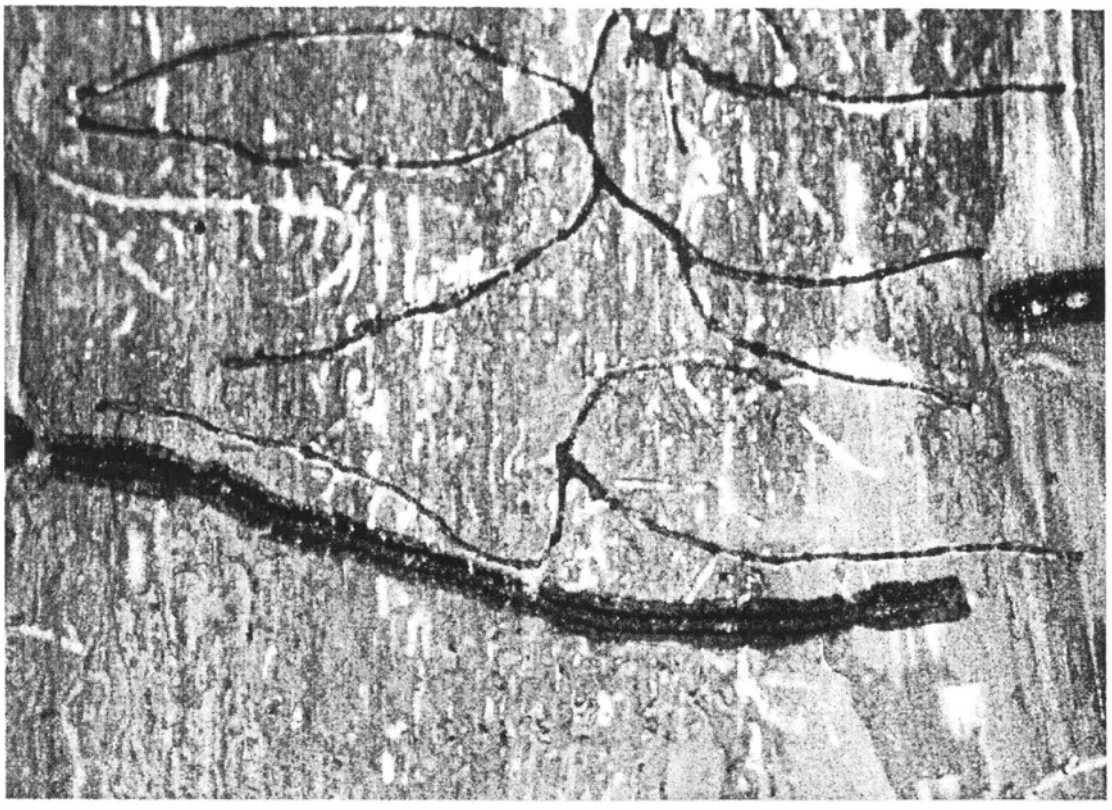
In three fallen trees (28, 60 cm long bolts) 12 of 206 (5.8 %) of *P. elegans* galleries exposed by debarking were initiated directly from galleries made by *S. ventralis* (Figure 13). Seven of these were near the entrance hole to the adult gallery and five were derived from exit tunnels made by emerging brood beetles.

Observations on the succession of species attacking fallen material showed that *P. elegans* and *P. sericeus* flew at least twice a year, one flight occurring from April to May and a second from July to August. *Scolytus ventralis* had only one flight period in mid-summer. On two occasions when *S. ventralis* brood beetles were emerging from a tree attacked the previous year, *P. elegans* and *P. sericeus* arrived and bored galleries into the still available phloem of the same tree. In no case was *S. ventralis* observed attacking areas of bark that were already infested with heterospecific bark beetles.

4.3.2 Comparative Resource Exploitation by *S. ventralis* and *P. elegans*.

Because emergence by *P. elegans* from co-attacked bolts in 1993 was very low, no relationship between numbers of emergence holes made by *P. elegans* and *S. ventralis* could be observed (Figure 14). Data from 1994 revealed significant negative relationships between

Figures 12 and 13. Photographs of galleries of *S. ventralis* and *P. elegans* etched on the sapwood of a naturally-colonized grand fir, showing typically intermingling galleries without contact between maternal tunnels (Figure 12) and of a more rare example in which a *P. elegans* gallery has been initiated off one arm of the *S. ventralis* gallery (Figure 13). Sv = *S. ventralis* galleries; Pe = *P. elegans* galleries. Galleries of both species marked by felt pen.



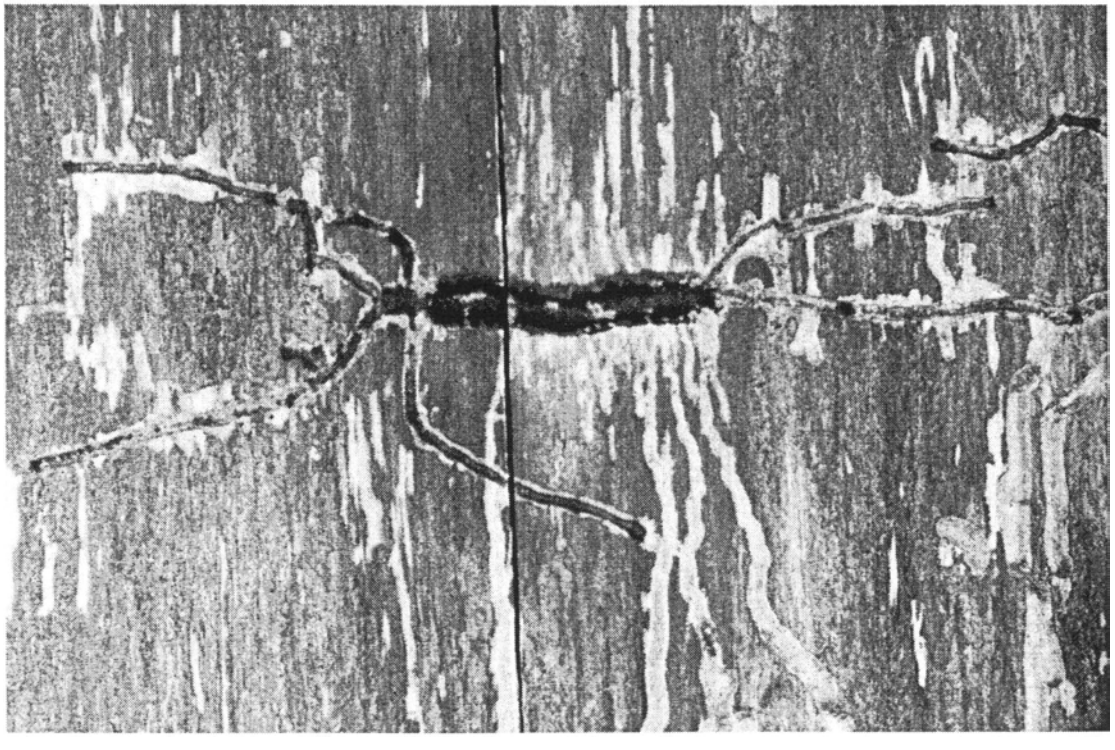
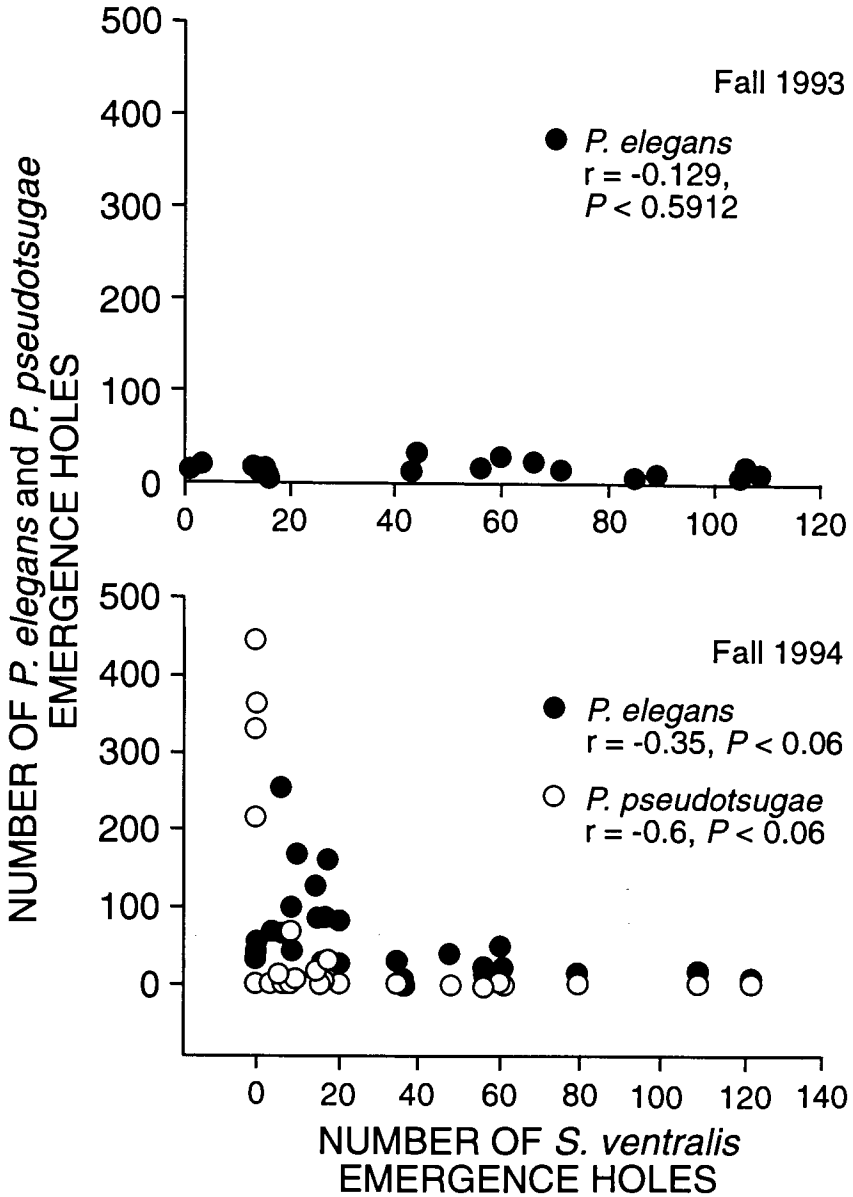


