Southern Pine Beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae): Quantitative Analysis of Chiral Semiochemicals

by

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SOUTHERN PINE BEETLE, *Dendroctonus frontalis* ZIMMERMANN (COLEOPTERA:SCOLYTIDAE): QUANTITATIVE ANALYSIS OF CHIRAL SEMIOCHEMICALS

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Abstract

Semiochemicals released from logs infested by southern pine beetle (SPB), *Dendroctonus frontalis*, from a total of eight infestations located in Texas, South Carolina, and Virginia were collected four to eight days after initial attack. The quantities and chiralities of most semiochemicals, as analyzed by gas-liquid chromatography, showed geographic and temporal variations. Changes in the quantities of α-pinene (aP), frontalin (F), and *endo*-brevicomin (eB), are believed to result from responses of the host and the beetle to each other's activity at a given time and differences in their respective health. The chiralities of aP, F, and eB at all locations generally remained stable over time, yet variation across the insect's geographic range, particularly for aP and F, is believed to be due to genetic variation of individuals. Geographic and temporal variations in the quantities and chiralities of *cis*-verbenol (cV), *trans*-verbenol (tV), and verbenone (V) are presumed to be due to the multiple pathways of origin (SPB, autoxidation, and microorganisms).

Analysis of the same semiochemicals isolated from hindguts of individual beetles from Texas, South Carolina, and North Carolina showed

quantities of cV and tV to be substantially greater in females than in males; whereas, males contained much greater amounts of V. Geographic differences were found in quantities of tV and V in both sexes and in aP and F in males only. The chiralities of most semiochemicals present in SPB hindguts differed markedly from those released from infested logs. Males produced predominantly (+)-F and (-)-eB, (-)-cV, and (-)-V; however, the chirality of tV varied considerably among areas. In contrast, females produced predominantly (+)-cV and (+)-V and (-)-F, (-)-eB, and (-)-tV. The (+) enantiomer of aP predominated in both sexes, but the proportion of (+)-aP was generally lower than that released from SPB-infested logs from the same areas. Geographic differences in chirality of tV and V were significant in males and for eB in females.

In laboratory trials, aP autoxidized under ambient temperatures to form tV, myrtenol (M), V and to a lesser extent, cV. Both the quantities and chiralities of these compounds were dependent on the chirality of the aP precursor. Significantly greater amounts and proportions of the (+) enantiomer of each compound were produced when (+)-aP was predominant than when the antipode of the precursor was predominant. The extent to which autoxidation products play a role in bark beetle behavior is expected to be dependent on the proportion of aP in pine resin and its chirality present in a pine species.

The information gained from this research was used to elaborate on previously proposed behavioral sequences occurring during the mass attack of host trees by SPB and to suggest new avenues to improve the use of semiochemicals in pest management efforts.

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Chapter 1

Introduction

The pine forests of the southeastern United States provide many valuable resources: timber, recreation, aesthetics, hydrologics, and wildlife. The economic value of some resources, i.e. timber, can be easily determined, but for the others, the value is not as easily calculated. Many factors which occur naturally or artificially including, fire, disease and insects, can reduce the value of these resources. Insects alone caused damage and mortality to trees amounting to $75.6 million per year from 1985 to 1992 (1993 Report on Losses Caused by Forest Insects, Southern Forest Insect Work Conference proceedings).

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), is the most important cause of pine mortality in the southeastern United States. From 1960-1990, this species alone caused pine damage and mortality valued at more than $900 million (Price et al. 1991). In addition, the frequency and severity of SPB outbreaks has increased throughout the South (Hedden 1978). However, SPB does not just impact the timber industry, it also has a significant impact on recreation, water, and wildlife resources (Leuschner 1980).

Currently accepted control tactics to reduce losses due to SPB include cut-and-salvage, cut-and-leave, fell-and-spray with insecticides, and fell, pile and burn (Billings 1980). However, such tactics are not always applicable due to infestation size, access, timber markets, and environmental concerns (Billings 1980, Swain and Remion 1980).

There is strong interest in the use of semiochemicals for sampling, manipulating, and control of insect pests (Borden 1993). Two pheromones of SPB, frontalin and verbenone, are in operational use for surveying and predicting regional populations levels (Billings 1988) or are being evaluated for effectiveness in suppressing the growth of infestations (Payne et al. 1992), respectively.

Pheromone chirality is reported to be important in maintaining bark beetle species integrity and reduce competition for resources (Lanier 1974, Seybold 1993). Studies have shown that *Ips pini* Say, which inhabits a large geographic ranges (i.e., nearly 4800 km) and use chiral compounds as pheromones, produce and respond to different enantiomeric ratios of those compounds at different geographic locations (Herms et al. 1991, Lanier et al. 1972, Lanier et al. 1980, Miller et al. 1989). In other studies, the enantiomeric ratios of *cis*- and *trans*-verbenols released by bark beetles are directly related to the enantiomeric ratio of the host terpene precursor, α-pinene (Byers 1981,1983; Klimetzek and Francke 1980), which in turn, is quite variable among trees of different genetic origin (Lindstrom et al. 1989, Mirov 1961).

Recent evidence indicates that SPB may also exhibit geographic variation in the production of and response to its pheromones (Berisford et al. 1990). Additional information is needed on the mechanisms of chiral pheromone production which may account for such variation.

The goal of the research presented in this dissertation was to provide a better understanding of the role of semiochemicals in the process of host colonization by SPB and provide insight into the variability of olfactory cues with reference to geographic variation in a species. This information is in

support of the long-term goal of building our understanding of semiochemical-based communication in bark beetles and for improving the use of semiochemicals in control efforts. Specific objectives to fulfill this research goal are:

1. Determine the extent of geographic (local and regional) and temporal variation in the quantities of semiochemicals released from logs obtained from naturally SPB-infested host trees.
2. Determine the extent of geographic (local and regional) and temporal variation in the chirality of semiochemicals released from logs obtained from naturally SPB-infested host trees.
3. Determine the potential chiral and quantitative contribution of semiochemicals produced by SPB to the overall semiochemical blend released from SPB-infested host material.
4. Determine the potential chiral and quantitative contribution of semiochemicals produced by autoxidation of α-pinene to the overall semiochemical blend released from SPB-infested host material.

Chapter 2

Literature Review

Biology of the Southern Pine Beetle and Its Hosts

Taxonomic Status

The genus *Dendroctonus* Erichson (1836) (Coleoptera: Scolytidae) is widely distributed, with representatives occurring in North and Central America, Europe, and Asia. Hopkins (1909) listed 24 species; however, 19 *Dendroctonus* species are currently recognized (Wood 1982); 17 of which are restricted to North and Central America.

The SPB has been studied since late in the nineteenth century. Zimmermann (1868) initially described this species, placing it in the family Hylurgidae, in the tribe Hylurgi, and synonymized it with *Bostrichus frontalis* Fab. The beetle was later placed in the family Scolytidae (LeConte 1868). Wood (1963) synonymized *D. arizonicus* Hopkins and *D. mexicanus* Hopkins with SPB on the basis of anatomical characteristics. However, in spite of their morphological and biological similarities, the pest populations in the southeastern United States and Arizona, and Mexico differ greatly in host preference (Rose 1966). Vité et al. (1974) suggested that SPB and *D. mexicanus* were separate species, because Texas and Mexico beetles failed to interbreed. They also identified differences in their morphology, pheromone chemistry and, to a lesser degree, their behavior. In response to this study, Wood (1974) restored *D. mexicanus* as a valid species. Recently, studies have confirmed SPB and *D. mexicanus* as separate species based on karyology, breeding experiments, male genitalia, and external morphology, supporting the synonym of *D. arizonicus*

with SPB (Lanier 1981, Lanier et al. 1988). The SPB is considered to be one of the more advanced *Dendroctonus* species based on morphological, ecological, chromosomal, and electrophoretic evidence (Bentz and Stock 1986, Lanier 1981, Wood 1963, 1982)

Genetic Variability

The SPB from several regions in the southeastern United States, Arizona, Mexico and Honduras have produced fertile hybrids in all combinations tested (Lanier et al. 1988, Vité et al. 1974,); however, significant genetic differences in isozymes have been found in beetles from these regions, particularly between populations from the southeastern United States and Arizona and Mexican populations (Anderson et al, 1979, Namkoong et al. 1979). The data appear to indicate that the Arizona and Mexican SPB populations differ genetically from populations in the southeastern United States. Though not as significant, populations in the southeastern United States have also shown divergence in isozyme genes. Virginia SPB are different from Texas beetles, but neither population is different from a Georgia population (Anderson et al. 1979). Evidence of genetic divergence has also been found among populations of other *Dendroctonus* spp. including: *D. ponderosae* Hopkins (Stock and Guenther 1979, Stock and Amman 1980), *D. terebrans* (Olivier) (Anderson et al. 1983), and *D. psuedotsugae* Hopkins (Stock et al. 1979). All such examples of genetic variation appear to be the first consequence of genetic separation and are the result of adaptations of separated populations to different environments, genetic drift, and/or selection in the founding of a colony (Mayr 1954).

Genetic variation in SPB has also been found to occur locally. Florence and Kulhavy (1981) studied five gene loci in SPB collected from several trees in a

single Texas infestation. They reported high levels of genetic variation among beetles from a single tree and significant spatial and temporal differences between trees separated by short distances. They concluded that genetic divisions found among the beetles examined likely reflected genetic differences mediated by variations in the SPB pheromone system. Also in a single infestation, Florence et al. (1982) looked at the differential response of the SPB to the aggregation pheromone, frontalin, during dispersal among genotypes of an esterase locus. The differences predict increased genetic diversity with increasing distances from the source population.

Distribution

The distribution of SPB in the southeastern United States roughly corresponds to the distribution of its two preferred hosts, loblolly pine, *Pinus taeda* L., and shortleaf pine, *P. echinata* Mill.; from as far north as central New Jersey south to Florida and from the Atlantic seaboard west to Missouri and eastern Texas (Payne 1980) (Fig. 2.1). Outbreaks of beetle infestations, however, commonly occur in an area from Texas east to Georgia and north to Virginia - a span of nearly 2,200 km. Isolated populations of *D. frontalis* have been found in regions of Arizona and New Mexico and the mountainous regions of Mexico and Honduras (Lanier et al. 1988, Payne 1980, Vité et al. 1974).

Hosts and Their Genetic Variability

The SPB is reported to be capable of attacking and killing all pine species within its range (Hopkins 1909b, St. George and Beal 1929). In the southeastern United States the preferred hosts are loblolly and shortleaf pine; however, SPB will also colonize pitch pine, *P. rigida* Mill., Virginia pine, *P. virginiana* Mill., table-mountain pine, *P. pungens* L., longleaf pine, *P. palustris* Mill., slash pine, *P.*

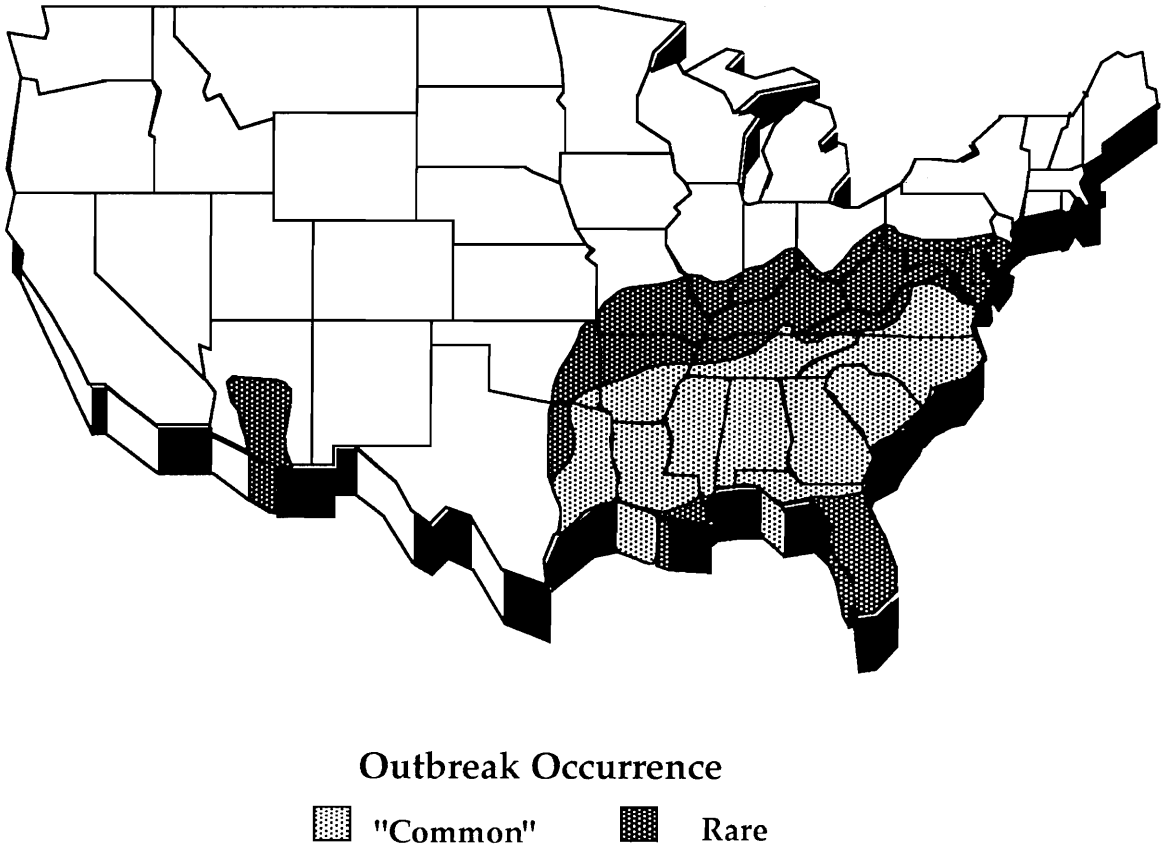

Figure 2.1 General distribution range and outbreak occurrence of the southern pine beetle, *Dendroctonus frontalis*, north of Mexico.

elliotti Engel., and spruce pine, *P. glabra* Walt (Payne 1980). During epidemics, SPB may even attack eastern white pine, *P. strobus* L., red spruce, *Picea rubens* Sarg., and Norway spruce, *P. abies* L. (Hopkins 1909b). In Arizona and New Mexico SPB can be found in Apache pine, *P. engelmanni* Carr., chihuahua pine, *P. leiophylla* Schiede and Deppe, and ponderosae pine, *P. ponderosae* Laws. In Central America, SPB attacks *P. leiophylla*, montezuma pine, *P. montezumae* Lamb., aztec pine, *P. teocote* Schiede and Deppo, pinabeta, *P. pseudostrobus* Lindl., *P. oocarpa* Schieda, pringle pine, *P. priglei* Shaw, Lawson pine, *P. lawsonii* Roezl, and Mexican white pine, *P. ayacahuite* Ehrenb. (Lanier et al. 1988, Mirov 1961, Wood 1963).

Pine oleoresin (also referred to as resin or pitch), is a complex mixture of monoterpenes (turpentine) and diterpenoid resin acids (rosin). Mirov (1961) provided a comprehensive report on the composition of gum turpentine from 92 pine species. Major components of oleoresin from both loblolly and shortleaf pines are α-pinene and β-pinene, with minor amounts of camphene, myrcene, limonene, and β-phellandrene.

Monoterpene composition varies considerably within a conifer species (Chang and Hanover 1991, Zavarin and Cobb 1970). With regard to loblolly pine, α-pinene composition is generally lowest (15 to 30%) in the west and northern portions of its range (Louisiana and inland areas) and then increases toward the east and along the coastal areas (40 to 59%) (Squillance and Wells 1981). Coyne and Keith (1972) and Gilmore (1971) found a similar geographic pattern; however, they found the α-pinene composition in stem xylem oleoresin to range from 56 to 95%. The α-pinene composition can also vary seasonally, at

different stem heights, and between stem xylem and branch cortex (Gilmore 1975, Rockwood 1973).

Monoterpene composition appears to be under strong genetic control (Hanover 1966, 1971). This implies that the evolution of a tree species by natural selection may result from geographical differences, as has been found for ponderosae pine (Smith 1964, 1977). In addition, environmental differences east and west of the Mississippi River appear to have caused considerable genetic evolution within the loblolly pine species (Florence and Rink 1979).

The release of oleoresin upon injury serves as both a physical and chemical barrier to macro- and microorganism invasion (Smith 1965). The viscous substance produced from a healthy tree can "pitch-out" invading beetles (Payne 1980), is toxic to both beetles and associated microorganisms (Berryman 1972), and ultimately seals the wounds created by invaders. Monoterpenes, such as α-pinene, myrcene, camphene and terpinoline, in turn, may be used by bark beetles as primary attractants in the location of the host or as synergists of pheromones in the aggregation of individual beetles (Borden 1982, Payne et al. 1978). The terpene components, α-pinene and myrcene, also have been found to serve as precursors in the biosynthesis of pheromones i.e., *cis*-and *trans*-verbenol, verbenone, myrtenol, and mytenal from α-pinene by *Dendroctonus* and *Ips* species (Francke and Vité 1983; Hughes 1973, 1975) and ipsdienol and ipsenol from myrcene by *Ips* species (Hendry et al. 1980, Hughes 1974).

Life History

Detailed reviews of the life history of SPB have been presented (Flamm et al. 1988, Fronk 1947, Payne 1980, Thatcher 1960). The SPB is a multivoltine

species that attacks living trees and whose successful reproduction is contingent upon host mortality. The ability of this beetle to colonize live hosts is dependent on how quickly the host is mass attacked and is also facilitated by its mutualistic relationship with pathogenic fungi, which are thought to be important in overcoming host resistance, larval nutrition, and kairomone production (Barras and Perry 1975, Beaver 1989). The SPB requires 26-140 days to complete development, depending on temperature (Billings and Kibbe 1978, Thatcher and Pickard 1967), with 3-8 generations per year in the southern United States. The SPB's life cycle can be divided into four general phases: 1.) dispersal , 2.) host selection, 3.) colonization, and 4.) brood development (Coulson and Witter 1984, Stark 1982, Wood 1982).

Dispersal

Upon emergence, SPB take flight for durations which depend on a beetle's physiological condition, the distribution of pheromone sources, and the season. Hedden and Billings (1977) found fat content and body size to be greater in spring and fall, suggesting that beetles are physiologically ready to disperse long distances to widely distributed pheromone sources. Conversely, during the summer, SPB generally encounter pheromone sources near brood trees; therefore, smaller fat reserves are required for the short flights to pheromone sources (Gara 1967, Payne et al. 1978).

Host Selection

Some investigators suggest that SPB locate suitable host trees by randomly landing on vertical objects (Gara et al. 1965) or by responding to primary attractants, although the latter case has never been demonstrated for SPB. The host volatile, α-pinene, does appear to function in SPB host selection by

arresting beetles after landing on a host (Payne et al. 1978, Renwick and Vité 1969). More recently, it has been suggested that random landing is coupled with non-directed orientation (chemokinesis) in response to arrestment-causing host kairomones (primary attractants) (Moeck et al. 1981, Payne 1986, Payne and Coulson 1985). Lightning-struck trees are particularly attractive as they provide a vertical profile and source a of host volatiles (kairomones) (Payne and Coulson 1985).

Upon landing on a tree, the "pioneer" beetle (e.g. females in the genus *Dendroctonus*) bites into the outer bark in response to chemical stimuli (Thomas et al. 1981). If the tree is suitable, the female initiates boring activity and begins releasing aggregation pheromones (secondary attractants) which attract conspecifics to the host. If the tree is found to be unsuitable, the beetle resumes flight and continues its search for a host (Bunt et al. 1980, Dickens et al. 1992).

Colonization

The reproduction of SPB is contingent upon the death of all or part of its host. The SPB has evolved a complex pheromone system which enables a large number of beetles to converge on the host rapidly enough to overcome the tree's defenses. In addition, as mentioned previously, the beetles ability to colonize living trees is augmented by their symbiotic relationship with fungi. These two aspects of the beetle's biology will be discussed in detail below.