Figure 14. Relationships between emergence holes of *S. ventralis* and *P. elegans* in 0.0396 m^2 sections of bark removed from each of 20 bolts taken from five field-attacked grand firs near Coeur d'Alene, Idaho on October 1993 and of *S. ventralis, P. elegans* and *P. pseudotsugae* emerging from identical bark sections from 27 bolts from five trees sampled on 15 October 1994. Spearman's correlation coefficients given for each relationship.


Fall 1993
P. elegans
r = -0.129,
P < 0.5912
Fall 1994
P. elegans
r = -0.35, P < 0.06
P. pseudotsugae
r = -0.6, P < 0.06
NUMBER OF P. elegans and P. pseudotsugae EMERGENCE HOLES
NUMBER OF S. ventralis EMERGENCE HOLES
0
100
200
300
400
500
0
20
40
60
80
100
120
140

emergence of *S. ventralis* and *P. elegans*, and particularly between *S. ventralis* and *P. pseudotsugae* (Figure 14). Analysis of gallery establishment by *S. ventralis* and *P. elegans* also disclosed a negative interspecific relationship (Figure 15), regardless of whether or not unsuccessful galleries were eliminated from analysis. The patterns of attack distribution along the boles of 10 different trees were so variable that no consistent relationship between the two species was evident (Figure 16). However, for the pooled data there was a negative relationship between the numbers of galleries built by *S. ventralis* and *P. elegans,* but no such relationship between gallery numbers and diameters of the bole (Figure 17). In all of the relationships between emergence or galleries of the two species (Figures 14, 15, 17), there were numerous instances of moderately high values for both beetle species, indicating a fair measure of tolerance between the species and a concordantly weak degree of resource partitioning.

4.3.3 Semiochemical-Based Interactions

In Experiment 21 (Figure 18), unattacked logs baited with *P. elegans* pheromone sustained similar numbers of *S. ventralis* attacks as unbaited control logs, indicating that (±)-ipsenol and (±)-ipsdienol had no inhibitory effect on attack by *S. ventralis*. Attack by *P. elegans* occured only and significantly on baited logs. Both baited and unbaited logs were attacked at low levels by *S. opacus*.

Bolts taken from trees that were baited with *P. elegans* pheromone at the time they were under initial attack by *S. ventralis*, produced significantly lower numbers of emergent *S. ventralis* than bolts from unbaited control trees (Figure 18, Experiment 22). The opposite effect occurred for *P. elegans*. Small numbers of *Crypturgus borealis* Swaine emerged from the baited trees, while equivalent numbers of *S. opacus* emerged from the control trees. *Pityokteines elegans* was strongly attracted to the blend of (±)-ipsenol and (±)-ipsdienol in Experiments 23 and 24 (Figure 19), verifying the identification of the principal pheromone components in Chapter 3. Neither SB3 (Experiment 23) nor *exo*-brevicomin (Experiment 24) caused a significant change in the catches of *P. elegans* in traps baited with its pheromone (Figure 19). The predator *E. sphegeus*

Figure 15. Relationships between densities of all galleries and successful galleries (with larval mines) of *S. ventralis* and *P. elegans* in 0.0496 m^2 bark sections removed from 38 bolts (60 cm long) taken from five naturally-attacked grand firs near Coeur d'Alene, Idaho on October 1993 (10 bolts) and from three trees on 16 July 1996 (28 bolts). Spearman's correlation coefficients given for each relationship.

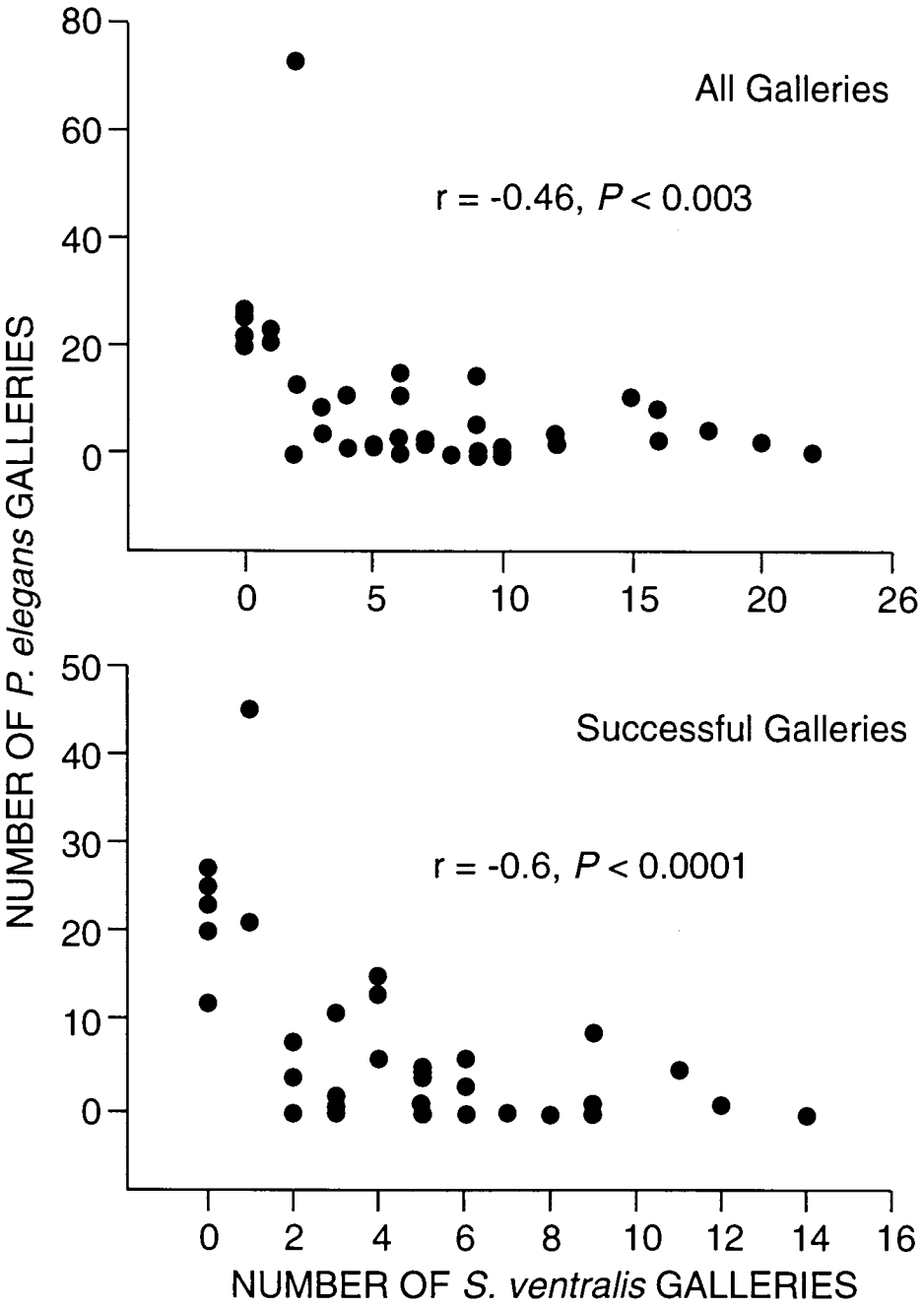

All Galleries
r = -0.46, P < 0.003
Successful Galleries
r = -0.6, P < 0.0001
NUMBER OF P. elegans GALLERIES
NUMBER OF S. ventralis GALLERIES
0
5
10
15
20
26
0
2
4
6
8
10
12
14
16
0
20
40
60
80
0
10
20
30
40
50

Figure 16. Relationships between number of gallery systems of *S. ventralis* and *P. elegans* with respect to tree diameter. Data obtained from ten fallen grand firs on July 17 - 19, 1997, 10 km north of Coeur d'Alene, Idaho.