In most cases, particularly during outbreaks, SPB attack the central portion (3-4 m) of the tree bole first and at higher densities than the portions above and below (Dixon and Payne 1980). During endemic periods, SPB may attack a host after *Ips* beetles and, therefore, may be relegated to the lower bole and crown area.

An individual female SPB tunnels through the outer bark to the phloem region where she constructs a nuptial chamber (Flamm et al. 1988, Payne et al. 1980). Each female is joined by a single male and mating takes place. After mating, the female begins construction of a characteristic serpentine gallery with egg niches cut in the sides of the gallery wall. The male guards the entrance and assists in the redistribution of boring dust and frass. Parent adults may reemerge to construct a second and even a third gallery in the same or a neighboring host tree. While constructing additional galleries, the female may mate with other males.

Brood Development

Detailed descriptions of the SPB life stages were presented by Hopkins (1909b) and added to by Thatcher (1960) and Dixon and Osgood (1961). Eggs are deposited in niches along the wall of the gallery and hatch in 3-27 days. The larvae pass through four instars in 13-64 days and pupate in a chamber constructed in the outer bark. Pupation lasts 3-36 days, after which callow adults remain in the bark until the cuticle hardens and conditions are favorable for emergence and dispersal (Kinn 1978). In the United States, the number of generations per year range from three in Virginia to eight in Texas (Payne 1980, Thatcher 1960). The ability of the beetles to overcome host tree resistance and successfully colonize the host is due, in part, to these multiple overlapping generations and their ability to mass attack trees over a short period of time.

Semiochemical Communication System

Bark beetles possess elaborate semiochemical communication systems which they use to orientate to host material to feed, mate, and reproduce. Many

detailed reviews have been provided in this area (Birch 1978, 1984; Borden 1974, 1977, 1982, 1984, 1985, 1989; Brand et al. 1979; Byers 1989; Geizler and Gara 1978; Geizler et al. 1980; Renwick and Vité 1970, 1980; Rudinsky and Ryker 1977; Ryker 1984; Smith et al. 1993; Vité and Francke 1976; Wood 1970, 1973, 1982; Wood and Bedard 1977). Semiochemicals are natural compounds produced and released by individuals of a species which elicit a behavioral response in members of the same or different species (Nordlund 1981). Semiochemicals which are used in intraspecific communication are referred to as pheromones. Behavioral responses to pheromones include searching for mates by one sex (e.g., sex pheromones), aggregation of both sexes at a host plant (e.g., attractant or aggregation pheromones), and dispersal of both sexes away from a specific area (e.g., inhibitor or antiaggregation pheromones). Semiochemicals used in interspecific communication are referred to as kairomones when the species receiving the chemical message benefits and allomones when the emitter of the chemical message benefits at the expense of the receiver. There is considerable overlap with regards to the functions of a compound, i.e., the same compound may serve both intra- and interspecific functions. For example, frontalin serves as an pheromone to SPB and a kairomone to its natural enemy, *Thanasimus dubius* (Fabricius) (Vité and Williamson 1970).

A series of studies conducted at the Boyce Thompson Institute for Plant Research provided the foundation by which the semiochemical system of SPB was first described (Coster 1970; Coster and Gara 1968; Gara 1967; Gara and Coster 1968; Renwick 1970; Renwick and Vité 1969, 1970; Vité and Crozier 1968; Vité and Renwick 1968) and later revised (Vité and Francke 1976). Recently, Smith et al. (1993) presented an extensive historical review of research on the

semiochemical communication system of SPB and other members of the southern pine bark beetle guild.

Upon landing on a tree, "pioneer" females bite into the bark. If the host is found to be suitable, the females begin releasing the primary aggregation pheromone, frontalin, and the synergists, *trans*-verbenol and myrtenol (Fig. 2.2). These compounds, in combination with host volatiles, stimulate mass aggregation of conspecifics (predominantly males) to the host (Renwick and Vité 1969, Rudinsky 1973). Arriving males also release frontalin and myrtenol plus *endo*-brevicomin and verbenone. Verbenone and myrtenol at low concentrations enhance female response, thereby, balancing the sex ratio of responding beetles (Billings 1985, Rudinsky et al. 1974). The (+) enantiomer of *endo*-brevicomin is also reported to enhance attraction to frontalin (Vité et al. 1985). The resulting aggregation, along with the introduction of symbiotic fungi, enables SPB to successfully attack a host tree and produce brood which emerge to attack other trees. As the population of attacking beetles increases, the concentration of verbenone, (-) *endo*-brevicomin, and myrtenol released by males also increases. At some unknown threshold, these three compounds begin to inhibit beetle response to the aggregation pheromones and cause arriving beetles to switch their attack to neighboring trees (Payne et al. 1978, Rudinsky 1973, Rudinsky et al. 1974, Vité and Renwick 1971). It has been suggested that the switching of mass attack from one host tree to a neighboring tree may be the result of both the cessation of release of attractive compounds (frontalin and α-pinene) and the increased concentration of inhibitor pheromones (verbenone, *endo*-brevicomin, and myrtenol) released from the tree as was found for *Ips typographus* (L.) (Schlyter et al. 1987, 1989).

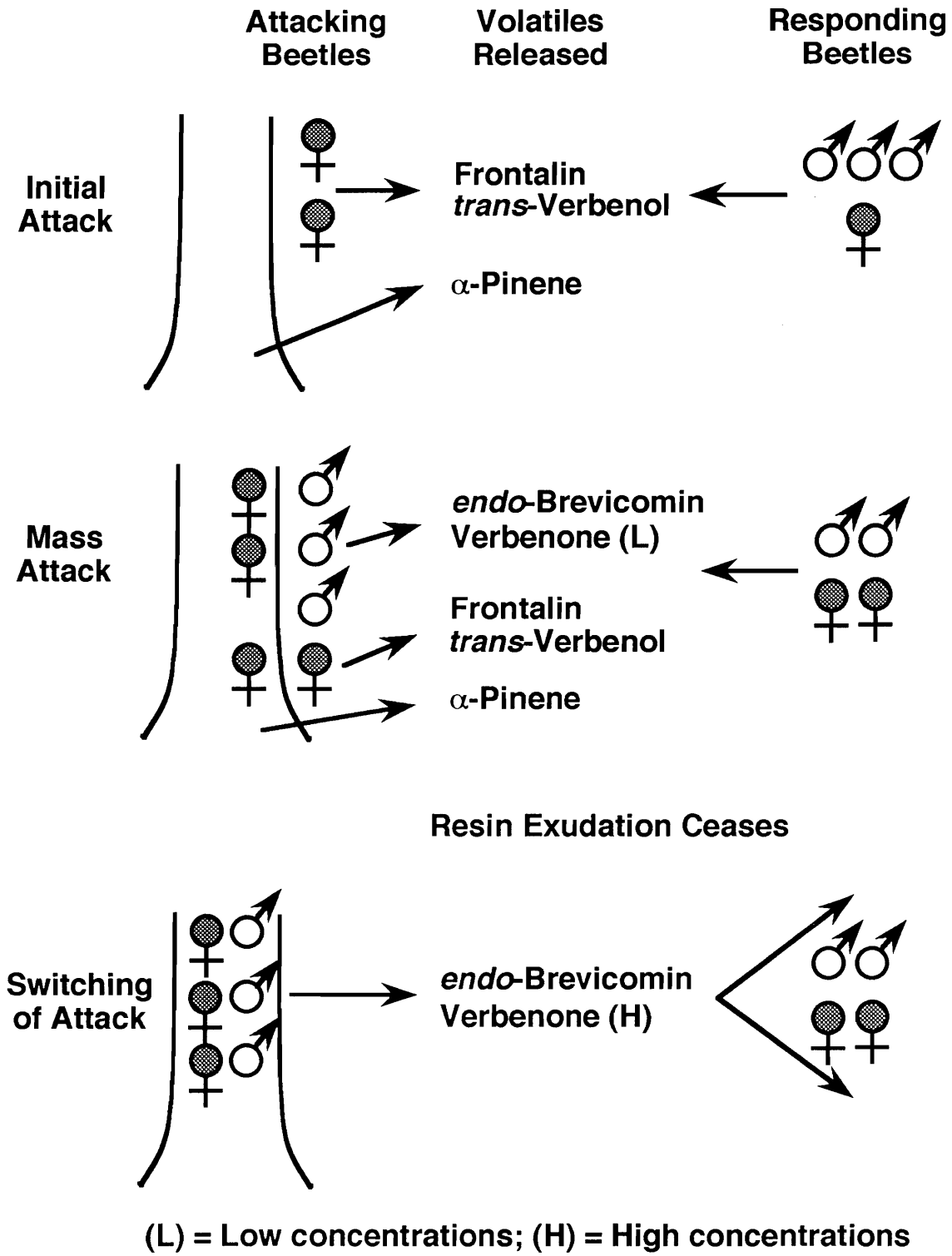

Figure 2.2 Mechanism of host tree colonization by the southern pine beetle, *Dendroctonus frontalis* (revised from Vite and Francke [1976]).

Although several host- and beetle-associated chemicals have been found to be produced and/or utilized by SPB as part of its communication system, behavioral responses of this beetle have only been determined for a few of these compounds (Table 2.1). A summary of known or speculated biosynthetic pathways and responses to some of the most studied semiochemicals are discussed below.

Host-produced kairomones

As the pioneer beetles begin to invade the host, conductive phloem tissues are cut and copious amounts of oleoresin exude from these wounds. The oleoresin of loblolly pine is largely composed of **α-pinene**, 2,6,6-trimethylbicyclo [3.1.1] hept-2-ene (Mirov 1961) (Fig. 2.3) which has been determined to be one of the more important host tree odors in the behavioral chemical complex of SPB (Renwick and Vité 1969). Although, α-pinene alone has not been shown to be attractive to field populations (Payne et al. 1978), it does synergize the attractiveness of frontalin (Kinzer et al. 1969). In combination with frontalin, α-pinene appears to function as a kairomone arrestant; whereby, the pheromone draws beetles to the tree and the kairomone arrests their flight so they land (Renwick and Vité 1970; Payne 1980). In laboratory studies, α-pinene also causes arrestment of walking beetles (McCarty et al. 1980, Payne 1979).

α-Pinene also plays a significant role in the production of other pheromones utilized by SPB. First, SPB larvae, pupae and adults exposed to α-pinene (either through consumption of phloem material or vapors) produce *cis*- and *trans*-verbenol, mytenol, and verbenone (Hughes 1973, 1975, Renwick et al. 1973). It has been hypothesized that the production of these pheromones

Table 2.1 - Southern pine beetle and host tree-associated compounds.[a]

Compound	Reference	Source	Behavioral Effect
Frontalin	1,2	A,B,C,D	Attractant (1,14,15)
endo-Brevicomin	3,4	A,C,D	Multifunct. Inhibitor/Synergist (14,16,17,21,22)
trans-Verbenol	5,2	A,B,C,D	Synergist
cis-Verbenol	6,2	B,C,D	
Verbenone	5,2	A,B,C,D	Multifunct. Inhibitor/Synergist (14,15,17,18,22)
α-Pinene	7,2	C,D	Synergist (1,7,14,15)
Myrcene	2	C,D	Attractant (19)
Myrtenol	8,9	A,B,C,D	Multifunct. Synergist/Inhibitor (20)
cis-myrtenol	2	B,C,D	
Isoamyl acetate	10	E	Synergist (10)
Isoamyl alcohol	10	E,F	Synergist (10)
2-phenylethanol	10	E	Synergist (10)
2-phenyethyl acetate	10,11	E	Synergist (10)
6-methyl-5-hepten-2-one	11	F	
6-methyl-5-hepten-2-ol	11	F	
4-methyl-2-pentanol	9	A,B	
6-hydroxlcamphene	12,2	A,B,C,D	
Pinocarvone	9,2	A,B,C.D	
trans-pinocarveol	9,2	A,B,C,D	
Myrtenal	9,2	A,B,C,D	
Acetophenone	2	A,C,D	
Chrysanthenone	2	A,C,D	
4-Allylanisole	2	C,D	Repellent (23)
cis-3-pinen-2-ol	13	B	
3-methyl-2-cyclohexen-1-ol (seudenol)	13	A,B	
1-methyl-2-cyclohexen-1-ol	13	A,B	
1-cyclohexenemethanol	13	A,B	
3-methyl-2,cyclohex-1-one (MCH)	13	A,B	
2-methyl-2-cyclohexen-1-ol	13	A,B	

[a] Modified from Payne 1980.

1-Kinzer et al. 1969
2-R.M. Siverstein and J.R. West, pers. comm. to T.L Payne
3-Silverstein et al. 1968
4-Pitman et al. 1969
5-Renwick 1967
6-Silverstein et al. 1966
7-Renwick and Vite 1969
8-Hughes 1973
9-Renwick et al. 1973
10-Brand et al. 1977
11-Brand and Barras 1977
12-Renwick et al. 1976
13-Renwick and Hughes 1975
14-Payne et al. 1978
15-McCarty et al. 1980
16-Vite and Renwick 1971
17-Richerson and Payne 1979
18-Rudinsky 1973
19-P.D. Billings, pers. comm. to T.L. Payne
20-Rudinsky et al. 1974
21-Vite et al. 1985
22-Salom et al. 1992
23-Hayes et al. 1994

A-Male hindguts
B-Female hindguts
C-Beetle-infested tree parts
D-Female frass
E-Yeast metabolite
F-Basidiomycete

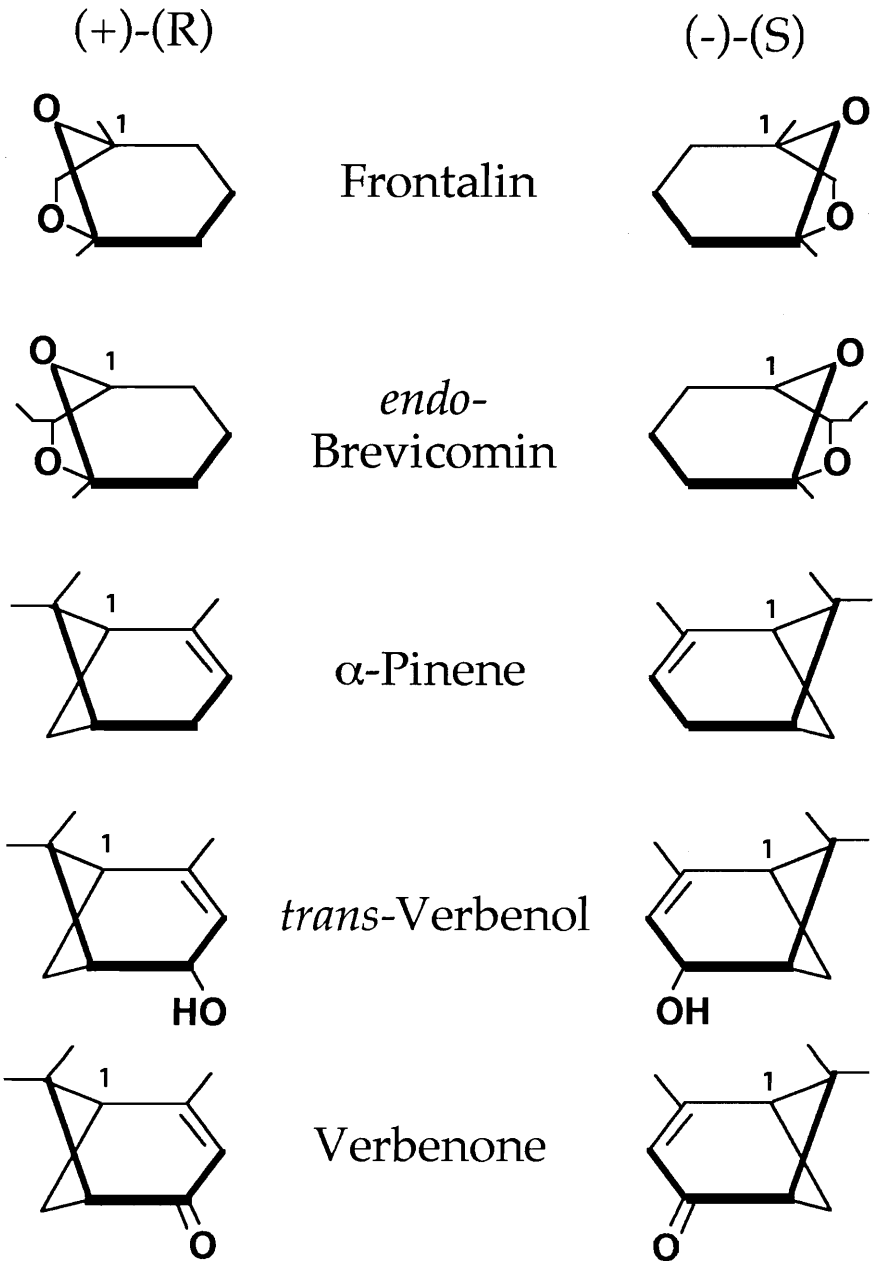

Figure 2.3 Chemical structures of the enantiomers of five principal semiochemicals components of the southern pine beetle, *Dendroctonus frontalis*, semiochemical communication system.

evolved from a need to detoxify noxious compounds absorbed or consumed by beetles while in the host (Hughes 1973, Francke and Vité 1983, White et al. 1980,). The biochemical pathway producing secondary alcohol pheromones (e.g., *cis*- and *trans*-verbenol and myrtenol) involves the allylic oxidation or hydration of the monoterpenes, but production of ketones (e.g., verbenone) may involve further oxidation, hydrogenation, or rearrangement of the carbon chain (Fig. 2.4) (Pierce et al. 1987, Vanderwell and Oehlschlager 1987). In addition, when α-pinene is exposed to air it autoxidizes to yield similar products, i.e., *cis*- and *trans*-verbenol, verbenone, and myrtenol (Hunt et al. 1989, Moore et al. 1956). The extent to which pheromones produced by autoxidation contribute to the SPB pheromone system is unknown.

The enantiomeric composition or chirality of α-pinene can differ among conifer species (Klimetzek and Francke 1980, Mirov 1961). Within the genus, *Pinus*, the composition can range from almost pure (+), as in *P. halipenis* $[\alpha]_D$ = +48.30°, to almost pure (-), as in *P. pinaster* $[\alpha]_D$ = -43.4° (Mirov 1961). Comyns and Lucas (1957) reported that pure (+)-α-pinene had $[\alpha]_D$ = +52.4°. α-Pinene in *P. taeda* is reported to be $[\alpha]_D$ = +20.17° (= 38.5% ee = 69.3% (+)) (Mirov 1961). The enantiomeric composition of α-pinene can also differ within conifer species of different genetic origin as was found in ponderosa pine (Mirov 1961) and Norway spruce (Lindstrom et al. 1989). The chirality of the α-pinene within a conifer has been found to be important with regards to the stereoselective production of oxygenated pheromones by bark beetles. Renwick et al. (1976) showed that *Ips paraconfusus* Lanier produces (+)-*cis*-verbenol from (-)-α-pinene; whereas (+)-α-pinene yields (+)- *trans*-verbenol. The chiral relationship between the enantiomeric ratios of oxygenated pheromones (*cis*- and *trans*-verbenols and

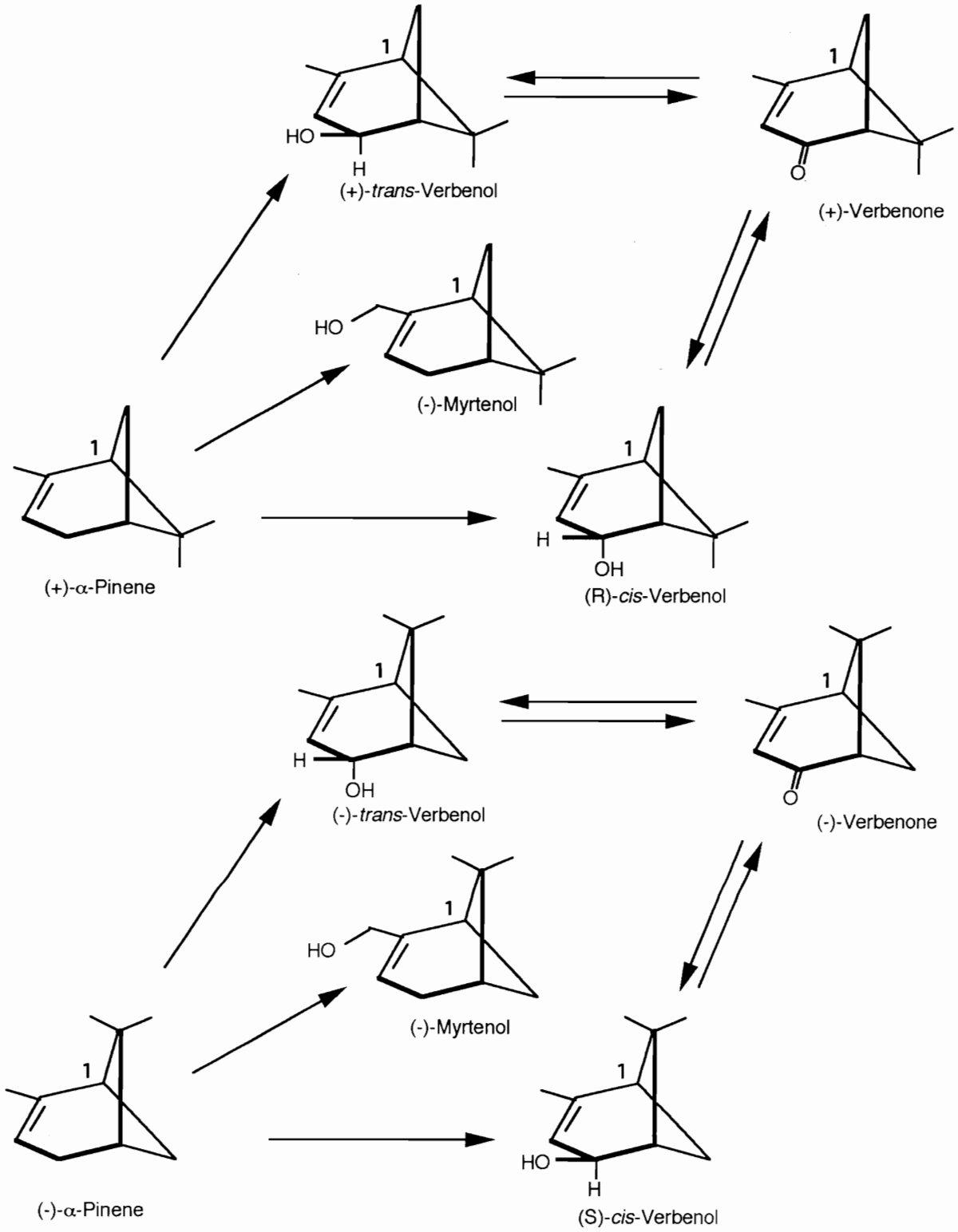

Figure 2.4 Oxidation of α-pinene to *cis*- and *trans*-verbenol, myrtenol, and verbenone (revised from Vanderwel and Oehschlager [1987]).

verbenone) produced by SPB and the enantiomeric ratio of α-pinene in their hosts is unknown.

Beetle-produced semiochemicals

Frontalin, 1,5-dimethyl-6,8-dioxabicyclo [3.2.1] octane (Fig 2.3), is a primary aggregation pheromone produced by several *Dendroctonus* species including SPB (Borden 1982, Kinzer et al. 1969, Payne et al. 1978). It is found in the hindguts of newly emerged SPB females (Coster and Vité 1972) and probably released by flatulation or in frass when they land on a tree and have determined it to be a suitable host. Males have also been found to produce frontalin (Rudinsky et al. 1974). Although the actual biosynthetic pathway has yet to be determined, White et al. (1980) has speculated that frontalin is likely biosynthesized *de novo* via a specialized hormone type of metabolic pathway. Silverstein (according to White et al. 1980), on the other hand, suggested that this pheromone is directly synthesized from short-chain dihydroxy ketones as precursors (e.g., 6-methyl-6-hepten-2-one) (Brand et al. 1979). It has been suggested that mycangial fungi may provide the beetles with the precursors of frontalin, as the fungi have been found to produce 6-methyl-6-hepten-2-one (Brand and Barras 1977, Brand et al. 1979). Studies have shown that the level of frontalin declines significantly after feeding (Coster and Vité 1972) and mating (Coster 1970), however, the authors reported that these activities do not irreversibly inhibit pheromone production as females are still attractive even after re-emergence from the host. The enantiomeric ratio of frontalin is 15% (+) : 85% (-) (Stewart et al. 1977). The beetle responds significantly more to the mixture containing > 50% (-) than to mixtures containing predominantly the (+) enantiomer (Payne et al. 1982). Frontalin likely plays a dual role, as sex

pheromone to males (McCarty et al. 1980) and aggregation pheromone to both sexes, functioning in close-range communication, to bring individual beetles together in sufficient numbers to overcome host tree defenses (Johnson and Coster 1978, Payne et al. 1978). The natural enemy, *Thanasimus dubius*, responds to frontalin as a kairomone, thereby allowing the predator to find its prey (Vité and Williamson 1970).

***endo*-Brevicomin**, *endo*-7-ethyl-5-methyl-6,8-dioxabicyclo [3.2.1] octane (Fig. 2.3) (Silverstein et al. 1968), is synthesized by male SPB in very small quantities (Pitman et al. 1969). The enantiomeric composition of naturally produced *endo*-brevicomin from beetles was 97% (+) and 3% (-) (Redlich et al. 1987). Vanderwell et al. (1992) recently demonstrated that (E)-6-nonen-2-one serves as the precursor to *endo*-brevicomin as the production of this pheromone by *D. brevicomis* LeConte and *Dryocoetes confusus* Swaine was significantly increased upon exposure of beetles to deuterium-labeled precursor.

Tests of racemic *endo*-brevicomin indicate that the compounds functions to inhibit the aggregation of both male and female SPB to attractive trees and thus switch the mass attack to new host trees (Payne et al. 1978, Vité and Renwick 1971). Other investigations have shown that (+)-*endo*-brevicomin significantly enhances response of both sexes of SPB to traps baited with frontalin and host volatiles (Vité et al. 1985). Low release rates of racemic endo-brevicomin also increased response, but to a lesser extent than did the (+)-enantiomer. In contrast, the (-)-enantiomer significantly reduced beetle response.

***trans*-Verbenol**, *trans*-2,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-ol (Fig. 2.3), is found in the hindgut of newly emerged female SPB (Renwick 1967). As mentioned previously, the production of this compound results from the

oxidation of α-pinene upon consumption of phloem material or exposure to vapors while in the host (Hughes 1973, 1975; Renwick et al. 1973). Byers (1983) showed that *D. brevicomis* convert the (+)- and (-)-enantiomers of α-pinene to the corresponding enantiomers of *trans*-verbenol, but it is unknown if such a conversion pathway occurs in SPB. It is known, however, that an enantiomeric ratio of 60%(+) :
40%(-) of *trans*-verbenol is naturally synthesized by SPB (Plummer et al. 1976). The effects of *trans*-verbenol chirality on SPB behavior has not been determined. Female beetles arrive at the host with a full load of pheromone; however, the level of *trans*-verbenol declines significantly after 48 hrs of feeding (Coster and Vité 1972). *trans*-Verbenol is also produced outside the beetle by the autoxidation of α-pinene upon exposure to air (Hughes 1975, Hunt et al. 1989, Moore et al. 1956). This may explain why *trans*-verbenol can substitute for α-pinene as a synergist for the pheromone frontalin (Kinzer et al. 1969; Payne et al. 1978; Renwick and Vité 1969, 1970). *trans*-Verbenol is also reported to be metabolized from α-pinene internally by bacteria in the beetle's gut (Brand et al. 1975) and externally by other microbial activity (Prema and Bhattacharyya 1962). As with α-pinene, *trans*-verbenol alone is unattractive to walking and flying beetles (Vité and Crozier 1968).

***cis*-Verbenol**, *cis*-2,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol, is also found in the hindgut of newly emerged female SPB (Pitman et al. 1968, Renwick et al. 1973), however, the enantiomeric ratio of the naturally produced compound has not been determined. As with *trans*-verbenol, this compound is a product of the oxidation of α-pinene in beetles or externally by autoxidation (Hunt et al. 1989) (Fig. 2.4) and microorganisms (Leufven et al. 1984). This compound is not

known to affect SPB behavior (T.L. Payne, personal communication); however, it is an important component in the pheromone systems of three sympatric *Ips* species (e.g., *I. calligraphus* (Germar) *I. grandicollis* (Eichhoff) and *I. avulsus* (Eichhoff)) (Smith et al. 1993, Vité et al. 1976).

Verbenone, 4,6,6-trimethylbicyclo [3.1.1] hept-3-en-2-one (Fig. 2.3), a multifunctional pheromone, is produced predominantly by male SPB (Renwick 1967) and other scolytid species (Borden 1985). The SPB derives verbenone from α-pinene upon the oxidation of *trans*-verbenol (Hughes, 1973, 1975). The enantiomeric ratio of verbenone isolated from Texas SPB was reported to be 15% (+) : 85% (-) (Payne and Billings 1989, citing J.P. Vité, personal communication). This compound is also produced outside the beetle in two ways. One is by the autoxidation of *trans*-verbenol in the presence of air (Hunt et al. 1989, Moore et al. 1956). Borden et al. (1986) showed that verbenone becomes the predominant oxidation product after 24 hours. It was not determined if the enantiomeric ratio of α-pinene affects the conversion rate to the corresponding enantiomers of *trans*-verbenol and verbenone. A second external source of verbenone is the symbiotic fungi introduced into the host tree by SPB (Brand et al. 1976). This chemical is believed to be a multi-functional population regulator (Renwick and Vité 1980, Rudinsky 1973); at low concentrations it balances the sex ratio of beetles attracted to the host by enhancing the attractiveness of aggregation pheromones to females and at high concentrations it inhibits the aggregation of both males and females to host trees (McCarty et al. 1980, Payne et al. 1978, Renwick and Vité 1969). Ryker and Yandell (1983) determined for *D. ponderosae* that verbenone must exceed the level of *trans*-verbenol (the primary aggregation pheromone of this species) by approximately 15 percent before it would exert its

antiaggregative properties. It is unknown if such a verbenone threshold level is required to inhibit SPB response to frontalin.

Beetle-Associated Microorganisms

There are many examples of mutualistic association of microorganisms (fungi, yeasts and bacteria) with bark and ambrosia beetles (Barras and Perry 1975, Beaver 1989). The primary benefit to the microorganism is their transport and introduction into new habitats suitable for their growth. Beetles appear to also benefit in several ways, such as 1) microorganisms serving as a food source for all ambrosia beetles and some bark beetles, 2) inoculated plant-pathogenic fungi help to reduce host defense mechanisms in the area of beetle attack (*Dendroctonus*) or are the sole means of killing the tree so that the beetles can colonize the host (as with some *Scolytus*), and 3) the conversion of host tree chemicals into beetle kairomones. Microorganisms are carried by many scolytid and platypodid beetles on the outer surface of the body or in specially evolved glandular cavities called "mycangia" (Batra 1963, Berryman 1989). In the SPB, the mycangium is located below the anterior margin of the pronotum (Francke-Grosmann 1965) and has been found to transport two fungal species (e.g., *Ophiostoma minor* (Hedgc.) and SJB-122, a basidomycete) and two yeasts (e.g., SJB-133 and an unidentified species) (Barras and Perry 1972). Metabolites of one yeast were found to enhance the attractive response of SPB to a 1:1:12 mixture of frontalin:*trans*-verbenol:turpentine (Brand et al. 1977). Whereas, one of the filamentous fungi (SJB-133), present as yeast phases in the mycangium, was found to be capable of converting *trans*-verbenol to verbenone through the oxidation of the precursor (Brand et al. 1976). Hunt and Borden (1990) obtained

similar results from two yeast species isolated from *D. ponderosae*. Brand et al. (1977) suggested that a complex conversion system may exist in which *cis*- or *trans*-verbenol, produced from α-pinene by one microorganism (Brand et al. 1975, Prema and Bhattacharyya 1962), may be the substrate for another. It had been suggested that verbenone, released as a result of microorganism activity in beetle-infested trees, may play a significant role in the termination of attack and switching of attack to a new host tree (Bakke 1981, Birgersson and Bergstrom 1989, Leufven et al. 1984, Leufven and Birgersson 1987, Raffa and Berryman 1983, Schlyter et al. 1989). However, a preliminary study suggests that the quantity of verbenone produced by SPB-associated fungi may not be as significant as was once thought (Brian Sullivan, Ph.D. candidate, University of Georgia, personal communication). The enantiomeric composition of verbenone produced by beetle-associated fungi is not known. Nor does it appear to be known if the composition of verbenone produced correlates to the enantiomeric composition of α-pinene from the host.

Chemical Structure, Blends and Concentration in Species Specificity

The activity of a semiochemical is dependent on many factors including its size, shape, functional groups, degree of saturation and chirality (Tumlinson and Teal 1987). Small molecules are used when a fast response is required (e.g., alarm pheromones), while large compounds, which tend to be less volatile, are used when long, extended exposure is required (e.g., sex, aggregation, and antiaggregation pheromones). Although the structure of pheromones differs greatly between insect orders and families, generally compounds are of similar structural types within genera as seen with terpene pheromones of *Ips* and

Dendroctonus bark beetles. The position, number, and geometry of double bonds and functional groups are also important with regard to the activity of compounds. Payne et al. (1988), evaluating the antennal olfactory and behavioral response of SPB to different frontalin analogs, showed that response to frontalin was significantly greater than to any of the analogs. Chirality, in turn, imparts a greater degree of specificity to a species' pheromone system. Figure 2.4 illustrates the structures of both enantiomers of the five principal components of the SPB semiochemical communication system. Silverstein (1979) described nine possible categories of behavioral response to enantiomers or diastereomers. At least two of these categories have evolved as part of the SPB semiochemical-based system . For example, SPB produce both enantiomers of frontalin, but is significantly more attracted to (-)- frontalin than to the (+)- antipode (Payne et al. 1982). On the other hand, Vité et al. (1985) showed that (+)-*endo*-brevicomin significantly enhanced attraction of SPB to frontalure, whereas (-)-*endo*-brevicomin inhibited response. Thus, one enantiomer may be active and the other inactive or each enantiomer may elicit different responses. Seybold (1993) provided an extensive review on the roles of chirality in olfactory-directed behavior.

Just after the discovery of the first pheromone, bombykol, from the silkworm moth, *Bombyx mori* (L.)., it was generally thought that each insect species produced and responded to a single pheromone (Kalson and Butenandt 1959). However, *Ips paraconfusus* was later found to produce and respond to a blend of three pheromones (e.g., (S)-(-) ipsenol, (S)-(+)-ipsdienol, and (S)-(-)-cis-verbenol) (Silverstein et al. 1966). It has since been discovered that most insects produce multicomponent blends of pheromones and that the single component

system is the exception rather than the rule. The blend of pheromones is important because some or all components may act as synergists; individually they elicit little or no attractiveness, but together they are highly attractive. The pheromone blend of *I. paraconfusus* is one example. Another example is the SPB attractant blend of frontalin, *trans*-verbenol, turpentine (containing α-pinene and other monoterpenes), verbenone, (+) *endo*-brevicomin, and myrtenol. Individual components of a blend may also function in concert to maximize steps in a behavioral sequence as has been found in several Lepidoptera species (Baker and Carde 1979, Linn et al. 1984, Teal et al. 1986). Blends and component ratios within blends play important roles in maintaining or increasing reproductive isolation of closely related species or reducing competition in sympatric species. A species may release a component which is inactive to conspecifics, but inhibitory to related species (Tumlinson and Teal 1987). On the other hand, a component, essential to reproductive behavior in the releasing species, may also be inhibitory to members of related species. Finally, two species may produce different component ratios of the same chemicals.

Variation in Production of and Response to Pheromones

Quantitative and qualitative differences in pheromone production and response can occur between regional and local populations (Berisford et al. 1990; Lanier et al. 1972, 1980; Miller et al 1989,) and between individual insects (Schlyter and Birgersson 1989). *Ips pini* (Say) from California and New York were significantly more attracted to their own pheromone blends containing almost 100% (-) ipsdienol and about 50% (-) ipsdienol, respectively (Lanier et al. 1972). In fact, California beetle response was inhibited by the New York

pheromone blend. It was later discovered that California *I. pini* response is inhibited by ipsdienol containing as little as 3% of (+)-enantiomer (Birch et al. 1980). Geographic variation in laboratory bioassay response to attractant extracts has also been shown in SPB from Texas, Georgia and Virginia (Berisford et al. 1990). Beetles from each region had significantly higher responses to the attractant extracts from their own region than to extracts from other regions. The authors hypothesized that possible variations in the quantity and quality of the pheromone components may account for the observed geographic differences in SPB response.

Lanier (1974) suggested that although there are many mechanisms which help to retain reproductive isolation of sympatric species, geographic variations in semiochemical production and response may evolve in insect species upon exposure to several possible selection pressures. Species which have large ranges may be exposed to different environmental conditions at the extremes of the range and even locally (Miller et al 1989). Similarly, the insects' host oleoresin composition, from which the insect may derive its pheromone, may differ at the extremes of its range and even locally (Miller et al. 1989, Mirov 1961). In *I. typographus* (L.), over 80% of the quantitative variation of *cis*- and *trans*-verbenol and myrtenol was explained by the variation in the amounts of α-pinene in the host (Birgersson et al. 1984). Pheromone composition may shift as a result of competition between sympatric species. For example, *I. pini* in California are sympatric with *I. paraconfusus* and both species utilize ipsdienol as pheromone. As a result, *I. pini* produce nearly 100% of the (-)-enantiomer (Birch et al. 1980). In New York, where *I. paraconfusus* is absent, *I. pini* produce about 50% (-)-ipsdienol (Lanier et al. 1980). Pheromone chirality may shift as beetles attempt to

escape natural enemies that show preferences to a certain ratio (Herms et al. 1991, Raffa and Klepzig 1989). Finally, genetic variability due to genetic drift may affect enzymatic pathways which in turn may affect pheromone chirality or other chemical properties (Miller et al. 1989). This may explain the fact that the enantiomeric composition of ipsdienol (derived from achiral myrcene) in a local population in British Columbia was nearly 100% (-) in half the individuals, about 90% (-) in 20% of the same population, and 5% of this population had as little as 65% (-)-ipsdienol (Slessor et al. 1985).

Pest Management of Bark Beetles

The history of methods used to manipulate or control SPB are varied and imaginative (Billings 1980). Some of the earliest tactics included rapid conversion of infested material into lumber (e.g., salvage) and burning the slabs (Hopkins 1909b, 1911); immersing unbarked logs in water; and exposing unsalvagable infested trees to solar heating (e.g. cut-and-leave) (St. George and Beal 1929). Some of these methods, i.e. salvage and cut-and-leave, are still used today. Other treatments, more recently evaluated, include pesticides, mechanical, silvicultural and behavioral chemicals. These are summarized below.

Pesticide Control

Several pesticides are or have been used for remedial or preventative control of bark beetles. Some chemicals, including ammonium fluoride, sodium fluoride, sodium arsenate, hydrocyanic acid gas, and ethyl monodichloroacetate, were introduced into the resin stream of freshly attacked trees. The treatments tended to reduce brood production, but only when the chemicals were

introduced before resin flow ceased (Thatcher 1960). Orthodichlorobenzene in kerosene sprays was successfully used in the 1940's. From the early 1950's to the late 1960's the standard control method was to spray infested trees and logs with lindane in fuel oil. The pesticide had an effectiveness ranging from 81 to 97% (Coulson et al. 1972), but was reported to kill beneficial insects and cause the beetle to disperse from the treated area and start new infestations elsewhere (Williamson 1970, Williamson and Vité 1971). Lindane has since been de-certified for use in forests because of its toxicity to nontarget organisms and persistence in the environment. Another control method, the "ground check control", involved baiting cacodylic acid treated host trees with frontalure (1:2 mixture of frontalin and α-pinene). The pheromone mixture drew beetles in the area to the trap tree where unfavorable conditions prevented development of the brood. Although, the treatment reduced brood survival by 84% (Coulson et al. 1973a, b) and did not detrimentally affect beneficial insects (Williamson 1970), the tactic was abandoned due to cost and complexity of the procedure.