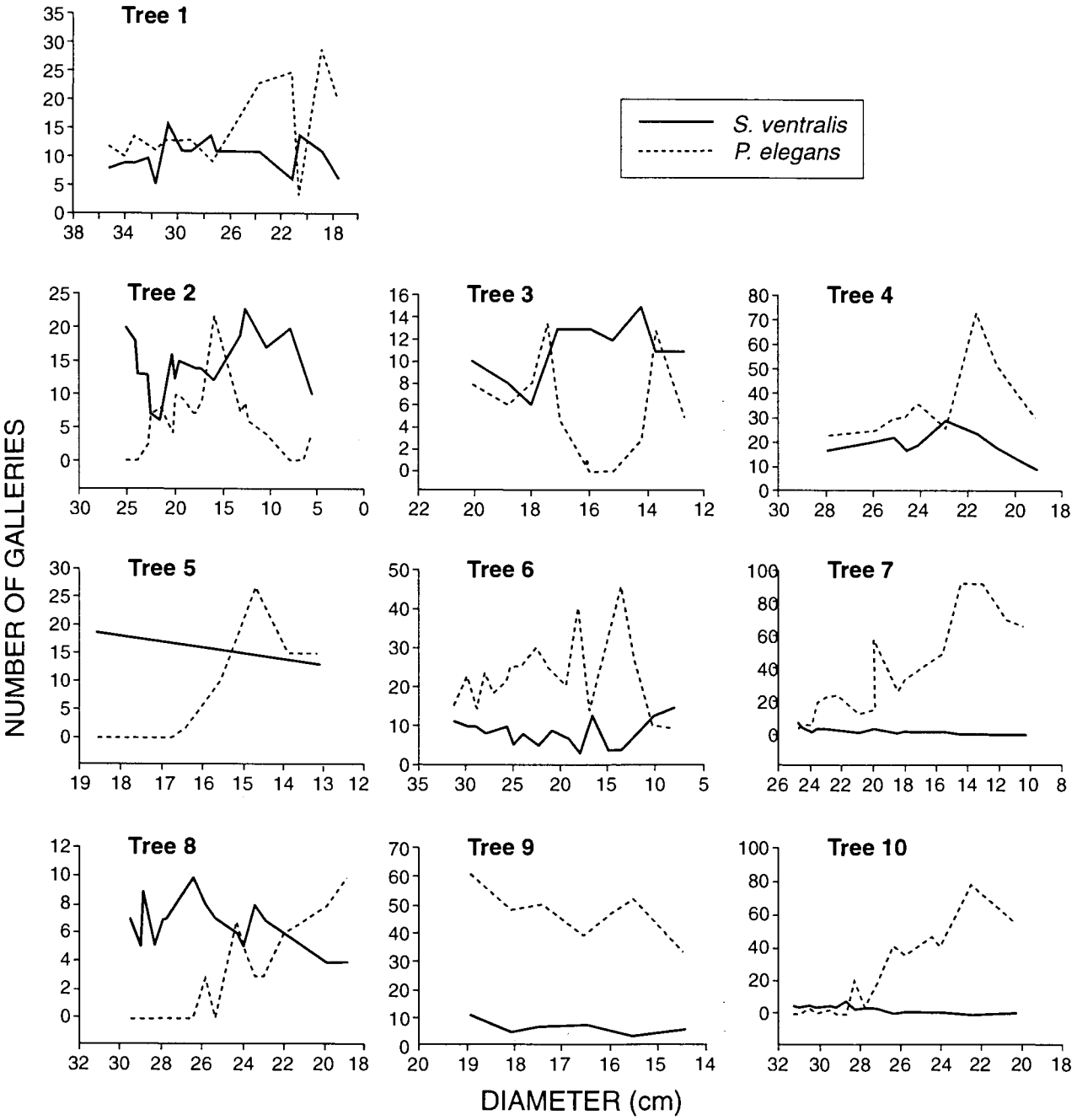
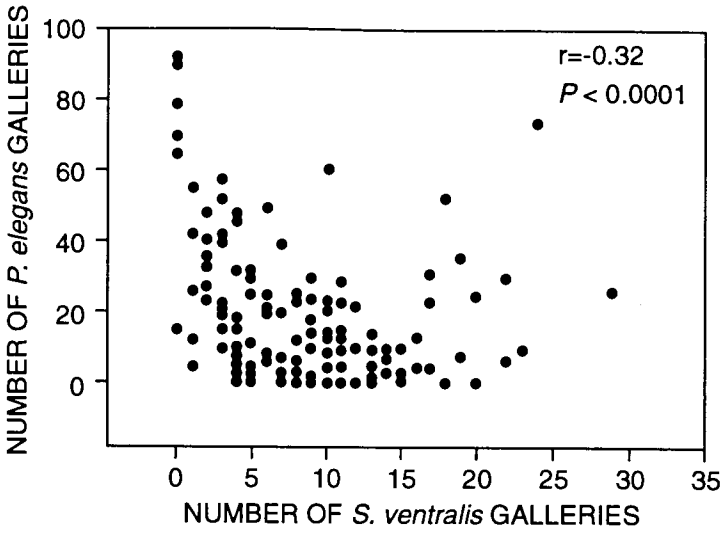
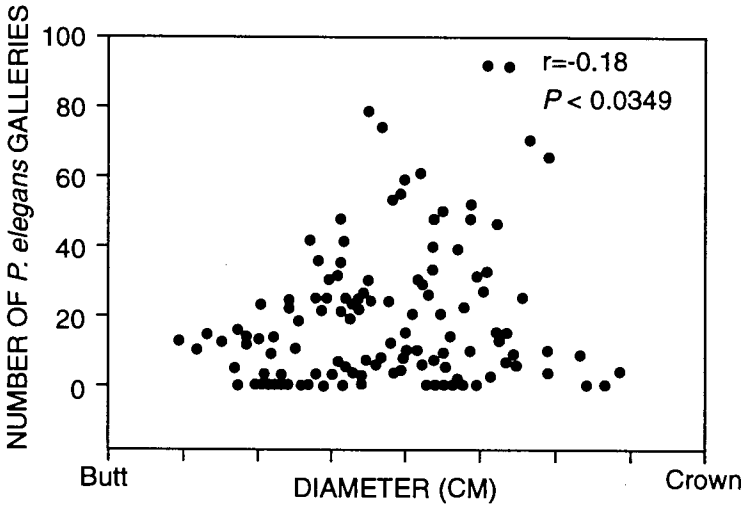

Tree 1
Tree 2
Tree 3
Tree 4
Tree 5
Tree 6
Tree 7
Tree 8
Tree 9
Tree 10
S. ventralis
P. elegans
NUMBER OF GALLERIES
DIAMETER (cm)

Figure 17. Relationships between 1157 galleries of *S. ventralis* and 2454 galleries of *P. elegans* counted along longitudinal transects of the boles of 10 different fallen grand firs (top), and for the same galleries between the numbers of galleries of *S. ventralis* (middle) and *P. elegans* (bottom) and the diameters of the bole in the 1 m intervals in which the galleries were counted.


NUMBER OF P. elegans GALLERIES
100
80
60
40
20
0
r=-0.32
P < 0.0001
0
5
10
15
20
25
30
35
NUMBER OF S. ventralis GALLERIES


NUMBER OF P. elegans GALLERIES
100
80
60
40
20
0
r=-0.18
P < 0.0349
Butt
DIAMETER (CM)
Crown