High cost and federal regulations currently prevent the treatment of large areas of forest with pesticides. Nevertheless, insecticides are still sometimes used to treat small infestations or prevent attack of high valued trees in commercial forests, seed orchards, naval stores or urban home and recreational areas (Thatcher et al. 1978).

Mechanical Control

Currently, forest managers and landowners have four direct control tactics at their disposal to control SPB. They include: 1) salvage removal, 2) cut-and-leave, 3) fell and spray with pesticides, and 4) fell, pile and burn (Billings 1980). The former two are the most commonly recommended to reduce losses

(Swain and Remion 1980). Salvage generally involves the rapid removal of all infested trees plus a buffer strip of uninfested trees from an infestation area (Texas Forest Service 1976). The removal of infested trees reduces beetle concentrations in the treated areas (Morris and Copony 1974), whereas removal of a buffer strip serves to disrupt spot growth. The cut-and-leave method is similar to the salvage treatment, except the felled trees are not removed (Ollieu 1969, Texas Forest Service 1975). Although the beetles developing in infested trees are not removed, this treatment has been shown to stop the expansion of infestations in several southern states. The biological rational to explain the success of this treatment is based on the understanding that infestation expansion requires emerging beetles, nearby host trees and a source of secondary attractants (Billings 1980). The attractant source is eliminated by felling of the most recently attacked trees, nearby trees are eliminated by felling a buffer strip, and emerging beetles from infested trees tend to disperse upon the elimination of the attractant source. Although these treatments have proven effective in reducing or halting infestation growth, they are not always applicable because of small infestation size, inaccessibility to infested areas, lack of wood market (e.g., salvage), and the reluctance by landowners to cut a 15-30 m buffer strip of uninfested trees.

Silvicultural Control

Research has documented that SPB outbreaks are associated with senescent forest stands occurring on poor sites (Hicks 1980). Often these stands have high basal areas and are composed of monocultures of large diameter, slow growing trees. Loblolly and shortleaf pine are considered to be the most susceptible to beetle attack. Several silvicultural treatments have been proposed

to promote individual tree and stand resistance to SPB attack including: management of tree species composition by favoring the most resistant species (e.g., slash, longleaf, Virginia, and eastern white pines); removal of high-risk trees, i.e., trees damaged by lightning, wind and ice; maintenance of proper stand density (80-90 ft^2/acre) through periodic thinnings or prescribed burns; planting of seedlings at wider spacings to reduce the number of thinnings, minimizing logging damage; and fertilization of stands to encourage growth (Belanger 1980). These treatments may be assigned according to economic considerations and management objectives, as each forest condition and locality presents different management problems.

Behavioral Chemicals

There is extensive literature regarding the use of semiochemicals in the management of insect pests (Beroza 1970, 1976; Birch 1974; Borden 1989, 1993; Mitchell 1981; Wood 1982,). Borden (1989) described five principal means by which semiochemicals can influence the population dynamics of bark beetles: mediation of aggregation and mass attack on new hosts; cessation of aggregation and shifting of attack to unexpoited region of the host or to new hosts; induction of aggregation by species that compete for the same host resource; inhibition of aggregation and attack by species that compete for the same resource; and mediation of host finding by commensal and entomophagous insects. The author listed six fundamental strategies for potential pest management of scolytids, including: 1) prevention of aggregation pheromone production, 2) disruption of olfactory perception, 3) exploitation of semiochemical-based secondary attraction (monitoring), 4) exploitation of repellent allomones, 5)

exploitation of the kairomonal response by entomophagous insects, and 6) exploitation of antiaggregation pheromones.

For the SPB, examples of Strategy 1 have involved removing sources of attraction (e.g. cut-and-leave or salvage) to disrupt aggregation (Swain and Remion 1981), or cause the tree to become toxic to the beetle (e.g., pesticides) (Berisford et al. 1981). Although, Strategy 2 has not been tested in the field, electrophysiological evidence (Payne and Dickens 1976) and behavioral data (Borden 1967) indicate that sensory adaptation or habituation occurs in pheromone-saturated environments. Examples of Strategy 3 tested to monitor or control SPB include: use of frontalin and turpentine-baited traps to determine predator : prey (*Thanasimus dubius* : SPB) ratios for predicting regional SPB population trends (Billings 1988); the trap tree method (Vité 1970); and deployment of elution devices containing frontalure (1:2 mixture of frontalin and α-pinene) on all non-host and host brood trees within an SPB infestation in order to draw beetles away from the active head (Richerson et al. 1980). A promising example of Strategy 4 involves the recent discovery that 4-allylanisole, a host-produced compound, repelled several bark beetle species from the allomone source in laboratory bioassays and significantly reduced capture of both sexes of SPB in attractant-baited traps in the field (Hayes et al. 1994). In addition, SPB may be repelled by a unknown compound released by *I. grandicollis* (Birch et al. 1980, Svihra et al. 1980), however this phenomena is in need of further study. In the case of Strategy 5, several natural enemies of SPB have demonstrated response to kairomones emitted from the beetles or associated fungi, including: the clerid, *Thanasimus dubius* F. to the aggregating beetle pheromone, frontalin (Vité and Williamson 1970); the predatory fly, *Medetera bistriata* L., to the

semiochemicals released from of the associated bark beetle (e.g., SPB and *I. grandicollis*) infested logs (Williamson 1971); the parasitoid, *Dinotiscus dendroctoni* (Ashmead) to a blend of compounds collected from beetle-infested trees (Salom et al. 1991); and numerous species whose arrival coincide with various stages of beetle attack and brood development (Camors and Payne 1973, Dixon and Payne 1980).

Recently, Strategy 6, i.e., exploitation of inhibitor or antiaggregation pheromones, has demonstrated the greatest potential for use in the control of the SPB. Both *endo*-brevicomin and verbenone each have been shown to significantly reduce capture of SPB in attractant-baited traps; however, combining the two compounds did not significantly reduce SPB capture over the reduction obtained with either inhibitor alone (Payne et al. 1978, Salom et al. 1992). Field tests of a 1:1 mixture of brevicomin isomers and verbenone caused reductions of 84% in beetle landing and 92% in galleries on treated trees (Payne and Richerson 1979, Richerson and Payne 1979). However, the treatment did not prevent the trees from succumbing to beetle attack even though mass attack by SPB did not occur. *Ips avulsus*, a sympatric species, was found to be capable of competitively replacing SPB (Payne and Richerson 1985). Ultimately, the treatment was considered successful, because the less aggressive *Ips* species could not sustain the growth of an infestation in the absence of SPB stressed trees.

The apparent equality of response of SPB to verbenone and *endo*-brevicomin alone or combined and the cost of pheromones led to the sole evaluation of verbenone in the suppression of SPB infestation growth (T.L. Payne, personal communication). The treatment of freshly attacked trees and uninfested trees at the active head of SPB infestations with verbenone-only has

shown considerable success in slowing or halting the growth of small to moderate-sized infestations (Billings et al. 1995; Payne and Billings 1988, 1989; Payne et al. 1992,). In large infestations, the verbenone-only treatment was less successful, but better success has been obtained when the verbenone treatment (treating a buffer strip only) is combined with the cut-and-leave tactic (felling freshly attacked trees only or felling all infested trees) (Billings et al. 1995).

Significance

Bark beetles of the genera *Dendroctonus*, *Ips*, and *Scolytus* are the most destructive pests of forests in the Northern Hemisphere. Damage from these insects causes losses of billions of cubic feet of timber valued at millions of dollars each year (Drooz 1985; Furniss and Carolin 1977). Tactics currently used to control bark beetles, such as salvage, cut-and-leave, or chemical control are not always successful and/or are of environmental concern.

The use of semiochemicals as management tools show considerable promise in reducing damage and mortality by bark beetles. Some of these compounds have already been successfully used to monitor population trends or as a mass trapping and/or disruption tactic (Borden 1993). The exploitation of semiochemicals as management tools requires a thorough understanding of the mechanisms involved in the production and release of and response to these chemicals by the target species. In addition, it is important to have an understanding of the effects of semiochemicals applied on the target species and associated organisms. Lanier et al. (1972) was one of the first to suggest that the indiscriminate use of semiochemicals could theoretically lead to resistance. Such a phenomenon has been studied with regards to use of pheromones in mating

disruption of the pink bollworm moth, *Pectinophora gossypilla* (Saunders) (Haynes et al. 1984, Haynes and Baker 1988). Just as there is the potential for the development of semiochemical resistance in the target insect, there is also the potential for the development of response "resistance" in natural enemies as many of these insects use host pheromones as kairomones.

Chapter 3

Southern Pine Beetle. 1. Geographic and Temporal Variation in the Quantity of Semiochemicals Released from Infested Logs

Introduction

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), is a multivoltine species which breeds in the subcortical region of mature pine trees. It is the most important mortality factor in stands of loblolly pine, *Pinus taeda* L., in the southeastern United States (Drooz 1985). Colonization of resistant hosts is accomplished by the mass aggregation of SPB on individual trees in response to semiochemicals.

The sequence of host colonization by SPB in response to beetle-produced pheromones and host volatiles was proposed by Renwick and Vité (1970) and later revised (Vité and Francke 1976). Upon location and acceptance of a host, "pioneer" females release primarily frontalin (F) and *trans*-verbenol (tV) (Rudinsky et al. 1974, Vité and Renwick 1968). These compounds, in combination with host volatiles, primarily α-pinene (aP), stimulate mass aggregation of conspecifics, with males predominant. Arriving males, in turn, release primarily verbenone (V) and *endo*-brevicomin (eB), both of which act as population regulators. Verbenone, at low concentrations, and also (+)-eB serve to enhance female response thereby balancing the sex ratio of arriving SPB (Billings 1985, Rudinsky et al. 1974). As SPB populations in the host increase, V concentrations exceed an unknown threshold at which point the pheromone, along with (-)-eB (Vité et al. 1985), inhibits response of both sexes to attractant semiochemicals. As a result of

this inhibition, mass attack switches to neighboring trees (Gara 1967, Payne et al. 1978, Rudinsky et al. 1974, Salom et al. 1992, Vité and Renwick 1971).

The amount of pheromone produced by individual insects can vary greatly as has been shown in several Coleoptera species, particularly in Scolytidae (Borden et al. 1986, Schlyter and Birgersson 1989 and references cited within). *Dendroctonus ponderosae* Hopkins females produced 0 to 2204 ng of tV after exposure to aP for 24 h (Borden et al. 1986). Studies of pheromones released from *Ips typographus* L. entrance holes showed large differences in quantities depending on the attack stage (Birgersson and Bergstrom 1989, Birgersson et al. 1984). Differences in quantities of pheromones produced by individual bark beetles are thought to be the result of genetic variation in enzyme systems used to convert precursors to pheromones or response of beetles to different environmental conditions (Birgersson et al. 1988). Understandably, bark beetles with large geographic ranges would be expected to have greater genetic variability and be exposed to a greater range of environmental conditions (Miller et al. 1989).

Although SPB also have a large geographic range, until recently the assumption was that the beetle and therefore its pheromone system was the same throughout its contiguous range (> 2200 km) in the United States. However, studies have shown significant genetic differences between Texas and Virginia SPB (Anderson et al. 1979, Namkoong et al. 1979). In addition, Berisford et al. (1990) showed, using laboratory bioassays, that SPB exhibits geographic differences in response to pheromone extracts collected from SPB-infested logs during the first two days of attack. For example, volatiles collected from Texas logs were most attractive to Texas SPB and significantly

less attractive to Virginia beetles. Virginia and Georgia SPB were similarly most attracted to extracts from their respective geographic areas. The authors suggested that the differences in SPB response amoung areas may have been due, in part, to quantitative differences in pheromones.

The objectives of this study were to 1) elucidate the possible local and regional variations in the quantity of six semiochemicals released from logs obtained from naturally SPB-infested host trees and 2) determine the extent to which changes in these semiochemicals occur over time. In particular, the study focused on the period of colonization when the host was most attractive to arriving SPB up until attraction was inhibited and the attack switched to neighboring trees.

Methods

Biological Materials. Four uninfested loblolly pine (approximately 28 cm diameter at breast height) were selected 1 to 3 m in front of the most recently attacked trees in each of three infestations in Virginia (Campbell, Pittsylvania and Bedford Counties) and Texas (Angelina National Forest, Angelina County), and two infestations in South Carolina (Union County) (Table 3.1) during the summer of 1993. Southern pine beetles were induced to attack trees by baiting each with racemic F and commercial southern pine turpentine (Klean Strip®). The baits were positioned on the bole of sample trees at approximately 3 m above ground to encourage the natural tendency of SPB to concentrate their attack at this height (Dixon and Payne 1979). After three days of mass attack, each tree was cut down and a 25 cm log section was cut from the 3 m height. The ends of each infested log were sealed with

Table 3.1. Southern pine beetle infestation, tree, and log characteristics observed in Texas (TX), South Carolina (SC), and Virginia (VA) in 1993.

State	Infestation Location	Approx. No. of Infested Trees	Pine BA[a] (m^2/ha)	Mean DBH[b] (cm)	Tree No.	Diameter of Tree at 3 m	Number of BP[c]/Log	Attacking BP/100cm^2
TX	**1. Angelina Co.**	250	3.57	26.9	1	24.1	14	2
	Lat. 94° 23' 07"				2	26.4	26	4
	Long. 31° 04' 25"				3	22.1	55	10
					4	27.2	58	8
	2. Angelina Co.	100	4.62	17.2	1	22.9	28	5
	Lat. 94° 15' 00"				2	24.4	37	6
	Long. 31°24' 44"				3	25.4	70	11
					4	25.9	54	8
	3. Angelina Co.	60	4.51	26.1	1	26.2	37	6
	Lat. 94° 15' 00"				2	24.9	32	5
	Long. 31° 23' 14"				3	25.1	28	4
					4	22.9	18	3
					State Mean	**24.8**	**38**	**6**
SC	**1. Union Co.**	150	5.08	15.1	1	20.2	28	5
	Lat. 81° 28' 00"				2	21.5	24	4
	Long. 34° 50 30				3	20.6	22	4
					4	20.9	31	6
	2. Union Co.	550	5.30	23.8	1	27.4	21	3
	Lat. 81° 39' 00"				2	23.1	42	7
	Long. 34° 53' 30"				3	22.5	42	7
					4	24.3	27	4
					State Mean	**24.3**	**33**	**5**
VA	**1. Campbell Co.**	250	5.26	15.1	1	23.9	57	9
	Lat. 78° 57' 30"				2	25.7	67	10
	Long. 37° 03' 30"				3	23.9	43	7
					4	24.4	117	19
	2. Pittsylvania Co.	750	3.95	20.9	1	25.7	80	12
	Lat. 79° 22' 30"				2	26.2	119	18
	Long. 37° 01' 30"				3	23.1	67	11
					4	26.9	60	9
	3. Bedford Co.	450	6.20	17.1	1	25.4	120	19
	Lat. 79° 22' 00"				2	23.4	82	14
	Long. 37° 24' 45"				3	23.9	90	15
					4	25.4	41	6
					State Mean	**24.8**	**79**	**12**

[a] BA - basal area of pines

[b] DBH - diameter at breast height (mean of all trees in stand)

[c] BP - beetle pairs (= female and male)

paraffin to reduce moisture loss and to prevent excess quantities of host volatiles from being collected during the aeration process as described below.

Collection of Volatiles. Each log section was placed in a 19 liter, tightly sealed metal bucket outfitted with inlet and outlet ports. Air, filtered through a desiccant (Drierite®, W.A. Hammond Drierite Company, Xenia, Ohio) and activated charcoal, was drawn through the bucket and over the log at 0.5 l/h by a small suction air pump (Berisford et al., 1990, T.L. Payne, personal communication). Volatile-laden air, exiting each bucket, passed through glass tubing (10 cm by 0.5 cm ID) containing 2.0 g of Poropak Q ® (80-100 mesh; Waters Chromatography Division, Millipore Corporation, Milford, MA). Preliminary studies have shown that the Poropak Q plugs absorbed ca. 98% of all compounds released from SPB-infested logs (Grosman, unpublished data and M. Dalusky, personal communication). C-flex® (Fisher Scientific) connectives led from the filter to the Poropak tubes. Compounds released from each log were collected for 5 consecutive days. Temperature during entrapment ranged from 24 to 27 °C. Every 24 h, old tubes were replaced with tubes containing clean Poropak Q. Poropak Q tubes containing collected volatiles were sealed with teflon tape and stored at -60 °C. After aeration, the bark was pealed from the logs and all SPB adults were counted and sexed.

Semiochemical Analysis. The collected volatiles were extracted from the Poropak Q with 5 ml of n-pentane : ether (80 : 20) in the direction opposite of gas flow. Preliminary trials showed that this volume of solvent was sufficient to remove all entrapped volatiles from the polymer, as no volatiles were detected by gas-liquid chromatography (GLC) analysis of a second 5 ml rinse. Rinses were measured for volume, transferred to clean 6 ml vials and

stored at -60 °C. 2-Octanol, at 10 μg/ml of n-pentane, was used as an internal standard.

All extracts were treated with about 3 g of anhydrous sodium sulfate granules to remove water contaminants. Due to the large concentration differences between the host compound aP and SPB pheromones, the extracts were first diluted to 10 ml for analysis of aP. Subsequently, the extracts were concentrated under nitrogen to 5 ml for pheromone evaluation.

Volatile extracts were analyzed on a Shimadzu GC-14A (split/splitless injector and FID detector equipped) using a Chiraldex G-TA capillary column (40 m X 0.25 mm ID) (Advanced Separation Technologies Inc., Whippany, NJ). The column, capable of separating the enantiomers of chiral semiochemicals, was connected to a methyl phenyl guard column (5 m X 0.53 mm ID, Restek, Inc.) to prevent degradation by water or high MW compounds. High purity helium was used as a carrier gas (160 kPa) and the temperature was programed from 32 °C for 1 min., 2 °C/min. to 68 °C for 4 min., 5 °C/min. to 115 °C for 5 min., 8 °C/min. to 140 °C for 7 min. A standard dilution series with concentrations of 0.5, 1.0, 5.0, 10.0, 50.0, and 100 ug enantiomer/ml was initially made from a stock solution containing equal amounts of racemic aP, F, eB, cV, tV, and V. This series produced linear standard curves (R^2 = 0.99 - 1.00). In subsequent weeks of analysis, however, only the concentration series containing 0.5, 10, and 50 μg/ml were run, as concentrations of semiochemicals within extracts generally fell within this range. In all series runs the curves were linear (R^2 = 0.99 - 1.00). Identification of host- and beetle-produced volatile peaks was made through comparisons with standard peak retention times and gas chromatography-mass

spectrometry (GC-MS) analysis. Quantification of semiochemical enantiomers was made by internal standards and the equation:

$$\frac{\text{Area of extract peak X Conc. of std.}}{\text{Area of the std. peak}} = \mu g/\text{ml X extract vol.} = \mu g/\text{extract}$$

In most cases, both enantiomers of an evaluated compound were detected from a log on all days; however, in some extracts only one enantiomer was below the limit of detection (LOD = the lowest area count obtained for an enantiomer from all extracts analyzed; LOD for (+)-cV = 0.068 μg/sample; (-)-cV = 0.205 μg; (+)-tV = 0.207 μg; (-)-tV = 0.147 μg). The quantity of the compound was then estimated by replacing zero counts with the LOD. This substitution was made only if both enantiomers were detected from a log on two or more days. No estimate was made if both enantiomers of a compound were not detected in a single extract.

Statistical Analysis. The experiment design is a multivariate nested repeated measure design for the blend of six semiochemicals with geographic area and infestations within a geographic area as between subject effects and day as the within subject effect. A univariate nested repeated measure design was used to evaluate individual compounds. Data were analyzed using analysis of variance [Abacus Concepts, SuperANOVA. (Abacus Concepts Inc., Berkley, CA, 1989)]. Semiochemical quantity data distributions were found to be nonnormal. Therefore, data were transformed by the equation $y = \log 10 (x + 1)$. Differences among geographic areas and infestations within an area were determined using Tukey's Compromise (P = 0.05). However, because in a repeated measures design, the within subject variables (i.e., days) are correlated with each other, quantitative comparisons among days for each

compound could not be made using the standard post-hoc tests. Therefore, trends in temporal data are discussed.

Coefficients of variation (CV) (Sokal and Rohlf 1981) were calculated using transformed quantities of each semiochemical. The corrected coefficients of variation (CCV) were then calculated using the equation $(1 + 1/4n) \times CV$ to reduce the bias of CV which result from small samples. Regression was used to evaluate the relationship between the quantities of semiochemicals released from logs and the number of beetle pairs per log (Abacus Concepts, SuperANOVA). Relationships among the semiochemical quantity and other variables relating to site and tree characteristics (e.g., latitude and longitude of infestation location, number of days after initial attack, and total number of beetle pairs (BP) within a log section) were determined by stepwise regression using the maximum R^2 improvement (SAS Institute Inc. 1985). This model selection method uses forward selection to fit the best 1, 2, 3, or 4 variable model. The variables are switched so that R^2 is maximized. The best model was selected when all variables within the model explained greater than 4% of the response variable.

In an unpublished study, T.L. Payne quantified F, tV and V released from cut logs during the first three to four days after initial attack. These figures were used as a comparison to quantities collected in our study four to eight days after initial attack.

Results

Infestation characteristics including size, basal area and tree diameter at breast height (DBH) were quite variable among the sites (Table 3.1).

Infestations were generally larger and more active in VA, resulting in higher attack densities per log. Only the quantities of aP ($R^2 = 0.351$; $P = 0.0004$) and F ($R^2 = 0.275$; $P = 0.0021$) emitted from individual logs were dependent on the number of beetle pairs (= number of entrance holes) per log. The quantities of these two compounds were subsequently adjusted to reflect the number of beetle pairs present in each log.

Semiochemical Blend: Storage problems resulted in the loss of most day 6 extracts; therefore, data from day 6 were not included in these analyses. The multivariate nested repeated measures analysis showed geographic area ($F_{12,38} = 10.45$; $P \leq 0.0001$), infestation within an area ($F_{30,78} = 2.56$; $P \leq 0.0005$), and temporal ($F_{18,187} = 5.35$; $P \leq 0.0001$) differences in the blend of the six compounds. There also were a significant interaction between day and geographic area ($F_{36,293} = 3.26$; $P \leq 0.0001$) and between day and infestation within an area ($F_{90,378} = 2.28$; $P \leq 0.0001$). MANOVAs of separate days showed significant differences among geographic areas in blend quantities for each day: day 4 ($F_{12,38} = 6.51$; $P \leq 0.0001$); day 5 ($F_{12,38} = 7.19$; $P \leq 0.0001$); day 7 ($F_{12,38} = 9.00$; $P \leq 0.0001$); and day 8 ($F_{12,38} = 11.22$; $P \leq 0.0001$). Differences in infestations within a geographic area also were significant for all days: day 4 ($F_{30,78} = 1.62$; $P \leq 0.047$); day 5 ($F_{30,78} = 4.88$; $P \leq 0.0001$); day 7 ($F_{30,78} = 2.55$; $P \leq 0.0005$); and day 8 ($F_{30,78} = 1.77$; $P \leq 0.023$).

Individual Compounds: Univariate analysis of individual compound quantities showed several with significant interactions. Quantity data showed day by geographic area interaction in aP ($F_{6,72} = 3.48$; $P \leq 0.0045$), eB ($F_{6,72} = 2.68$; $P \leq 0.021$), cV ($F_{6,72} = 3.86$; $P \leq 0.0022$), and tV ($F_{6,72} = 4.49$; $P \leq 0.0006$) and day by infestation within an area interaction in F ($F_{15,72} = 3.36$; $P \leq$

0.0003), eB ($F_{15,72} = 2.19$; $P \leq 0.014$), cV ($F_{15,72} = 2.97$; $P \leq 0.001$), and tV ($F_{15,72} = 3.65$; $P \leq 0.0001$). Due to these interactions, geographic areas, infestations within an area, and days were evaluated separately for each compound.

α-Pinene: All logs produced large amounts of this monoterpene hydrocarbon ranging from 151 to 878 μg/BP per 24 h and were at least a 100 fold greater than the most abundant pheromone, frontalin. The corrected coefficient of variation (CCV) averaged 44%, with a range of 12 to 201%. No differences were found among infestations within a geographic area on any one day (Table 3.2). Although, SC logs averaged the fewest number of beetle pairs (BP) (Table 3.1), the amounts released per entrance hole (= BP) were significantly higher than from TX and VA logs on day 4, but only greater than VA logs on day 5. Temporal patterns of aP within geographic areas were generally similar (Fig. 3.1A); however, there were large differences among areas, particularly from days 4 to 5. Whereas, aP quantities declined in VA and SC during this period, TX tended to increase. Infestation location (i.e., longitude) explained the most variability in aP quantity at almost 12% (Table 3.3). No more than 2% was explained by any other site or compound characteristic.

Frontalin: This compound was the most abundant pheromone with mean quantities ranging from 0.99 to 4.89 μg/BP per 24 h (CCV averaged 28%; range 4 to 90%). This range overlaps, but was wider than the range of 0.03 to 3.74 ug/BP/24 h. collected by T.L. Payne (unpublished data) from infested logs during the first four days after initial SPB attack. This study indicates that amounts of F released from infested trees may continue to increase even after

Table 3.2. Variation within geographic areas in the semiochemical quantities[a] released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack in 1993.

			Day							
			4		5		7		8	
Semiochem.	State	Infest.	Mean	SE	Mean	SE	Mean	SE	Mean	SE
α-Pinene	TX	1	393.2 a[b]	212.3	694.8 a	354.5	616.5 a	173.4	729.8 a	258.9
		2	288.0 a	65.6	445.4 a	36.5	248.6 a	31.4	332.7 a	93.6
		3	534.1 a	60.4	878.1 a	170.3	565.0 a	135.6	611.6 a	90.9
	SC	1	739.1 a	69.6	648.0 a	69.2	551.6 a	41.7	464.4 a	183.1
		2	707.3 a	242.4	626.8 a	74.5	552.3 a	128.8	743.9 a	118.5
	VA	1	575.9 a	131.5	340 a	99.6	407.9 a	73.1	382.6 a	87.5
		2	269.7 a	58.1	304.3 a	44.8	285.6 a	64.7	439.1 a	70.5
		3	349.4 a	45.5	150.4 a	20.6	351.6 a	99.8	410.1 a	106.4
Frontalin	TX	1	1.2 b	0.6	1.1 b	0.5	2.2 a	0.5	3.7 a	1.2
		2	1.5 ab	0.3	2.2 ab	0.3	1.6 a	0.1	2.3 a	1.1
		3	3.1 a	0.4	3.0 a	0.5	3.0 a	0.5	3.5 a	0.4
	SC	1	4.9 a	0.2	3.9 a	0.3	2.6 a	0.1	4.3 a	0.6
		2	2.9 b	0.6	2.5 a	0.5	3.7 a	0.5	3.4 a	0.8
	VA	1	2.8 a	0.7	2.1 a	0.3	2.5 a	0.3	2.6 a	0.5
		2	1.7 a	0.3	1.5 a	0.4	1.6 a	0.2	3.1 a	0.6
		3	2.0 a	0.4	1.0 a	0.2	1.5 a	0.4	1.9 a	0.4
endo -Brev.	TX	1	8.0 a	0.5	7.3 a	1.8	9.0 a	1.5	11.6 a	1.7
		2	15.7 a	6.9	22.5 b	2.6	13.3 a	2.1	19.0 a	9.1
		3	12.5 a	1.4	13.3 b	1.7	12.4 a	2.1	11.2 a	1.2
	SC	1	35.2 a	4.8	26.9 a	2.1	24.6 b	1.4	39.5 a	3.7
		2	24.0 a	2.5	20.3 b	1.4	38.8 a	2.5	32.1 a	3.8
	VA	1	22.4 a	3.0	16.2 ab	0.8	23.8 a	2.1	26.5 a	2.5
		2	16.1 a	1.3	19.8 a	4.5	16.5 b	1.7	31.6 a	7.0
		3	34.7 a	7.9	9.8 b	1.3	23.1 a	1.5	27.9 a	4.0

[a] α-pinene and frontalin presented as ug/beetle pair/24 h; *endo* -brevicomin, *cis* -verbenol, *trans* -verbenol, and verbenone presented as ug/24 h.

[b] For each column, within each state, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

Table 3.2 cont. Variation within geographic areas in the semiochemical quantities[a] released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack in 1993.

			Day							
			4		5		7		8	
Semiochem.	State	Infest.	Mean	SE	Mean	SE	Mean	SE	Mean	SE
cis -Verb.	TX	1	2.7 a[b]	1.1	2.9 b	1.3	5.4 a	2.4	11.3 a	3.5
		2	2.9 a	1.0	8.9 a	1.8	9.7 a	3.2	12.3 a	3.8
		3	6.1 a	1.4	1.0 b	0.4	5.3 a	1.1	8.0 a	1.3
	SC	1	2.6 a	0.7	3.2 a	1.0	1.2 a	0.3	8.3 a	2.8
		2	2.3 a	0.6	0.6 b	0.4	2.7 a	1.1	0.3 b	0.3
	VA	1	2.2 a	0.4	1.8 a	0.9	1.0 a	0.6	2.4 a	0.6
		2	5.4 a	2.7	4.0 a	2.6	2.6 a	1.3	5.7 a	2.7
		3	8.4 a	2.1	2.5 a	0.9	2.4 a	1.0	2.8 a	1.1
trans -Verb.	TX	1	7.1 a	3.8	9.3 a	4.1	16.5 a	7.1	3.6 a	3.4
		2	2.7 a	1.2	28.3 a	12.0	0.2 b	0.2	0.5 a	0.3
		3	5.7 a	1.8	7.9 a	1.3	11.0 a	3.2	2.0 a	0.6
	SC	1	4.4 a	0.8	12.6 a	0.5	7.3 a	0.7	7.0 a	3.4
		2	6.9 a	2.7	8.0 a	3.0	8.6 a	4.0	3.4 a	1.7
	VA	1	8.0 a	4.2	1.5 b	0.1	2.0 b	0.4	4.4 a	0.5
		2	13.6 a	4.3	23.4 a	11.4	16.0 a	6.6	18.9 a	10.2
		3	15.9 a	4.9	9.7 a	2.3	4.6 ab	1.7	4.8 a	1.6
Verbenone	TX	1	24.4 a	12.3	22.8 a	9.2	21.5 a	8.4	39.1 a	2.3
		2	19.8 a	5.5	23.7 a	5.6	14.3 a	2.7	23.0 ab	4.1
		3	15.1 a	1.1	19.4 a	6.3	17.5 a	5.6	19.7 b	4.4
	SC	1	10.6 a	1.8	9.8 a	1.8	6.9 a	0.5	19.5 a	4.6
		2	7.6 a	1.7	11.9 a	4.8	20.8 a	9.1	17.1 a	6.8
	VA	1	12.2 a	0.7	8.3 a	1.0	10.1 b	1.5	11.7 b	2.6
		2	14.1 a	6.0	17.4 a	6.2	14.7 b	3.2	27.2 ab	9.0
		3	28.4 a	6.1	11.9 a	2.1	26.4 a	2.9	30.4 a	4.9

[a] α-pinene and frontalin presented as ug/beetle pair/24 h; *endo* -brevicomin, *cis* -verbenol, *trans* -verbenol, and verbenone presented as ug/24 h.

[b] For each column, within each state, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

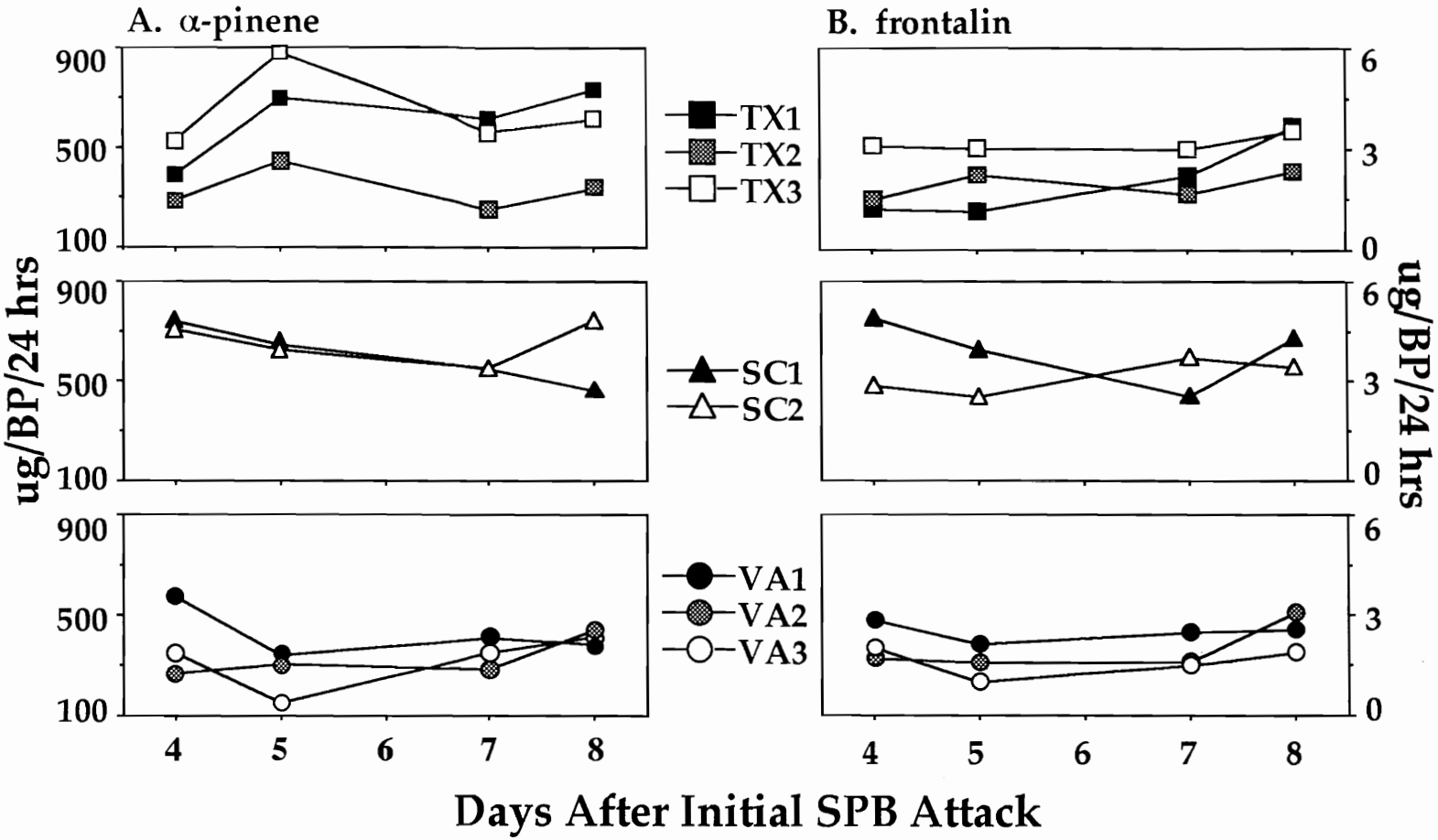

Figure 3.1. Temporal variation in quantities of (A) α-pinene and (B) frontalin released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC), and Virginia (VA). Standard error bars are omitted for clarity.

Table 3.3. Relationships among quantities of semiochemicals released from southern pine beetle-infested logs and site, log, and semiochemical characteristics using stepwise regression (Maximum R^2).

	Dependent Variable - Semiochemical Quantity					
Independent Variables [a]	α-Pinene (aP)[b]	Frontalin (F)	*endo* -Brevicomin (eB)	*cis* -Verbenol (cV)	*trans* -Verbenol (tV)	Verbenone (V)
aP quantity		0.094 ***[c]	-	-	-	-
aP chirality		0.059 ***	-	0.139 ***	-	0.058 **
cV quantity					-	0.233 ***
cV chirality					-	-
tV quantity				-		0.043 *
tV chirality				-		-
V quantity				0.227 ***	0.086 ***	
V chirality				-	-	
BP	-	-	-	-	-	-
Day	-	-	-	-	0.080 ***	0.049 **
Latitude	-	-	0.317 ***	-	0.067 **	-
Longitude	0.119 **	0.279 ***	0.062 ***	-	-	-
Maximum R^2 [d]	**0.119**	**0.432**	**0.379**	**0.366**	**0.230**	**0.383**

[a] BP- no. of beetle pairs; Day- days after initial SPB attack; Latitude- latitude of infestation location; Longitude-longitude of infestation location.

[b] aP, α-pinene; F, frontalin; eB, *endo* -brevicomin; cV, *cis* -verbenol; tV, *trans* -verbenol; V, verbenone.

[c] Significant relationships (r^2) are represented by * for $P < 0.1$; ** for $P < 0.01$; and *** for $P < 0.001$

[d] Calculated by the forward selection to fit the best 1, 2, 3, or 4 variable model. The variables are switched so that R2 is maximized. The best model was selected when each variable within the model explained greater than 4% of the response variable.

the average peak mass attack period of three to four days (Dixon and Payne 1979).

SPB logs from SC released significantly larger amounts of F per BP on day 4 than did logs from VA or TX (Table 3.4). Differences among geographic areas eventually disappeared as a result of a gradual increase in quantities released from TX and VA logs. Differences among infestations within the TX and SC were also most pronounced during the first few days of aeration, whereas no differences were found among VA infestations for all days. Generally, F quantities were relatively stable over time, but showed modest increases only in TX1 and VA2 (Fig. 3.1B). In contrast, amounts from SC1 logs declined by day 7 before rebounding. Three factors (longitude, aP amount, and aP percent) were found to explain ca. 43% of the variability in F quantities (Table 3.3); however, no other characteristic contributed more than an additional 4%.

endo-Brevicomin: Release rates of eB were not found to be dependent on BP numbers in individual logs. This was surprising because no source of eB, other than SPB, has been reported. Mean quantities of eB per infestation ranged from 7.4 to 39.51 ug/24 h with CCV averaging 12% (range 4 to 56%) As with frontalin, the quantity of eB released was higher in SC than in TX for all days (Table 3.4). Quantities of eB from VA generally ranged in between SC and TX. Differences among infestations within a geographic area only occurred on day 5 and 7 (Table 3.2). Quantities released from TX logs were remarkably consistent over time (Fig. 3.2A). In contrast, SC and VA infestations showed much greater variability, with most experiencing a decrease in amounts released on day 5, but then recovered by day 8. Almost

Table 3.4. Variation among geographic areas in the semiochemical quantities[a] released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack.