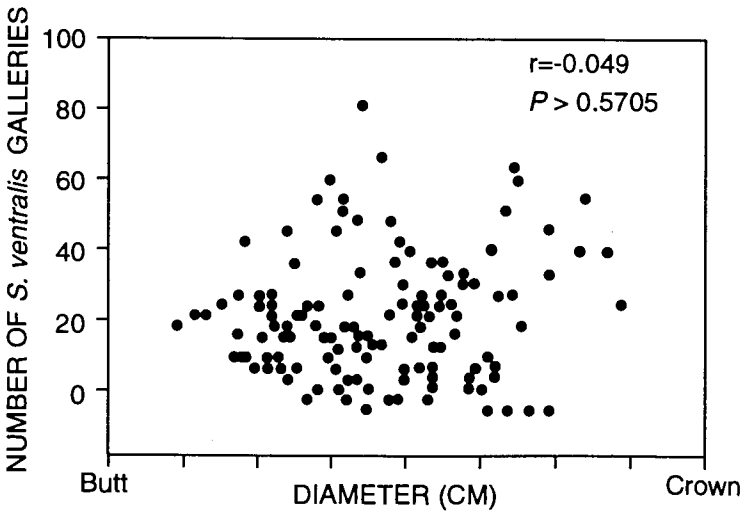
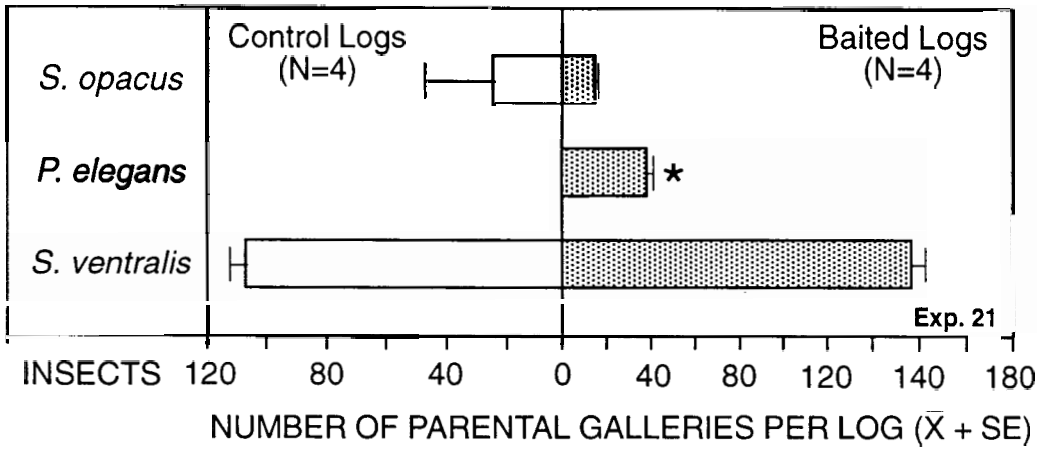

NUMBER OF S. ventralis GALLERIES
100
80
60
40
20
0
r=-0.049
P > 0.5705
Butt
DIAMETER (CM)
Crown

Figure 18. Numbers of galleries of three species of bark beetles in unbaited grand fir logs or logs baited with *P. elegans* pheromone (Experiment 21), and numbers of *S. ventralis* and *P. elegans* (Experiment 22) that emerged from bolts taken from grand fir trees that were under initial attack by *S. ventralis* and then were baited 2 and 4 m high on the bole with *P. elegans* pheromone. Baits contained (±)-ipsenol and (±)-ipsdienol released at 0.8 and 0.4 mg per 24 h, respectively. Bars with asterisks indicate significant differences in paired catches between baited and control, *t*-test, $P < 0.05$.


Control Logs
(N=4)
Baited Logs
(N=4)
S. opacus
P. elegans
S. ventralis
*
Exp. 21
INSECTS 120 80 40 0 40 80 120 140 180
NUMBER OF PARENTAL GALLERIES PER LOG (X̄ + SE)

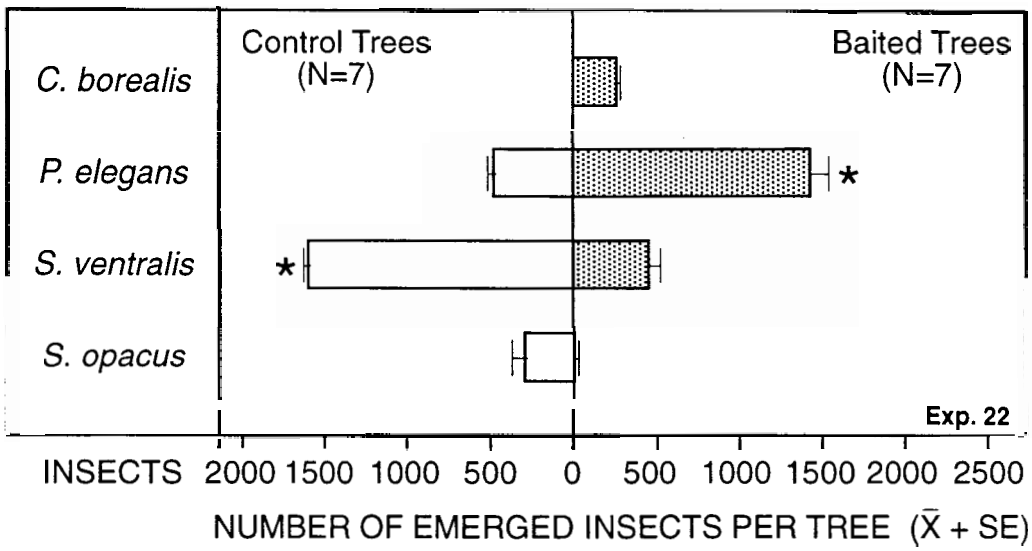
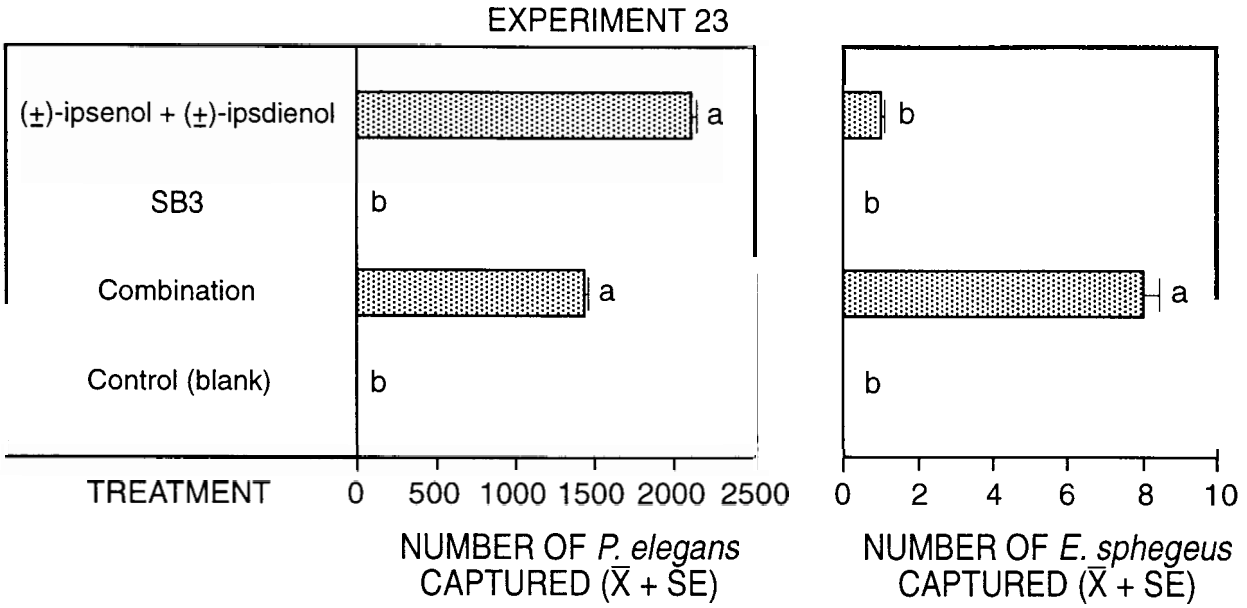
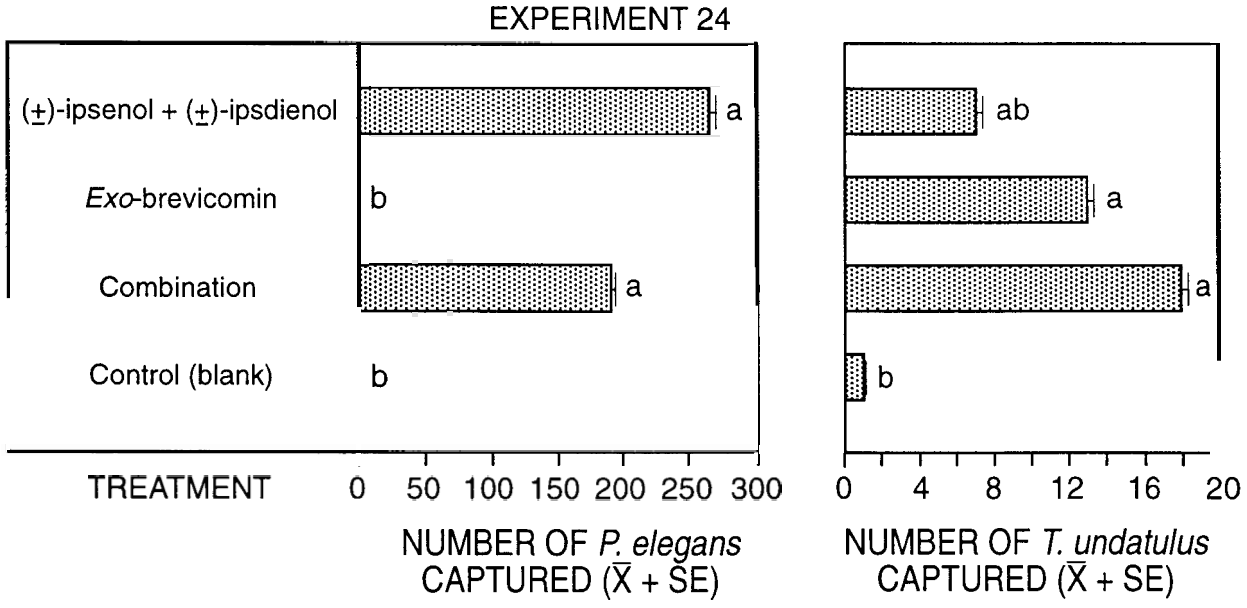