		Day							
		4		5		7		8	
Semiochem.	State	Mean	SE	Mean	SE	Mean	SE	Mean	SE
α-Pinene	TX	405.1 b[b]	75.8	672.8 a	130.6	476.7 a	83.1	558.1 a	100.8
	SC	723.2 a	116.9	637.4 a	47.2	552 a	62.7	604.1 a	114.0
	VA	398.3 b	60.0	264.9 b	41.7	348.4 a	44.7	410.6 a	47.2
Frontalin	TX	1.9 b	0.3	2.1 ab	0.3	2.3 ab	0.3	3.2 a	0.5
	SC	3.9 a	0.5	3.2 a	0.4	3.1 a	0.3	3.8 a	0.5
	VA	2.2 b	0.3	1.5 b	0.2	1.8 b	0.2	2.5 a	0.3
endo -Brev.	TX	12.1 b	2.3	14.4 b	2.2	11.6 c	1.1	13.9 b	3.0
	SC	29.5 a	3.3	23.6 a	1.7	31.8 a	3.0	35.8 a	2.8
	VA	24.4 a	3.5	15.3 b	1.9	21.1 b	1.4	28.7 a	2.6
cis -Verb.	TX	3.9 a	0.8	4.3 a	1.2	6.8 a	1.4	10.5 a	1.7
	SC	2.4 a	0.4	1.9 a	0.7	2.0 b	0.6	4.3 b	2.0
	VA	5.3 a	1.3	2.7 a	0.9	2.0 b	0.6	3.6 b	1.0
trans -Verb.	TX	5.2 b	1.4	15.2 a	4.8	9.2 a	3.1	2 b	1.1
	SC	5.6 ab	1.4	10.3 a	1.7	7.9 a	1.9	5.2 ab	1.9
	VA	12.5 a	2.5	11.5 a	4.5	7.6 a	2.8	9.4 a	3.8
Verbenone	TX	19.8 a	4.2	22.0 a	3.8	17.8 a	3.3	27.3 a	3.2
	SC	9.1 b	1.3	10.8 b	2.4	13.9 a	5.0	18.1 a	4.0
	VA	18.2 a	3.4	12.5 ab	2.3	17.1 a	2.5	23.1 a	4.0

[a] α-pinene and frontalin presented as ug/beetle pair/24 h; *endo* -brevicomin, *cis* -verbenol, *trans* -verbenol, and verbenone presented as ug/24 h.

[b] For each column, within each semiochemical, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

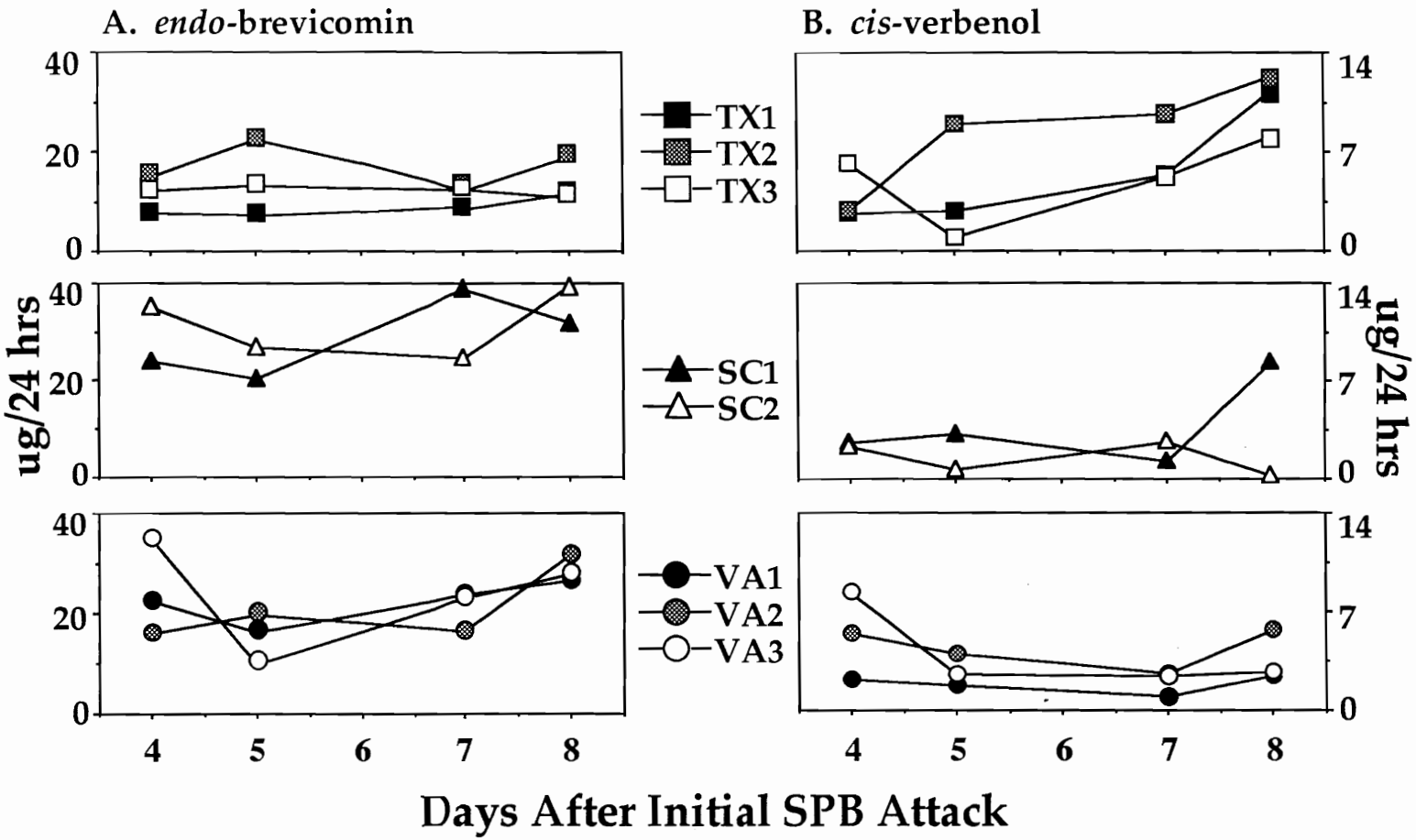

Figure 3.2. Temporal variation in quantities of (A) *endo*-brevicomin and (B) *cis*-verbenol released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC) and Virginia (VA). Standard error bars are omitted for clarity.

40% of the variability in eB quantity was explain by the latitude and longitude of each infestation (Table 3.3). Less than three percent was explained by any one of the other site or compound characteristics.

cis-Verbenol: cV showed considerable variability among infestations with means ranging from 0.26 to 12.33 ug/24 h (CCV averaged 56%; range 15 to 212%) (Table 3.2). No cV was detected from several logs from TX and SC on days 5 and 7 (Fig. 3.2B). Generally, mean quantities did not appear to differ appreciably among infestations within each geographic area on a given day (Table 3.2). In addition, area quantities tended to be similar except on days 7 and 8 where TX amounts were significantly higher than in SC and VA (Table 3.4). Although, there was no consistent trend in quantity of cV over time among infestations, all TX infestations released the highest amounts by day 8 (Fig. 3.2B). In contrast, quantities remained relatively constant in most eastern infestations. Almost 37% of cV quantity variability was explained by two compound characteristics (Table 3.3). Somewhat surprisingly, the greatest contributor was V amount (23%) which is known to be produced from the oxidation of cV. However, it has been reported that a reverse hydration reaction can revert V back to cV (Vanderwel and Oehlschlager 1987).

trans-Verbenol: As with cV, quantities of tV also showed considerable variability among infestations within a geographic area, with means ranging from 0.2 to 28.3 ug /24 h (CCV averaged 50%; range 3 to 213%) (Table 3.2). This quantitative range encompasses the amount of 2.4 ug/24 h collected by T.L. Payne (unpublished data) from infested logs during the first three days after initial attack. Differences were significant in VA on days 5 and 7, and among infestations within a geographic area on day 7 in TX (Table 3.2).

Geographic areas differed only on days 4 and 8 with VA quantities being higher than in TX (Table 3.4). Generally, there was no consistent trend over time among infestations (Fig. 3.3A). Quantities of tV peaked on or before day 4 in infestations VA1 and VA3; peaked on day 5 in infestations TX2, SC1, and VA2; or peaked on day 7 in infestations TX1 and TX2. The variability in peak occurrence over time likely reflects differences in the stage of attack on a particular day. Three variables contributed significantly to tV quantity variability; however, all explained less than 9% (Table 3.3). As with cV, V amount, surprisingly, explained the greatest percentage of tV quantity variability.

Verbenone: Mean quantities of verbenone released from infested logs ranged from 6.92 to 39.12 ug/24 h with CCV averaging 18% (range 3 to 42%) (Table 3.2). These quantities are substantially higher than the 0.27 ug/24 h collected from infested logs during the first three days after initial attack by T.L. Payne (unpublished data). These data support the hypothesis of Vité and Francke (1976) that females initially release small amounts of verbenone which enhance the attraction of females to hosts. Upon arrival of males, verbenone concentrations increase dramatically and serve to switch attack to neighboring trees.

Differences in verbenone quantities among geographic areas occurred early on days 4 and 5 (Table 3.4). Differences among infestations within an area, however, only occurred on days 7 and 8 (Table 3.2). Amounts released in most infestations, with the exception of VA3 and SC2, remained stable until day 8, at which time quantities increased (Fig. 3.3B). This may reflect the increased contribution of SPB-associated microorganisms. Nearly 40% of the

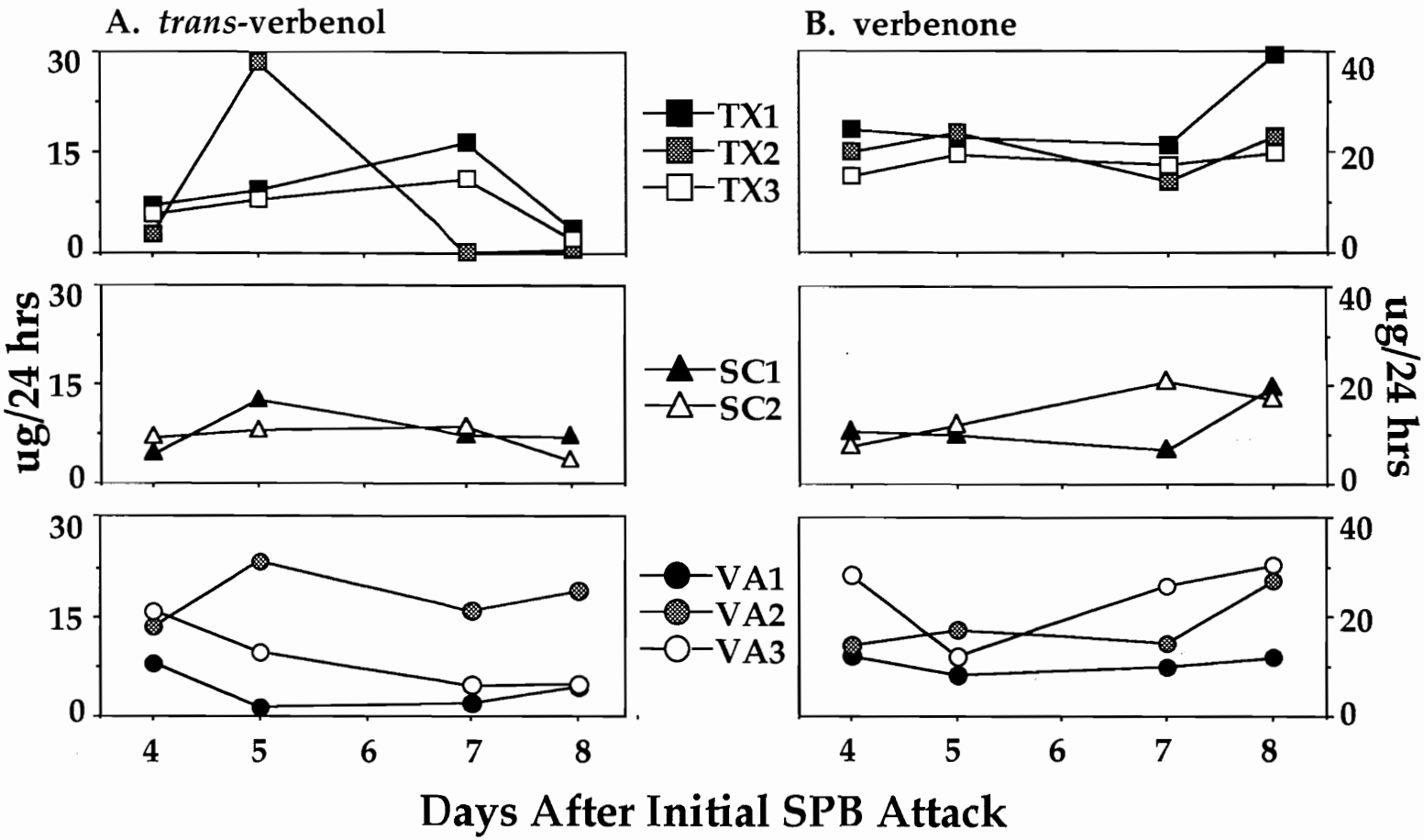

Figure 3.3. Temporal variation in quantities of (A) *trans*-verbenol and (B) verbenone released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC), and Virginia (VA). Standard error bars are omitted for clarity.

variability in V was explained by four variables (Table 3.3), of which cV amount was by far the greatest contributor at 23%. No other significant variable explained more than 5%.

Discussion

Many bark beetle species utilize several semiochemicals for host selection and colonization (Borden 1982). However, most studies evaluating the semiochemical systems of these beetles have focused on specific characteristics of these systems. Some studies have addressed variation in the quantities of compounds present in individual beetles or released from individual entrance holes over time (Birgersson and Bergstrom 1989, Birgersson et al. 1984). Others studies examined geographic variation in the quantity and chirality of a single compound in individual beetles (Miller et al. 1989). This is the first reported study that evaluates both geographic and temporal variations in the quantity of a blend of semiochemicals released from naturally-infested host material. It was not my intent to measure the complete release of semiochemicals from SPB-infested logs from the point in time when the first SPB begin boring into the host to complete shut down of aggregation. However, the volatile collection technique I used permitted the determination of release rates of six semiochemicals emitted from SPB-infested log sections four to eight days after initial attack. This is the transition period during which attractive semiochemicals of SPB predominate followed by increasing amounts of inhibitory semiochemicals. Discussion, herein, will focus on the extent to which beetle, host, and other

environmental factors may contribute to temporal and geographic variations in the quantities of the six semiochemicals.

Semiochemical Quantity. The baiting procedure was used to induce SPB attack of selected trees in a short period of time and to synchronize mass attack on each host tree. However, infestations within and between geographic areas varied considerably in size and stand density (Table 3.1). These factors, along with different environmental and host conditions, are known to influence local SPB population levels and vigor (Coulson 1980). Although most baited trees showed evidence of attack (pitch tubes or boring dust at the tree base) within 24 h, the rate at which SPB arrived at each baited tree varied as is evident from the variable number of attacking BP/100 cm^2 (Table 3.1).

The quantity of semiochemicals released from a section of log is dependent, in part, on the number of beetle pairs (= entrance holes) present. However, only aP and F quantities showed significant relationships with the number of beetle pairs per log. It is surprising that eB quantities did not exhibit this dependence as this semiochemical, like F, is reported to be produced only by bark beetles (Vanderwel and Oehlschlager 1987). However, Vanderwel et al. (1992) indicated that the precursor of eB, (E)-6-nonen-2-one, may be biosynthesized by one or more sources including the beetle itself, the host, and/or beetle-associated microorganisms. Similarly, the lack of dependence of the oxygenated monoterpene (cV, tV and V) quantities on BP numbers is likely due to the fact that each is directly converted from precursors by two or more of these same sources.

Temporal Variation. Most compounds evaluated in this study exhibited temporal trends similar to those found for semiochemicals present in phloem surrounding entrance holes of *I. typographus* (Leufven and Birgersson 1987) or released from these holes (Birgersson and Bergstrom 1989). This suggests that tree health (as determined by levels of moisture, competition, soil nutrients, etc. in a geographic area), plays a large role in determining the quantities of semiochemicals released from SPB-infested logs. Quantities of the host monoterpene aP declined by day 4 (Fig. 3.1A), apparently as a result of depletion of primary resin at the entrance hole (Leufven and Birgersson 1987). As the attack proceeded, the subsequent increase of aP by day 8 may be the result of an increase in the production of secondary resin by affected phloem cells [1] and/or of the construction by SPB adults of preemergence holes to the surface of the bark (personal observations). These holes may allow semiochemicals trapped in previously plugged galleries to be released.

The SPB pheromones F and eB also exhibited similar temporal patterns in most trees sampled from SC and VA, i.e., declining quantities on day 5 and subsequent increases by day 8 (Figs. 3.1B and 3.2A). This pattern is somewhat surprising as quantities of F in female hindguts are known to decline significantly after 48 h (Coster and Vité 1972). One explanation for the continuous high release of this compound over time is that most of the F is

[1] In response to an attack by bark beetles and associated microorganisms, conifers use two types of resin as the first line of defense (Berryman 1972, Reid et al. 1967). The attacking beetles initially encounter primary resin, which is stored preformed in cells and resin-containing cavities. Thereafter, host cells begin production of secondary resin in response to beetle-microorganism activity. (Christiansen and Horntvedt 1983).

released by SPB in frass within 48 h of the initiation of attack. Frontalin is then released from the frass slowly, but at a constant rate. Alternatively, the temporal trend observed in this study may reflect the SPB response to the host's defenses. Birgersson et al. (1984) found substantially higher quantities of most pheromones in hindguts of *I. typographus* collected from trees with rich resin flow (trees in good health) than in beetles from trees of poor heath. As F and eB are produced *de novo* by SPB upon contact with an acceptable host, the degree of host resistance experienced by attacking beetles would determine the quantity of pheromone produced. Raffa and Berryman (1983) found that bark beetles attacking a healthy host generally produce greater quantities of pheromones and for a longer period of time. The greater and longer production of these compounds attract greater numbers of beetles, which are required to overcome a healthy tree's defenses.

The oxygenated monoterpenes tV and V also exhibited quantitative patterns over time similar to those found for *I. typographus* (Leufven and Birgersson 1987). Generally, quantities of tV peaked 3 to 5 days after initial attack and then declined and leveled off by day 8 (Fig. 3.3A). In contrast, quantities of V were stable until day 7 at which time quantities increased substantially by day 8 (Fig. 3.3B). Interestingly, cV temporal patterns from SPB generally differed from those of *I. typographus*. Whereas cV quantities from *I. typographus*-infested trees peaked within the first 1-3 days and then declined to relatively low levels (Birgersson and Bergstrom 1989, Leufven and Birgersson 1987), quantities from SPB-infested logs were low initially, but continued to increase after 8 days (Fig. 3.2B). However, two infestations, TX3 and VA3, did exhibit patterns similar to those observed for *I. typographus*.

The differences in cV, tV, and V quantities over time reflect the possible differences in the contributions of at least three potential sources. First, SPB are capable of detoxifying host tree monoterpene hydrocarbons, such as aP, by the addition of oxygen to form tV, cV, and subsequently V (Hughes 1973, 1975; Renwick et al. 1973). The beetle then releases these compounds, a portion of which is emitted from the entrance hole and the rest absorbed on the gallery walls (Leufven and Birgersson 1987). Second, the latter portion of the two terpene alcohols is used by microorganisms colonizing the gallery walls to form V (Brand et al. 1976). A third pathway is the autoxidation of monoterpene hydrocarbons to cV and tV, and further oxygenation by this pathway to V (Hunt et al. 1989). Leufven and Birgersson (1987) suggested a fourth pathway in which oxygenated monoterpenes are formed by enzymes released from plant cells damaged by bark beetles or microorganisms. A fifth source are sympatric bark beetle species. In the case of SPB, three *Ips* species, *I. calligraphus* (Germar), *I. grandicollis* (Eichhoff) and *I. avulsus* (Eichhoff), will normally colonize the same host just after SPB attack and produce *cis*- and *trans*-verbenol as part of their pheromone system (Smith et al. 1993). However, none of these species was present in any of the sample logs and so could not be a contributing source of pheromones in this study. Lastly, Vanderwel and Oehlschlager (1987) indicated that V may undergo a hydration reaction and revert back to either cV or tV. This may explain the significant dependence of some precursor quantities on product quantities (Table 3.3).

Oxygenated monoterpenes produced by SPB and autoxidation are believed to be the sole contributors to the semiochemicals released from SPB

entrance holes during the first few days. After 6 to 7 days of attack, however, SPB contributions would decline, autoxidation would likely increase as a result of secondary resin production, and microorganisms would begin to make appreciable contributions to V concentrations.

Geographic variation. Latitude and longitude of infestation location explained significant proportions of the variability of the semiochemicals, aP, F, and eB (Table 3.3). These compounds were released initially at higher rates in SC than in other geographic areas (Table 3.4). The higher release rates of aP in SC may indirectly reflect the better defenses of host trees in this area at that time. These trees responded to SPB attack by attempting to "pitch out" invading beetles with resin and produced characteristic pitch tubes. Trees under attack in VA and TX appeared to be stressed prior to attack as they rarely produced pitch tubes upon attack by SPB. Birgersson and Bergstrom (1989) also collected higher amounts of host volatiles from trees with pitch tubes as compared to trees with poor resin flow. When present, the pitch tubes apparently provide a greater surface area from which aP is released.