Control Trees
(N=7)
Baited Trees
(N=7)
C. borealis
P. elegans
S. ventralis
S. opacus
*
*
Exp. 22
INSECTS 2000 1500 1000 500 0 500 1000 1500 2000 2500
NUMBER OF EMERGED INSECTS PER TREE (X̄ + SE)

Figure 19. Numbers of beetles captured in multiple funnel traps in Experiments 23 and 24. Experiment 23 (25 May - 24 June, 1997) tested the effect of synthetic host volatiles (SB3) and Experiment 24 (26 June - 7 July, 1997) tested for the effect of *exo*-brevicomin on the response of *P. elegans* to its own pheromone. N = 10 for each Experiment. Release rates as follows: (±)-ipsenol and (±)-ipsdienol, 0.8 and 0.4 mg per 24 h, respectively; SB3 762 mg per 24 h; *exo*-brevicomin, 0.28 mg per 24 h.


EXPERIMENT 23
(±)-ipsenol + (±)-ipsdienol
SB3
Combination
Control (blank)
TREATMENT
a
b
a
b
0 500 1000 1500 2000 2500
NUMBER OF P. elegans CAPTURED (X̄ + SE)
b
b
a
b
0 2 4 6 8 10
NUMBER OF E. sphegeus CAPTURED (X̄ + SE)


EXPERIMENT 24
(±)-ipsenol + (±)-ipsdienol
Exo-brevicomin
Combination
Control (blank)
TREATMENT
a
b
a
b
0 50 100 150 200 250 300
NUMBER OF P. elegans CAPTURED (X̄ + SE)
ab
a
a
b
0 4 8 12 16 20
NUMBER OF T. undatulus CAPTURED (X̄ + SE)

was caught at significant levels in Experiment 23 in traps baited with the combination of *P. elegans* pheromone and SB3, and in Experiment 24 *T. undatulus* was captured at significant levels in traps baited with *exo*-brevicomin combined with *P. elegans* pheromone or by *exo*-brevicomin alone (Figure 19).

4.4 Discussion

The interspecific relationships for emergence holes (Figure 14), gallery establishment (Figure 15) and attack distribution (Figures 16 and 17), are consistent with a moderate level of competitive interaction between *S. ventralis* and *P. elegans*. This interaction apparently takes place primarily through exploitative competition (Brian, 1956; Keddy, 1989) and occurs in two ways. In the first type of interaction *P. elegans* would arrive at a new host before *S. ventralis* and would pre-empt the phloem resource. Because *S. ventralis* was never observed to superimpose its attacks on those of any other species, it would then be relegated to attack unexploited portions of the bole, or another host. In the second type of interaction, *P. elegans* would arrive on a new host at the same time as *S. ventralis* or shortly after, and would compete directly by occupying tissue which *S. ventralis* would then avoid. Also my data (Figures 14 -16) show a certain degree of tolerance by *P. elegans* of *S. ventralis* infestation. The ability to coexist is reflected by my observations of *P. elegans* using *S. ventralis* entrance and/or exit holes (Figure 13), and of the attack by *P. elegans* and *P. sericeus* of a tree infested in the previous year by *S. ventralis* and still occupied by the latter species. Although coexistence is possible, neither species thrives to the same extent as when each is the sole occupant of the phloem resource (Figures 14, 15).

My results do not support the hypothesis that semiochemical-based interference competition occurs between the two species. *Scolytus ventralis* colonized *P. elegans* pheromone-baited logs to the same extent as unbaited control logs (Figure 17, Experiment 21) and neither *exo*-brevicomin produced by *S. ventralis* nor SB1-3 (to which *S. ventralis* responds) had any effect on *P. elegans* (Figure 17, Experiment 22).. My results are contrary to those of Poland and Borden (1997) who found that response by the spruce beetle, *D. rufipennis,* to traps baited with

its pheromone was reduced by the presence of the pheromones of the two secondary bark beetles *Dryocoetes affaber* Mannerheim and *Ips tridens* Mannerheim. In addition, attack by the spruce beetle on felled spruces was reduced by the presence of pheromones of the secondary species, a clear evidence of interference competition (Poland, 1997).

The attack distribution by *P. elegans* and *S. ventralis* in and among trees can be elucidated in part by examining the seasonal flight periods for both species. In the early spring (May), *P. elegans* flies and colonizes mainly winter-fallen material. In one instance I found it to have mass attacked a severely weakened standing tree that bore no sign of having resisted attack. Since *P. elegans* utilizes an aggregation pheromone, it can concentrate its attack in very defined hosts or areas therein. If there is some host material not colonized, it could be either used by re-emergent parent *P. elegans* as occurs with *P. sparsus* (Hosking and Knight, 1976), or by early-emerging fir engravers in June. My observations indicate that *S. ventralis* would avoid attacking bark previously attacked by *P. elegans*, and that the presence of *P. elegans* galleries nearby would limit the size of the expanding *S. ventralis* galleries, accounting in part for the negative association between successful galleries and emergence of the two species (Figures 14, 15). In the middle of the summer most of the host material remaining from the winter would be drying out very fast and *S. ventralis* during its peak flight period would visit and attempt to colonize standing trees throughout the stand. At the end of the summer, numbers of flying *S. ventralis* would be decreasing but a partial or complete emergence (third flight) of current-year-brood *P. elegans*, as occurs for *P. sparsus* (Hosking and Knight, 1976), would provide beetles that would either attack areas of a tree not infested by *S. ventralis* or would superimpose attacks on areas colonized by *S. ventralis*, if the latter still have phloem available. These late attacks by *P. elegans* would further account for the negative relationship between the two species.

The mechanisms of recognizing suitable hosts also differ between the two species, and probably are associated with resource partitioning. Neither species segregates its attacks strongly on the basis of diameter (Figures 16, 17). *Scolytus ventralis* strongly responds (in laboratory), to host volatiles from living trees, with a preference for those weakened by root rot (Figure 2). On other hand, *P. elegans* did not respond at all to extracted or synthetic bark oil, suggesting that it is

adapted to attack only deteriorating bark, including that attacked by *S. ventralis*. Such bark is apparently recognized and avoided by the latter species. For example, I observed two trees that were completely colonized (last larval instars) by *P. elegans*, on which many *S. ventralis* adults landed, and left soon thereafter, presumably because they recognized a deteriorated phloem resource that was already colonized by a heterospecific competitor. When *S. ventralis* attacks a vigorous tree at low density, causing the tree to create reaction zones around the sites of beetle and fungal invasion (Raffa, 1991), *P. elegans* and other bark beetles do not superimpose their attacks on these areas from outside the tree and recognize and avoid them when mining through the phloem.

It is evident from the above observations that *S. ventralis* and *P. elegans* occupy overlapping, but not identical niches, and thus neither species will competitively exclude the other (Begon and Mortimer, 1982). *Scolytus ventralis* requires unblemished phloem, vectors a lethal fungus, and with the aid of this fungus can kill a host, although it also exploits recently dead or dying hosts. *Pityokteines elegans* is more of an opportunist, that will attack fresh phloem in very weakened hosts, but will also superimpose its attacks on those of *S. ventralis*. Because resource partitioning is only partial, competitive interaction through exploitative competition is possible, and can have a roughly equal negative effect on both species. In this sense, the partially tolerant relationship is more like that of *I. pini* and *P. knechteli* (Poland and Borden, 1994) than the intolerant relationship between *I. pini* and *I. paraconfusus* (Light et al. 1983). Based on this interpretation of the interaction between *P. elegans* and *S. ventralis*, pheromone -induced competitive displacement (Poland, 1997) (Figure 18, Experiment 22) could have very limited potential for regulating the size of *S. ventralis* populations.

5 CONCLUSIONS

The study of semiochemical-based communication in the grand fir, *S. ventralis* and *P. elegans* system has proven to be a very challenging subject. The conspicuous aggregative behavior of the fir engraver in grand fir and the fact that other *Scolytus* species do have a pheromone, constantly pulled my research toward the hypothesis of pheromone-driven host colonization, leading to a plethora of negative results. In addition, *P. elegans* pheromone proved to be complex and chirally specific, and the interaction between the two species lacked the expected strong competitive nature found in several other interspecific associations in the Scolytidae. Nonetheless, several pertinent conclusions can be drawn from my research.

1. Exhaustive experiments do not reveal the presence of an aggregation pheromone during the process of aggregation and host attack by *S. ventralis*.
2. GC-EAD analyses of volatiles from uninfested grand fir, which demonstrated that *S. ventralis* can perceive numerous host volatiles, and laboratory and field bioassays that demonstrated strong attraction to blends of these volatiles, coupled with the evidence that *S. ventralis* has no aggregation pheromone, lead to the conclusion that this species exclusively uses host kairomonal signals during host selection and colonization.
3. The primary attraction response of *S. ventralis* relies on a complex blend of host kairomones, probably requiring the presence of bornyl acetate, and is stronger in response to the bark volatiles of root rot-infected trees in the laboratory than uninfected trees.
4. Videotape evidence of probable pheromone-based calling and marking behavior by female *S. ventralis* (Appendix 2) suggests that close-range chemical communication, such as that promote by a sex pheromone, may occur in this species.
5. Inaccuracy of determining the sex of *S. ventralis* using traditional methods involving morphology of the frons and the abdominal declivity, and complete accuracy when the length of the fifth sternite is included as a sex-specific trait, indicates that the latter characteristic should be used when experiments or data analysis demand complete separation of the sexes (Appendix 1).

6. Bioassay results with *P. elegans* clearly indicate that this species utilizes an aggregation pheromone during host colonization, but the nature of primary attraction for *P. elegans* remains unclear.
7. The results of GC-EAD analyses, as well as laboratory and field bioassays demonstrate that *P. elegans* requires the synergistic interactions between (-)-ipsenol, (+)-ipsdienol, and (-)-ipsdienol to induce a positive orientation response.
8. Analysis of the biology of *S. ventralis* and *P. elegans* and the relationships between them when they attack hosts, either separately or together, indicate that they do not occupy the same niche, and would not be expected to exclude competitively each other from a host in nature.
9. Based on experiments that reveal no evidence of semiochemical-based interference competition between *P. elegans* and *S. ventralis*, only exploitative competition can account for the negative relationship between the two species in such parameters as attack distribution and successful emergence.
10. Responses by the clerid predators *T. undatulus* and *E. lecontei* to the same volatiles as used to mediate aggregation by *S. ventralis* and *P. elegans* provide new evidence to support the hypothesis that natural selection favors the evolution of host-based ecotypes of bark beetle predators.

6. Appendix 1

Sex-specific traits in *S. ventralis*

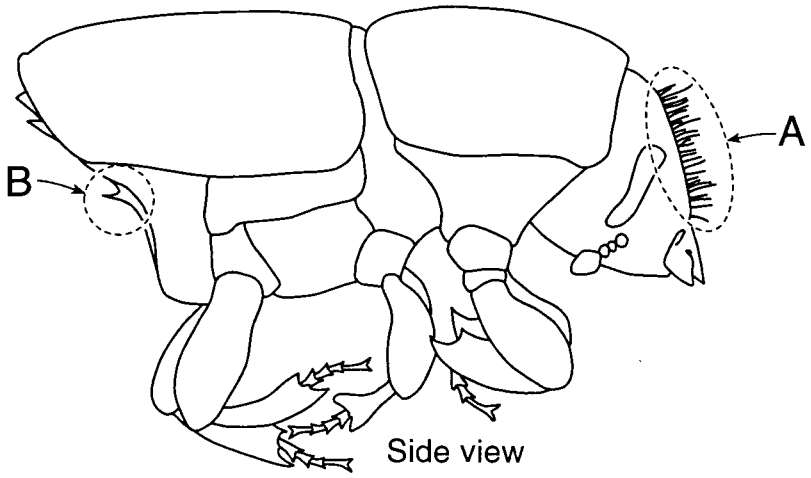
Investigation of the chemical ecology of any insect requires accurate determination of sex. Sex determination in *S. ventralis* is based on these external morphological characteristics (Figure 20): 1) length of fifth sternite in females greater than the combined length of the third and fourth sternite; in males the lengths are about the same (Blackman, 1934); 2) a small, definite, carinated median tubercle with a pointed apex located on the posterior margin of the second sternite in males, such structure poorly developed or absent in females (Edson, 1967; Bright, 1976; S. L. Wood, 1982); and 3) frons of males relatively flat with a hair brush; in females frons slightly convex with few hairs (Edson, 1967; Ashraf and Berryman, 1969).

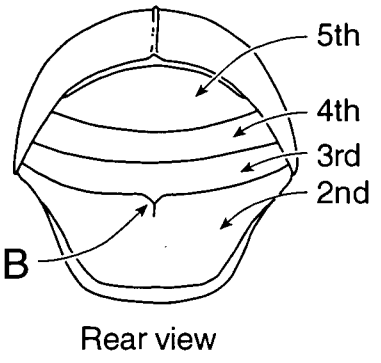
Morphological characteristics 2 and 3 were used to separate by sex several hundred *S. ventralis* that emerged from logs of grand fir collected near Coeur d'Alene , Idaho, with the male group being the most distinct. From the female's group, 100 beetles were dissected and inspected for internal sexual organs. Sixteen proved to be males, with external morphological characters resembling those of females. The frons was slightly convex with a very thinned and spread vestiture; the tubercle in the abdominal declivity was absent or very poorly developed. Since the beetles were recently emerged, these atypical males had not lost hairs from their frons due to boring or courtship, and their abdominal declivities were clear of frass which could have hidden the tubercle.

A second approach was to use characteristic 1, the length of the fifth sternite (Blackman, 1934). I separated another sample of 100 insects by sex using this characteristic, with 100 % accuracy as confirmed by dissection.

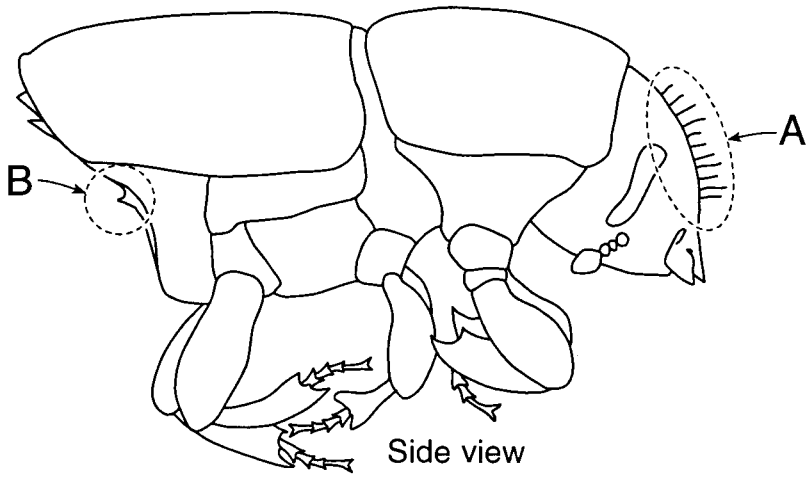
Another possible sex-specific trait is the pair of vaginal palpi found in *Scolytus scolytus* and *S. multistriatus*, which are a pair of sclerotized conical tubules extended posteriorally from

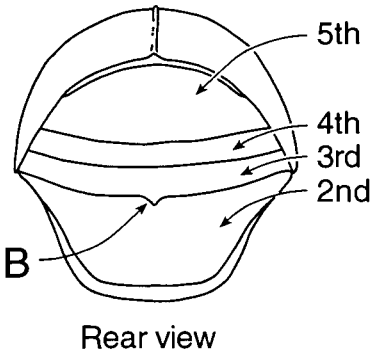
Figure 20. External morphological characteristics used to sex *S. ventralis*. (A) Frons, (B) tubercle, abdominal declivity showing 2nd to 5th sternites.





♂ *Scolytus ventralis*





♀ *Scolytus ventralis*

an accessory gland between the anal and genital openings to the exterior of the beetle (Fisher, 1937; Gore at al, 1977). Chemical and morphological evidence link the vaginal palpi and the accessory gland with the production, storage and release of the pheromone α-multistriatin by female of *S. multistriatus* (Gore et al., 1977). Vaginal palpi have been reported also in *S. scolytus* (Fisher, 1937) and also appear in photographs of *S. quadrispinosus* (Goeden and Norris, 1964). No other *Scolytus* spp have been examined for this female-specific trait. I readily observed vaginal palpi in female *S. ventralis* and *S. opacus*.

Accurate determination of sex of the fir engraver was also achieved by gently squeezing the insect's abdomen until its tip was protruded. In females the vaginal palpi were exposed, and in males the aedeagous was extruded. Based on these observations, I recommend sexing *S. ventralis* by the sex-specific length of the fifth abdominal sternite and by the presence in females of vaginal palpi.

7. Appendix 2

Summary of Experiments and Observations Yielding Negative Results on the Presence of an Aggregation Pheromone in *S. ventralis*

This appendix provides detailed methodology in support of the experiments and observations summarized in Table 4 (Chapter 2).