The higher amounts of F and eB initially collected from SC logs may, in turn, reflect SPB response to the host's defenses. As mentioned previously, bark beetles have been observed to produce larger quantities of pheromones for longer periods of time as host resin flow increases. As long as host resin flow continues, it is necessary for the beetle to continue releasing pheromones for recruitment of additional conspecifics to overcome the host defenses (Alcock 1982). An alternate hypothesis by Birgersson et al. (1988) suggests that at high population levels, as observed in VA, there would be a weak selection pressure for high pheromone production because individual

SPB producing small quantities of pheromone may "hitch-hike" on the signal of the large group. Conversely, at low population levels (i.e., SC), there is stronger selection pressure to produce large quantities of pheromones because a larger proportion of individuals would be pioneers or members of small groups. Data for both the quantity and chirality of pheromones present in SPB hindguts from different geographic areas are presented in Chapter 5.

Quantities of oxygenated monoterpenes, particularly in cV and tV, varied geographically (Tables 3.2 and 3.4). As mentioned previously, several pathways may contribute to the production of these compounds. It appears likely that the contribution of cV, tV and/or V by a given pathway will differ depending on geographic area, infestation, tree, and even portion of the tree. Individual bark beetles are known to be quite variable with regard to the quantities of pheromones present in hindguts (Birgersson et al. 1984, 1988; Miller et al. 1989). Resin flow can differ between neighboring trees (Hodges et al. 1977); resulting in differences in rate of autoxidation. Individual SPB are reported to carry different microorganism species of varying abundance (Barras and Perry 1972, Bridges et al. 1984). It may be that some microorganisms are more common in certain geographic areas than in others, although, this hypothesis has yet to be investigated. The presence or absence of certain microorganisms associated with a SPB population would likely have a dramatic affect on the quantities of oxygenated monoterpenes released from trees in a given infestation during the later stages of attack.

Behavioral Implications. The information gained in this study can be used to elaborate on previously proposed behavioral sequences occurring during the mass attack of host trees, particularly from the period when the

host is most attractive to SPB to the point in time when attack switches to neighboring trees.

Upon landing on a host and initiating attack, pioneering females release frontalin (Kinzer et al. 1969, Renwick and Vité 1969, 1970). Frontalin, along with (+)-aP released in defensive host resin, attracts large numbers of SPB, predominately males (Renwick and Vité 1969). At the same time, females appear to release large amounts of tV and small quantities of V and eB (Rudinsky et al 1974, Vité and Renwick 1968, T.L. Payne, unpublished data), all of which enhance SPB response to F (Payne et al. 1978, Renwick and Vité 1969,). Although F quantities in SPB hindguts were found to decrease significantly within 48 h after feeding (Coster and Vité 1972), my observations indicate that large quantities of F continue to be released by SPB, their frass, and/or from gallery walls even longer than 8 days after initial attack.

Dixon and Payne (1979) reported the number of SPB landing on a host is greatest (i.e., the host is most attractive) 3 to 5 days after initial attack. Because F quantity levels continue to remain high during this period, SPB response to the aggregation pheromone must be inhibited in order to prevent overpopulation of the host. The inhibition of SPB response and subsequent switching of mass attack to neighboring trees appears to be controlled by several events. First, the multiple beetle attack sites appear to deplete primary resin reserves in the host thus reducing the quantity of aP available for use by SPB as a kairomone. Second, the quantity of tV often declined to low levels after day 5. At these reduced levels, the attractiveness of F to arriving SPB may be reduced. Third, upon locating the entrance hole of a female, males begin releasing V and eB (Renwick and Vité 1969).

At low male densities, V enhances female response to F, thereby balancing the sex ratio of arriving SPB (Renwick and Vité 1969, Salom et al. 1992). At higher male densities, both V and eB inhibit response of all arriving beetles. Based on average concentrations of F, eB, and V released from logs 4 and 5 days after initial attack, it appears that eB and V quantities must each reach levels of greater than 15% of F quantities before SPB response is inhibited. This situation differs from *D. ponderosae*, where V levels must be greater than 30% of tV levels (the primary aggregation pheromone of this species) before V inhibits *D. ponderosae* response (Miller et al. 1995).

Although a study by Salom et al. (1992), evaluating the effects of V and eB on response of SPB to attractant-baited traps, showed no additional inhibitory effects of eB when included in traps already containing V, it should be noted that the concentration of V released in this study was almost 40 times higher than that of eB. In contrast, Payne et al. (1978) found that traps baited with V and eB together, at a ratio of 20:1, captured significantly fewer SPB than traps baited with either inhibitor alone. Therefore, it seems likely that the inhibitory effect of V would be significantly enhanced by the addition of more equal quantities of eB.

The knowledge gained in this study concerning variation in the quantities of semiochemicals released from naturally-infested host material is but one step leading to a better understanding of bark beetle semiochemical communication systems. A semiochemical message detected by receptive beetles in flight provides information on the condition of potential hosts and mates (Miller et al. 1989). Specific information obtained by receiving beetles, however, is not just encoded in the quantity of individual compounds, but

also in the chirality of these compounds (Seybold et al. 1993). Relatively little is currently known about the chirality of SPB semiochemicals released from naturally infested hosts. It is possible that beetle, host, and other environmental factors which may influence the variability in chirality of a compound are not necessarily the same as those influencing the variation in quantity. As semiochemicals become increasingly important as pest management tools, greater understanding is needed on the variation and the causes there of of all characteristics of these compounds.

Chapter 4

Southern Pine Beetle. 2. Geographic and Temporal Variation in the Chirality of Semiochemicals Released from Infested Logs

Introduction

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), utilizes semiochemicals to mediate mass attack of host pine trees (Payne 1980). The principal aggregation pheromone, frontalin (F), is produced predominately by females and released upon locating a host tree suitable for colonization (Kinzer et al. 1969). The attractiveness of F is enhanced by the host terpene, α-pinene (aP), and by *trans*-verbenol (tV), which is produced by two sources; SPB and autoxidation of aP (Hunt et al. 1989; Payne et al. 1978; Renwick and Vité 1969, 1970). Two additional pheromones produced predominately by male SPB, *endo*-brevicomin (eB) and verbenone (V), serve in population regulation by switching mass attack from one tree to another (Payne et al. 1978; Pitman et al. 1968; Renwick and Vité 1969, 1970; Vité and Renwick 1971,). Verbenone also is produced from *cis*-verbenol (cV) and tV through autoxidation (Hunt et al. 1989) and by SPB-associated microorganisms (Brand et al. 1976).

Both the quantity and chirality of semiochemicals are important in conveying information about the condition and genetic quality of potential hosts and mates (Miller et al. 1989, Seybold et al. 1993). However, the amount of pheromone produced by individual insects can vary greatly as has been shown in several Coleoptera species, particularly in Scolytidae (Borden et al. 1986, Schlyter and Birgersson 1989 and references cited within). Pheromones released from *Ips typographus* L. entrance holes showed large differences in

quantities depending on the attack stage (Birgersson and Bergstrom 1989, Birgersson et al. 1984,). Quantities of SPB semiochemicals released from infested host material not only exhibited temporal variations, but also geographic variations (see Chapter 3).

Superimposed on the differences in the amount of semiochemicals produced and released is the potential for variation in the enantiomeric ratios of chiral semiochemicals. Such qualitative differences are known to occur within and between populations of insects. *Ips pini* (Say), a scolytid with a geographic range spanning the North American continent, produces and responds to nearly 100% R-(-) ipsdienol in most western populations (California and British Columbia), whereas eastern populations (New York) produce and respond to racemic ipsdienol [about 50% of the (-) enantiomer] (Lanier et al. 1972, Miller et al. 1989). Furthermore, western populations of *I. pini* are only attracted to the (-) enantiomer while S-(+) is inhibitory even in small amounts (Birch et al. 1980, Lanier et al. 1980,), even though 34% of *I. pini* collected from a western population had 6 to 40% S-(+) enantiomer (Borden et al. 1986).

Southern pine beetle also have a large geographic range, yet until recently the assumption was that the beetle and its pheromone system were the same throughout its contiguous range (> 2200 km). However, studies have shown that genetic differences exist between Texas and Virginia beetles (Anderson et al. 1979, Namkoong et al. 1979). In addition, Berisford et al. (1990) showed that SPB exhibits geographic differences in laboratory response to pheromone extracts collected from SPB-infested logs during the first two days of attack. For example, volatiles collected from Texas logs were most

attractive to Texas beetles and significantly less attractive to Virginia beetles. Virginia and Georgia SPB were similarly most attracted to extracts from their respective geographic areas. The authors suggested that differences in beetle response among areas may have been due, in part, to qualitative differences in semiochemicals.

Relatively little is known about the chirality of semiochemicals used by SPB, with regard to the overall ratio released from infested host material. However, female SPB hindguts are known to contain predominately (-)-F, (-)-tV, and (+)-cV, whereas males contain (-)-V, and (-)-eB (see Chapter 5). The chirality of cV, tV and V, produced by autoxidation is dependent on the ratio of the precursor, aP, with (+)-aP yielding (+)-tV and (+)-V (see Chapter 6). The chirality of V produced by microorganisms is unknown, but may differ from that produced by SPB and autoxidation. In loblolly pine, *Pinus taeda* L., resin was reported to contain (+)-aP (Mirov 1961). However, semiochemicals collected from insect or plant tissue may in fact contain precursors or by-products, which distort the composition of the compound(s) identified and thus have little bearing on what is released into the air to guide behavior (Silverstein 1985). Semiochemicals released from naturally-infested host material and collected from the air would provide the most realistic representation of stimuli that evoke olfactory behavior. Only one previous study has attempted to evaluate quantitative characteristics of semiochemicals actually released from bark beetle-infested host material (Birgersson and Bergstrom 1989).

The objectives of this study were to: 1) elucidate the possible local and regional variations in the chirality of a blend of six semiochemicals released

from logs obtained from host trees naturally-infested by SPB and 2) determine the extent to which changes in this semiochemical characteristic occur over time. In particular, the study focused on the period of colonization from when the host was most attractive to arriving beetles to when attraction was inhibited and the attack switched to neighboring trees.

Methods

Biological Materials. Four uninfested loblolly pine were selected 1 to 3 m in front of recently attacked trees in each of three infestations in Virginia and Texas, and two infestations in South Carolina. Sample trees were baited with racemic F and commercial southern pine turpentine (Klean Strip®) to induce mass attack by SPB. After three days of attack, each tree was cut down and a 25 cm log section were cut from a height of 3 m. The ends of each infested log were sealed with paraffin.

Collection of Volatiles. Individual logs were placed in 19 liter, tightly sealed metal buckets outfitted with inlet and outlet ports. Air, filtered through a desiccant and activated charcoal, was drawn through the bucket and over the billets at 0.5 l/h. Volatile laden air, exiting each bucket, passed through glass tubing (10 cm by 0.5 cm ID) containing 2.0 g of Poropak Q ® (80-100 mesh; Waters Chromatography Division, Millipore Corporation, Milford, MA). Compounds released from each billet were collected for 5 consecutive days. Temperature during entrapment ranged from 24 to 27 °C. Old tubes were replaced with tubes containing clean Poropak Q every 24 h during this period. Poropak Q tubes containing collected volatiles were sealed with teflon

tape and stored at -60 °C. After aeration, the bark was pealed from the logs and all SPB adults were counted and sexed.

Semiochemical Analysis. Collected volatiles were extracted from the Poropak Q with 5 ml of n-pentane : ether (80 : 20) in the direction opposite of gas flow. Rinses were measured for volume, transferred to clean 1 dram vials and stored at -60 °C. 2-Octanol, at 10 μg/ml of n-pentane, was used as an internal standard. All extracts were treated with about 3 g of anhydrous sodium sulfate granules to remove water contaminants. Due to the large concentration differences between the host compound aP and SPB pheromones, the extracts were first diluted to 10 ml for analysis of the enantiomeric ratio of aP. Subsequently, the extracts were concentrated under nitrogen to 5 ml for pheromone evaluation.

Volatile extracts were analyzed on a Shimadzu GC-14A (split/splitless injector and FID detector equipped) using a Chiraldex G-TA capillary column (40 m X 0.25 mm ID) (Advanced Separation Technologies Inc., Whippany, NJ) connected to a methyl phenyl guard column (5 m X 0.53 mm ID, Restek, Inc.). High purity helium was used as a carrier gas (160 kPa) and the temperature was programed for 32 °C for 1 min., 2 °C/min. to 68 °C for 4 min., 5 °C/min. to 115 °C for 5 min., 8 °C/min. to 140 °C for 7 min. A dilution series with concentrations of 0.5, 1.0, 5.0, 10.0, 50.0, and 100 μg/ml/enantiomer was made from a stock solution containing equal amounts of racemic aP, F, eB, cV, tV, and V. To reduce column contamination and check for accuracy, pentane and a standard (10 μg/ml per enantiomer), respectively, were injected (1 μl/injection) before and after a series of four extract injections. Identification of host- and beetle-produced volatile peaks was made through comparisons

with standard peak retention times and gas chromatography-mass spectrometry (GC-MS) analysis. Quantification of semiochemical enantiomers was made by internal standards and the equation:

$$\frac{\text{Area of extract peak X Conc. of std.}}{\text{Area of the std. peak}} = \mu g/\text{ml X extract vol.} = \mu g/\text{extract}$$

In most cases, both enantiomers of an evaluated compound were detected from a log on all days; however, in some extracts one enantiomer was below the limit of detection (LOD = the lowest area count obtained for an enantiomer from all extracts analyzed; LOD for (+)-cV = 0.068 μg/sample; (-)-cV = 0.205 μg; (+)-tV = 0.207 μg; (-)-tV = 0.147 μg). The enantiomeric ratio of the compound was then estimated by replacing zero counts with the LOD. This substitution was made only if both enantiomers were detected from a log on two or more days. No estimate was made if both enantiomers of a compound were not detected in a single extract.

Statistical Analysis. The experiment was run as a multivariate nested repeated measures design with geographic area and infestations within an area as between subject effects and day as the within subject effect. The data were analyzed using analysis of variance [Abacus Concepts, SuperANOVA. (Abacus Concepts Inc., Berkley, CA, 1989)]. Semiochemical chirality data distributions were found to be nonnormal. Subsequently, proportions of (+) enantiomers were arcsine transformed. Differences among geographic areas and infestations within an area were determined using Tukey's Compromise (P = 0.05). However, because in a repeated measures design, the within subject variables (i.e., days) are correlated with each other, qualitative comparisons among days for each compound could not be made using the standard post-hoc tests. Therefore, trends in temporal data are discussed.

A hiearchial cluster analysis (Ludwig and Reynolds 1988), was used to compare local and area variations in chiral semiochemical blends. Corrected coefficients of variation (CCV) (Sokal and Rohlf 1981) were calculated using transformed quantities and percentages of each semiochemical. Relationships among semiochemical chirality and other variables relating to site and tree characteristics (e.g., latitude and longitude of infestation location, number of days after initial attack, and total number of beetle pairs (BP) attacking log section) were determined by stepwise regression using maximum R^2 improvement (SAS Institute Inc. 1985). This model selection method uses forward selection to fit the best 1, 2, 3, or 4 variable model. The variables are switched so that R^2 is maximized. The best model was selected when all variables within the model explained greater than 4% of the response variable.

Results

Infestation characteristics including size, basal area and tree diameter at breast height (DBH) were quite variable among the sites (Table 4.1). Infestations were generally larger and more active in VA, resulting in higher attack densities per log.

Semiochemical Blend: Storage problems resulted in the loss of most day 6 extracts; therefore, data from day 6 were not included in these analyses. The multivariate nested repeated measures analysis of chiral data showed geographic area ($F_{12,38} = 3.68$; $P \leq 0.001$), infestation within an area ($F_{30,78} = 2.19$; $P \leq 0.0031$), and temporal ($F_{18,142} = 5.85$; $P \leq 0.0001$) differences in the chiral blend of all six compounds. There also were significant interactions

Table 4.1. Southern pine beetle infestation, tree, and log characteristics observed in Texas, South Carolina, and Virginia.

Region	Infestation Location	Approx. No. of Infested Trees	Pine BA [a] (m^2/ha)	Mean DBH [b] (cm)	Tree No.	Diameter of Tree at 3 m	Number of BP[c]/Log
Texas	**1. Angelina Co.**	250	3.57	26.9	1	24.1	14
	Lat. 94° 23' 07"				2	26.4	26
	Long. 31° 04' 25"				3	22.1	55
					4	27.2	58
	2. Angelina Co.	100	4.62	17.2	1	22.9	28
	Lat. 94° 15' 00"				2	24.4	37
	Long. 31°24' 44"				3	25.4	70
					4	25.9	54
	3. Angelina Co.	60	4.51	26.1	1	26.2	37
	Lat. 94° 15' 00"				2	24.9	32
	Long. 31° 23' 14"				3	25.1	28
					4	22.9	18
					State Mean	**24.8**	**38**
South Carolina	**1. Union Co.**	150	5.08	15.1	1	20.2	28
	Lat. 81° 28' 00"				2	21.5	24
	Long. 34° 50 30				3	20.6	22
					4	20.9	31
	2. Union Co.	550	5.30	23.8	1	27.4	21
	Lat. 81° 39' 00"				2	23.1	42
	Long. 34° 53' 30"				3	22.5	42
					4	24.3	27
					State Mean	**24.3**	**33**
Virginia	**1. Campbell Co.**	250	5.26	15.1	1	23.9	57
	Lat. 78° 57' 30"				2	25.7	67
	Long. 37° 03' 30"				3	23.9	43
					4	24.4	117
	2. Pittsylvania Co.	750	3.95	20.9	1	25.7	80
	Lat. 79° 22' 30"				2	26.2	119
	Long. 37° 01' 30"				3	23.1	67
					4	26.9	60
	3. Bedford Co.	450	6.20	17.1	1	25.4	120
	Lat. 79° 22' 00"				2	23.4	82
	Long. 37° 24' 45"				3	23.9	90
					4	25.4	41
					State Mean	**24.8**	**79**

[a] basal area of pines

[b] diameter at breast height (mean of all trees in a stand)

[c] beetle pairs (= female and male)

between day and geographic area ($F_{36,222} = 2.40$; $P \leq 0.0001$) and day and infestation within an area ($F_{84,285} = 1.82$; $P \leq 0.0002$). Analysis of separated days again showed significant differences among geographic areas in chiral blend for each day: day 4 ($F_{12,36} = 2.60$; $P \leq 0.0132$); day 5 ($F_{12,30} = 11.64$; $P \leq 0.0001$); day 7 ($F_{12,20} = 3.92$; $P \leq 0.0035$); and day 8 ($F_{12,22} = 4.80$; $P \leq 0.0007$). Differences in infestations within an area were significant only on days 4 and 5: day 4 ($F_{30,74} = 1.82$; $P \leq 0.02$); day 5 ($F_{30,62} = 1.86$; $P \leq 0.02$); day 7 ($F_{30,42} = 1.63$; $P \geq 0.07$); and day 8 ($F_{24,40} = 1.21$; $P \geq 0.29$).

Separate cluster analyses of chiral semiochemical blend data for all infestations on each day showed infestations to be quite similar (Fig. 4.1). The range at which all infestations formed one group was 74% to 87% similarity. Figure 4.1A shows that on day 4 three clusters exist at 87% similarity. While two of the TX infestations group together, there were considerable overlap in other area infestation groups. On days 5 and 7, infestations within an geographic area tended to group together with other infestions in the same area (Figs. 4.1B and 4.1C). Infestations on day 5 showed the greatest similarity (87%) among the four days. The eastern (VA and SC) and western (TX) infestations cluster together except for TX3. SC1 forms a singular group, indicating a strong difference from other groups. The three groups cluster at > 90% similarity. The day 7 dendrogram shows a similar grouping of eastern and western area infestations as observed in day 5 dendrogram except that VA1 became a singular group. On day 8 (Fig. 4.1D) the infestations were most dissimilar (74% similarity) and geographic area grouping became more irregular. However, most TX and VA infestations exhibited area groupings.