7.1 Laboratory Aerations

Volatiles produced by groups of male, female or mixed sex *S. ventralis* were collected on Porapak Q following the aeration procedure described in Chapter 2. Unless otherwise noted, the aerations were performed under laboratory conditions (24 °C and 12:12 h L:D regime), and prior to the aerations all insects were kept for up to 4 days at ca 4 °C inside glass jars with moisten paper. Fresh grand fir logs were used as host material. In order to reveal possible variations in the volatile production by insects alone and /or insects plus host material, aerations were performed in the following sequence over a 36 mo. period.

1. Separate aerations of 104 unfed virgin females and 72 unfed virgin males in glass tubes (30 cm long and 3 mm diam.) for 312 h.
2. Aeration of 17 unfed virgin females and males in the same glass tube for 384 h.
3. Separate aerations of bolts attacked by 130 virgin females or 130 virgin male beetles introduced into preformed entrance holes inside metal drums (70 cm long and 45 cm diam.) for 168 h in darkness and at ca 25 °C.
4. Aeration of 130 females and 130 males boring together into a bolt under the same conditions as above, but for 120 h. Females were introduced into the drums three days before the males.

5. Aerations of 154 virgin females or 236 virgin males individually boring for 96 h into grand fir logs inside glass aeration chambers (Chapter 2). All beetles had emerged the same day and were not cold-stored. One uninfested control log (20 cm diam.) was aerated under the same conditions.
6. Aerations of 312 females and 175 males boring in grand fir bolts in separate glass chambers for 216 h, with Porapak Q capture devices replaced with fresh devices after 60 h.
7. Above aerations repeated with 303 females and 120 males.
8. Aeration of 184 females and 106 males insects boring together (only 97 couples) into bolts inside of a glass chamber for 192 h.
9. Aerations of males and females added daily to separate glass chambers containing grand fir bolts. First day, 84 females and 84 males. On second, third and fourth days, 83 and 32, 163 and 79, and 38 and 13 males and females, respectively, were added. Aerations were conducted inside of glass chambers and continued for 288 h. All insects had emerged on the same day they were used and were not cold-stored.
10. Aeration of 50 females and 50 males inside the same glass tube (as above) for 48 h. All insects had emerged the day they were used and were not cold-stored.
11. Aerations in glass chambers of beetles separated by a mesh from infested and uninfested grand fir bolts. First aeration: 50 females and 50 males in separate chambers with a screen-enclosed fresh uninfested grand fir bolt for 192 h. Second aeration: 25 males with logs infested by 25 females for 192 h. Third aeration: 26 females and 26 males in separate chambers boring into fresh grand fir logs (insects and bolts screen-enclosed), to which were added 26 females and 21 males, respectively, for 168 h. In this last aeration, the enclosed insects were placed on the logs two days before the introduction of the other insects. Porapak Q traps were replaced after 48 h and a second for the remaining 120 h.

Differential diagnosis (conditions in Chapter 2) of the volatile extracts obtained in the different aerations showed no conspicuous sex-specific peaks, as well as no peaks that indicated insect-produced compounds. This conclusion was supported by GC-EAD analysis, on male and female antennae, of the captured volatiles from aeration 9, which also revealed no candidate pheromones. The only clear trend was to observe high quantities of host volatiles from those aerations in which the insects were actively boring into bolts or branches of grand fir.

7.2 Field Aerations

Aerations of trees in the field were done in a mature *Abies grandis / Acer rubrum* forest with well represented Douglas-fir (Steel and Gier-Hayes, 1992), located 10 km North of Coeur d'Alene. Adapting the methodology of Browne et al. (1979), 1 m long sections of the bole of six standing grand fir trees ($\bar{X}$ dbh = 83.18 cm) under attack by *S. ventralis* were wrapped in a clear plastic sheet open at the top with the sealed bottom exiting in to a Porapak Q trap. Air was drawn at 1.5 L per min through the trap under vacuum from a portable pump connected to a power generator. Two unattacked trees ca. 3 m from the trees under attack were also wrapped and sampled as controls. The aerations lasted 49 ± 1.5 h. GC analysis (conditions as in Chapter 2) of the trapped volatiles showed no conspicuous differences between infested and uninfested trees, reinforcing the results of laboratory aerations. GC-EAD analysis of volatiles from one infested-tree aeration showed no differences from the antennal responses to volatiles from control trees.

7.3 Juvenile Hormone Treatments

Juvenile hormone (JH) and JH analogues have been reported to promote pheromone production in the following scolytid species: *Ips paraconfusus* (Borden et al., 1969; Chen et al., 1988), *Trypodendron lineatum* (Fockler and Borden, 1973), *Pityogenes chalcographus* L. (Francke et al., 1977), *Dendroctonus brevicomis* LeConte (Hughes and Renwick, 1977a, b), *Pityokteines* spp (Harring, 1978), *Scolytus scolytus* (Blight et al., 1979), and *Dendroctonus*

ponderosae Hopk. (Conn, 1981). These results led me to test the hypothesis that if *S. ventralis* produces an aggregation pheromone, treatment with JH should disclose evidence of that pheromone.

Accordingly, female and male *S. ventralis* that had emerged from logs within the past 24 h, or were excised from the bark after feeding in fresh grand fir logs for 48 h, were topically treated with 1 µg or 10 µg of methoprene (isopropyl 11-methoxy-2,6,11-timethyl-2,4-dodecadienoate), an juvenile hormone analogue, in 1 µl of pentane (Fockler and Borden, 1973; Pierce et al., 1986). Newly emerged control beetles were treated only with 1 µl of pentane. Each treatment was replicated with 20 beetles. Treated beetles were individually placed in gelatin capsules attached to a suitable grand fir log under room conditions. The insects were excised from the bark after 24 h, sorted by treatment, and their entire bodies were immediately extracted in dry ice-cold pentane. The extracts were filtered through glass wool and analyzed by GC (conditions in Chapter 2).

A second 20-replicate experiment was designed following a modification of the technique used by Chen et al. (1988), with 1 and 10 µg of methoprene or pentane applied just to the circular area of the log circumscribed by the perimeter of the gelatin capsule holding the beetle in contact with the bark. The beetles would contact or ingest the methoprene in the act of boring into the log. Only unfed insects were employed and they were placed on the log 2 h after the methoprene was applied to the bark surface. Pentane treatments were used as controls. Extracts were obtained and analyzed as above. In no instance did GC analysis of extracts from treated and control insects reveal any compounds that changed in amount following treatment, nor were there any evident sex-specific compounds.

7.4 Gland Extracts

Because the accessory glands with their associated vaginal palpi have been implicated in the production of α-multistriatin by *S. multistriatus* (Gore et al., 1977; Appendix 1), I examined female *S. ventralis* for similar evidence of pheromone production. Abdominal tips of 289 female

beetles that had fed in grand fir bark for 5 days were obtained by dissection. The abdominal tip was exposed by a gentle squeeze of the insect's body and removed from the rest of the body with microscissors. Confirmation, of the presence of the accessory gland with vaginal palpi in the cut off tip, was achieved by microscopic observations of 20 tips obtained in this manner. The tips were immediately immersed in ice-cold pentane, and the extract was analyzed by GC-EAD using male antennae.

GC-EAD analysis (see conditions on Chapter 2) revealed the presence of *exo*-brevicomin (exo-7-ethyl-5-methyl-6, 8-dioxabicyclo[3.2.1] octane) in trace amounts in the female extract. *Exo*-brevicomin is an important pheromone component in the genera *Dendroctonus* and *Dryocoetes* (Borden, 1985; Camacho et al., 1993). Very little is known about its biosynthesis (Vanderwel and Oehlschlager, 1987).

Laboratory and field trapping experiments, in which *exo*-brevicomin was tested for an effect on *S. ventralis* were inconclusive. However, there could be a link between the fact that *exo*-brevicomin is present in the female abdominal tip and the "marking" and "calling" behavior revealed by videotape analysis (see below) . It is possible that *exo*-brevicomin serves as a short range cue for male *S. ventralis* to detect a site with females in the vicinity, a courtship inducer, an epideictic or spacing pheromone (Prokopy, 1981) that accounts in part for the non-random distribution of *S. ventralis* attack (Berryman, 1968c), or as an allelochemic that suppresses attack by potential competitors.

7.5 Visual Observations and Videotaping

7.5.1 Materials and Methods

Laboratory observations were made on at least 40 individuals placed on cut bolts of grand fir and allowed to attack freely in the laboratory. Similar observations were made in the field from at least 80 individuals. Beetles that took flight during laboratory observations were replaced. However, when this behavior prevailed, the log was replaced because it was

considered not suitable. Sexes were not marked or tagged, but when a particular behavioral pattern was seen, the sex or sexes involved were determined.

Through the use of micro- and macro-videotaping techniques the behavior of 20 insect pairs was documented under laboratory conditions (22 °C and full ambient light) during 20 h of taping. Twenty insects of each sex were allowed to run freely and bore into fresh bolts (50 cm long and 15 cm diam) that were vertically (10 insect pairs) or horizontally (10 insect pairs) set on a laboratory bench (scenario 1). Ten more beetles of each sex were placed on one log (5 pairs) and on 10 green branches with needles (5 pairs), in which females (20 in the log and 20 in the branches) had been boring for 48 h (scenario 2).

The optical equipment was a Panasonic WV-CD 110 camera, fitted to either a Karl Zeiss surgical microscope or to a 50 mm (1:2.8) SMC Pentax-A macro lens, coupled to a 50 mm Vivitar 2x macro focusing teleconverter macro, depending on the degree of magnification needed. The camera was connected to a Panasonic GX4, Multifunctional Video Cassette Recorder AG-195, and the image was monitored with a Panasonic Color Video Monitor CT-1330-MC. Illumination was provided by a cold-light source (Schott KL1500).

Observations were also made in the field. On 2 July, 1996, five uninfested 1.3 m-long logs of grand fir were set up vertically in an open area of a grand fir forest near Coeur d'Alene, Idaho. When the first *S. ventralis* started to arrive at the logs, visual observations were made from 0900 h to 1800 h for three consecutive days. Behavior of the arriving insects, especially boring, courtship, and mating activities, were recorded.

7.5.2 Results and discussion

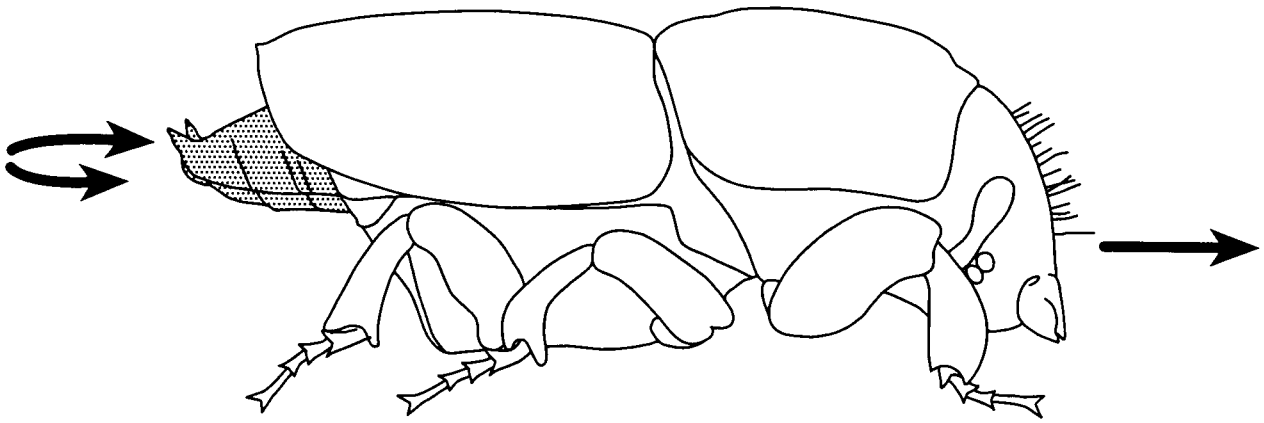
Videotaped observations, scenario 1. Most of the beetles were very mobile and walked very fast compared to other bark beetles (e.g. *D. pseudotsugae*) observed under the same conditions. Often when two individuals (sex not determined) approached each other a very brief "wrestling" occurred by means of rubbing their frons. After about 10 min on the log, many beetles appeared to select a spot where they would bore into or etch the bark. At this time several females were observed performing either one of two very conspicuous behaviors. The majority

were extruding the tip of the abdomen and rubbing it on the log surface as they walked in a zigzag fashion (Figure 21) that suggested a "marking" behavior as occurs in some moths (Swier et al., 1976; Colwell et al., 1978; Szocs and Toth, 199; Teal et al., 1981; West and Bowers, 1994). Each marking bout lasted about 30 sec and was repeated several times for up to 5 min. The second pattern, although displayed by only two females, may have been a variation of the previous behavior. These females extruded and raised the very swollen tip of their abdomens, (Figure 21) and walked in a zigzag fashion, emulating moth "calling" behavior (Figure 21) (Turgeon and McNeil, 1982; Alford and Diehl, 1985; West and Bowers, 1994). Such activity lasted < 30 sec, and was repeated several times in 5 min. Both "marking" and "calling" were not disturbed by nearby or walking beetles of either sex, and "marking" occurred whether the logs were placed horizontally or vertically. The "marking" behavior was seen again when two females were exposed to 0.25 mg of bark oil in an olfactometer (Chapter 2). To my knowledge these two behavioral patterns have not been reported for any other bark beetle.

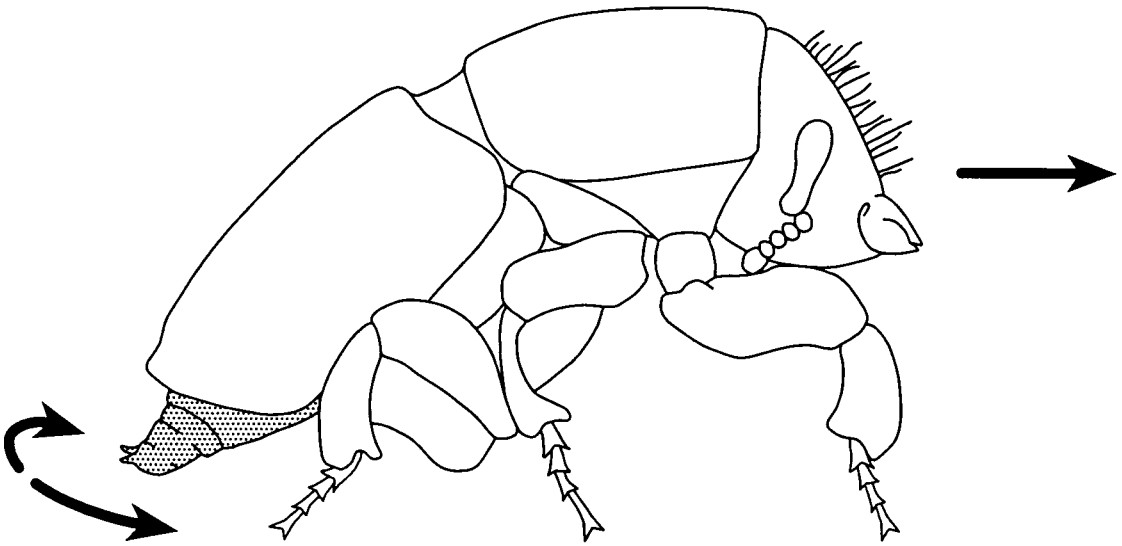
Videotaped observations, scenario 2. All 10 females that had bored for 48 h into the bolt were observed in courtship and later on in mating, as were the other 10 females boring into branches, mainly at the twig crotches. The courtship and mating observed was very similar to that found for *S. multistriatus* (Svihra and Clark, 1980), and confirmed the observations by Struble (1957) and Ashraf and Berryman (1966) for *S. ventralis*. All 40 couples mated outside the gallery, despite the length of female's gallery and the presence of the "nuptial" chamber, which is apparently used more as a turning point inside the gallery than it is for mating.

Each female waited for males within the gallery entrance, with her head facing inwards. Courtship started when the male began to nod his head rhythmically against the abdominal declivity of the female, possibly also stridulating. This action lasted up to 1 min. The male then turned around and without inverting himself, as in some other scolytid genera, where the male is upside down during mating (Francke-Grosmann, 1950; Reid, 1958), copulated with the apparently passive female who remained standing at the entrance hole. Defecation by females

Figure 21. Diagram of apparent "marking" and "calling" behavior observed in videotape analysis of female *S. ventralis* with conspecifics on the bark of grand fir logs. Arrows represent movement and direction.



"Calling" Behaviour



"Marking" Behaviour

occurred frequently during courtship. Sometimes during courtship the males extruded their aedeagus once or twice before they initiated copulation. In some cases females arrived at an entrance hole occupied by another female, and the resident female was induced by the intruding female to leave the entrance hole whereupon the intruder replaced her.

On several occasions when a female was deep within a gallery, a male was observed to enter the gallery possibly enticing the female to the entrance by means of "nodding" on female's abdomen, just as reported for *S. mali* (Doganlar and Schopf, 1984). This episode required 2-4 min and was followed inmediately by copulation. All females copulated at least twice with the same male. In periods of observation up to 3 h long, no male was observed to remain with a female after copulation. Males copulated on average with three different females. Copulation lasted an average of 45 ± 3.5 sec.

Field observations. Observations in the field confirmed the laboratory observations on courtship and mating behavior including the promiscuous behavior of both males and females. Two males were heard stridulating while in courtship. The rivalry displays between insects of the same sex were more pronounced, and females were seen stealing entrance holes more often. The speed at which 10 beetles walked up and down the logs was timed at 0.63 m per min. This walking speed, and the fact that the beetles never walked in a straight line, suggest that *S. ventralis* could easily visit several trees a day, and assess their suitability as hosts as well as their occupancy by potential mates.

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