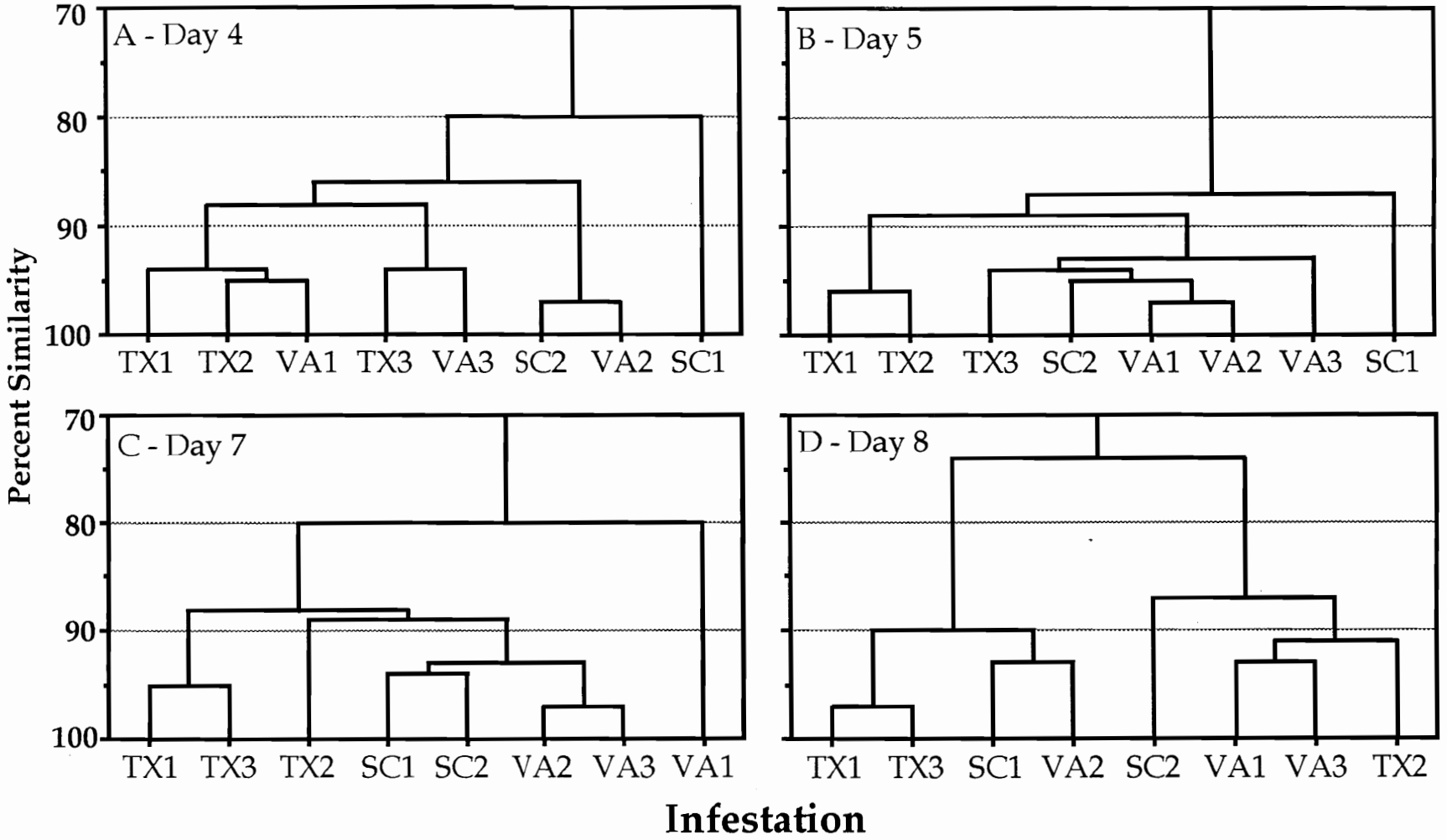

Figure 4.1. Dendrograms representing similarity among 6 semiochemical blend chiralities collected from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC), and Virginia (VA), (A) 4, (B) 5, (C) 7, and (D) 8 days after initial beetle attack. Clustering is by the flexible strategy.

Cluster analysis of semiochemical blend data for all sample trees with days pooled again showed relatively little dissimilarity with all trees forming a single group at 71% similarity (Fig. 4.2). A total of three groups exist at > 80% similarity. Generally there is little area grouping pattern except that 75% of the trees from TX grouped together.

Individual Compounds: Univariate analysis of individual compound chiralities showed several with significant interactions. Data showed day by geographic area interaction in tV ($F_{6,63} = 4.99$; $P \leq 0.0003$) and day by infestation within an area interaction in cV ($F_{15,62} = 2.54$; $P \leq 0.0053$); tV ($F_{15,63} = 5.02$; $P \leq 0.0001$) and V ($F_{15,72} = 2.16$; $P \leq 0.02$). Due to these interactions, geographic areas, infestations within an area, and days were evaluated separately for each compound.

α-Pinene: The mean enantiomeric ratios of aP per infestation ranged from 71.6%(+) : 28.4%(-) in TX3 to 90.6%(+) : 9.4%(-) in SC3 with CCV averaging 5% (range 1 to 15%). The percentage (+) of 71.6% is similar to the 69% (+)-aP isolated from loblolly pine by Mirov (1961), although it was not mentioned where the sample was collected. This compound showed no differences in chirality among infestations within a geographic area (Table 4.2), yet significant differences among areas existed between TX and the other areas on all days (Table. 4.3). These differences are supported by the fact that latitude alone explained 50% of the variability in aP chirality (Table 4.4). The aP percentages generally remained constant over time (Fig. 4.3A).

Frontalin: No differences in the percentages of (+)-F were found among infestations within a geographic area for all days (Table. 4.2); however, overall the percentage of (+) enantiomer in SC was significantly less than in

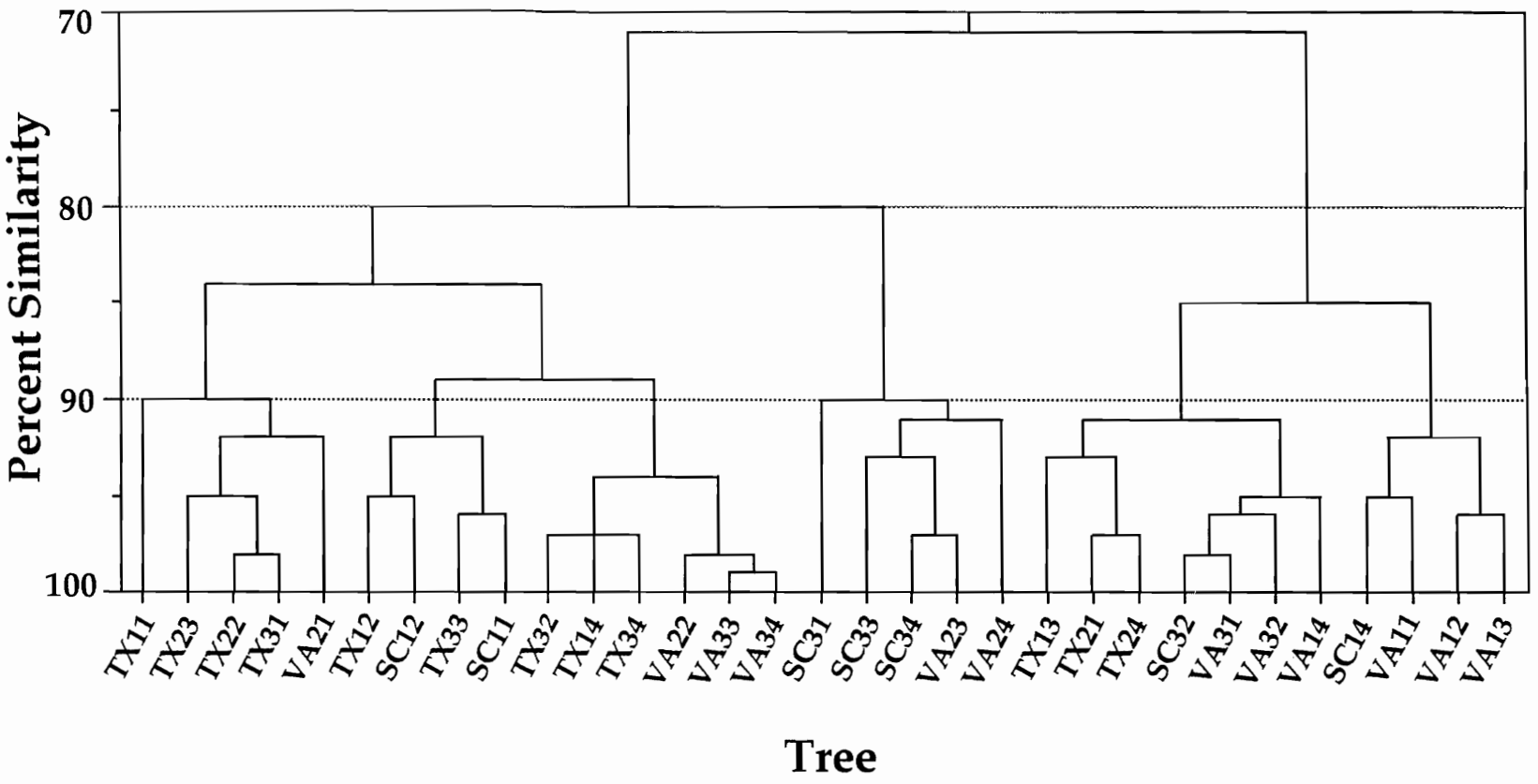

Figure 4.2. Dendrogram representing similarity among semiochemical blend chiralities from individual trees (days pooled) from eight southern pine beetle infestations in Texas (TX), South Carolina (SC), and Virginia (VA). Tree designation from left to right represents: state; infestation number; and tree number. Clustering is by the flexible strategy.

Table 4.2 Variation within geographic areas in the percent of the (+) enantiomer of semiochemicals released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack.

			Day							
			4		5		7		8	
Semiochemical	State	Infest.	Mean	SE	Mean	SE	Mean	SE	Mean	SE
α-Pinene	TX	1	80.3 a[a]	3.9	82.2 a	4.7	80.8 a	4.5	72.4 a	6.7
		2	78.7 a	0.5	77.9 a	0.6	77.3 a	0.6	78.6 a	2.2
		3	75.4 a	3.1	74.5 a	3.1	71.6 a	1.1	73.7 a	3.1
	SC	1	83.7 a	1.6	85 a	1.4	84.4 a	1.4	79.8 a	3.1
		2	86.1 a	0.9	88.4 a	2.5	86.7 a	2.6	90.6 a	3.1
	VA	1	89.6 a	0.7	90.4 a	0.8	88.1 a	1.0	89.2 a	0.9
		2	87.6 a	1.9	88.4 a	1.2	87.7 a	1.5	87.4 a	2.0
		3	85.3 a	2.1	89.2 a	1.2	87.9 a	1.3	87.5 a	1.4
Frontalin	TX	1	34.4 a	14.9	44.6 a	11.0	26.1 a	11.0	25.8 a	4.5
		2	35.8 a	8.3	39.3 a	2.0	44.5 a	8.3	37.7 a	5.2
		3	21.8 a	5.6	22.2 a	7.2	16.1 a	3.8	25.1 a	3.4
	SC	1	7.6 a	6.4	20.6 a	7.2	3.1 a	0.6	18.2 a	9.6
		2	13.1 a	4.6	18.1 a	11.2	15.2 a	6.4	18.0 a	8.8
	VA	1	35.9 a	7.2	27.7 a	7.0	27.5 a	3.3	21.8 a	3.2
		2	21.9 a	9.9	16.5 a	7.0	20.2 a	8.1	19.4 a	6.8
		3	23.8 a	3.2	12.7 a	4.1	27.5 a	5.5	25.8 a	4.8
endo -Brevicomin	TX	1	51.7 a	0.2	51.7 a	3.8	50.1 a	3.4	50.0 a	1.3
		2	57.0 a	2.5	55.1 a	2.5	55.6 a	4.1	48.6 a	2.6
		3	56.3 a	1.4	49.8 a	2.4	53.9 a	5.7	57.1 a	1.9
	SC	1	55.1 a	1.0	56.2 a	1.8	51.6 a	0.8	53.4 a	1.0
		2	55.2 a	1.7	56.1 a	3.0	54.0 a	1.3	55.2 a	1.6
	VA	1	55.4 a	1.2	49.8 a	1.4	48.8 b	0.3	50.4 b	0.2
		2	56.8 a	2.1	52.7 a	3.0	56.2 a	2.5	55.8 a	1.1
		3	59.6 a	2.2	53.2 a	1.4	50.5 ab	1.4	53.8 ab	1.5

[a] For each column, within each state, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

Table 4.2 cont. Variation with geographic areas in the percent of the (+) enantiomer of semiochemicals released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack.

			Day							
			4		5		7		8	
Semiochemical	State	Infest.	Mean	SE	Mean	SE	Mean	SE	Mean	SE
cis -Verbenol	TX	1	12.3 a[a]	4.1	2.1 a	0.5	3.4 a	1.8	1.0 a	0.5
		2	9.0 a	4.5	0.8 a	0.1	4.7 a	2.7	0.9 a	0.4
		3	4.0 a	0.9	19.3 a	13.0	1.5 a	0.3	0.9 a	0.2
	SC	1	6.4 b	2.4	29.3 a	15.2	9.4 a	3.1	6.9	3.5
		2	29.1 a	8.2	13.8 a	5.4	2.6 a	0.8	28.9 [b]	--
	VA	1	17.5 a	11.3	20.8 a	10.3	3.8 a	1.1	4.2 a	1.9
		2	38.9 a	12.2	23.8 a	13.5	12.7 a	8.0	8.0 a	4.3
		3	5.9 a	5.2	3.8 a	1.2	5 a	2.0	5.1 a	2.7
trans -Verbenol	TX	1	82.9 a	3.1	88.6 a	5.7	97.6 a	0.9	87.8 a	10.7
		2	76.2 a	1.7	97.9 a	1.4	65.6 [b]	--	20.9 b	1.6
		3	54.6 a	14.6	96.7 a	0.6	97.5 a	0.6	82.7 a	8.4
	SC	1	35.6 b	10.3	69.7 a	10.7	77.2 a	4.7	76.8 a	12.0
		2	84.9 a	4.8	82.2 a	3.9	70.1 a	5.7	27.7 a	16.1
	VA	1	70.0 a	12.4	90.1 b	0.4	23.5 b	13.0	15.5 a	5.9
		2	83.6 a	4.6	89.9 b	2.3	79 a	4.8	66.9 a	9.6
		3	72.5 a	8.5	97.3 a	0.6	77.9 a	3.7	38.4 a	19.0
Verbenone	TX	1	54.5 a	9.5	69.3 a	6.4	69.4 a	5.0	64.8 a	3.8
		2	66.4 a	3.1	73.7 a	2.0	76.3 a	1.4	76.3 a	5.0
		3	72.7 a	3.6	78.4 a	2.0	76.9 a	5.5	71.0 a	3.9
	SC	1	62.1 a	6.3	53.4 b	5.7	74.9 a	1.9	58.6 a	4.6
		2	74.0 a	3.7	78.5 a	5.0	70.5 a	7.6	70.8 a	5.3
	VA	1	63.8 b	2.1	74.8 a	4.6	75.9 a	3.5	74.1 a	2.6
		2	73.1 ab	2.4	72.0 a	1.5	79.6 a	0.7	78.0 a	0.5
		3	77.2 a	3.0	71.8 a	1.7	80.3 a	1.7	77.6 a	2.1

[a] For each column, within each state, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

[b] Percent determined from one observation.

Table 4.3. Variation among geographic areas in the percent of the (+) enantiomer of semiochemicals released from southern pine beetle-infested logs from Texas (TX), South Carolina (SC), and Virginia (VA), 4, 5, 7, and 8 days after initial attack.

		Day							
		4		5		7		8	
Semiochemical	State	Mean	SE	Mean	SE	Mean	SE	Mean	SE
α-Pinene	TX	78.1 b[a]	1.6	78.2 b	2.0	76.6 b	1.8	74.9 b	2.5
	SC	85.1 a	0.9	86.9 a	1.6	85.7 a	1.6	86.0 a	3.0
	VA	87.5 a	1.0	89.3 a	0.6	87.9 a	0.7	88.0 a	0.8
Frontalin	TX	30.6 a	5.7	35.4 a	4.9	28.9 a	5.6	28.8 a	2.8
	SC	10.4 b	3.8	19.4 a	6.2	9.1 b	3.7	18.1 a	6.0
	VA	27.2 a	4.2	19.0 a	3.8	25.1 a	3.3	22.3 a	2.8
endo -Brevicomin	TX	54.9 a	1.1	52.2 a	1.7	53.2 a	2.5	51.9 a	1.5
	SC	55.2 a	0.9	56.1 a	1.6	52.8 a	0.8	54.3 a	0.9
	VA	57.3 a	1.1	51.9 a	1.2	51.8 a	1.3	53.3 a	0.9
cis -Verbenol	TX	8.4 a	2.1	6.7 a	4.3	3.2 a	1.1	0.9 b	0.2
	SC	17.8 a	5.8	22.6 a	9.0	6.5 a	2.2	11.3 a	5.2
	VA	20.7 a	6.7	16.2 a	5.8	7.3 a	2.8	5.7 a	1.7
trans -Verbenol	TX	71.2 a	5.8	94.9 a	1.9	94.0 a	3.5	68.5 a	11.3
	SC	60.3 a	10.7	75.9 b	5.7	73.6 b	3.7	55.8 ab	13.3
	VA	75.4 a	5.1	92.4 a	1.3	58.5 c	9.6	40.2 b	9.2
Verbenone	TX	64.5 a	3.9	73.8 a	2.3	74.2 a	2.5	70.7 ab	2.6
	SC	68.0 a	4.1	66.0 a	5.9	72.7 a	3.7	64.7 b	4.0
	VA	71.4 a	2.1	72.9 a	1.6	78.6 a	1.3	76.6 a	1.6

[a] For each column, within each semiochemical, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

Table 4.4. Relationships among chiralities of semiochemicals released from southern pine beetle-infested logs and site, log, and semiochemical characteristics using stepwise regression (Maximum R^2).

	Dependent Variable - Semiochemical Chirality					
Independent Variables [a]	α-Pinene (aP)[b]	Frontalin (F)	*endo* -Brevicomin (eB)	cis-Verbenol (cV)	*trans* -Verbenol (tV)	Verbenone (V)
aP quantity	-	-	-	-	-	-
aP chirality		0.045 **	-	-	-	-
F quantity		0.045 **				
F chirality						
eB quantity			-			
eB chirality						
cV quantity				0.052 **	-	-
cV chirality					-	-
tV quantity				-	0.138 ***	-
tV chirality				-		-
V quantity				-	0.065 **	-
V chirality				-	-	
BP	-	0.099 ***	-	-	-	0.151 ***
Day	-	-	0.038 *	0.142 ***	-	0.047 *
Latitude	0.501 ***[c]	0.082 **	-	0.104 **	0.100 ***	-
Longitude	-	-	-	-	-	-
Maximum R^2 [d]	**0.501**	**0.271**	**0.038**	**0.298**	**0.303**	**0.197**

[a] BP- no. of beetle pairs; Day- days after initial SPB attack; Latitude- latitude of infestation location; Longitude-longitude of infestation location;

[b] aP, α-pinene; F, frontalin; eB, *endo* -brevicomin; cV, *cis* -verbenol; tV, *trans* -verbenol; V, verbenone.

[c] Significant relationships (r^2) are represented by * for $P < 0.1$; ** for $P < 0.01$; and *** for $P < 0.001$

[d] Calculated by the forward selection to fit the best 1, 2, 3, or 4 variable model. The variables are switched so that R^2 is maximized. The best model was selected when each variable within the model explained greater than 4% of the response variable.

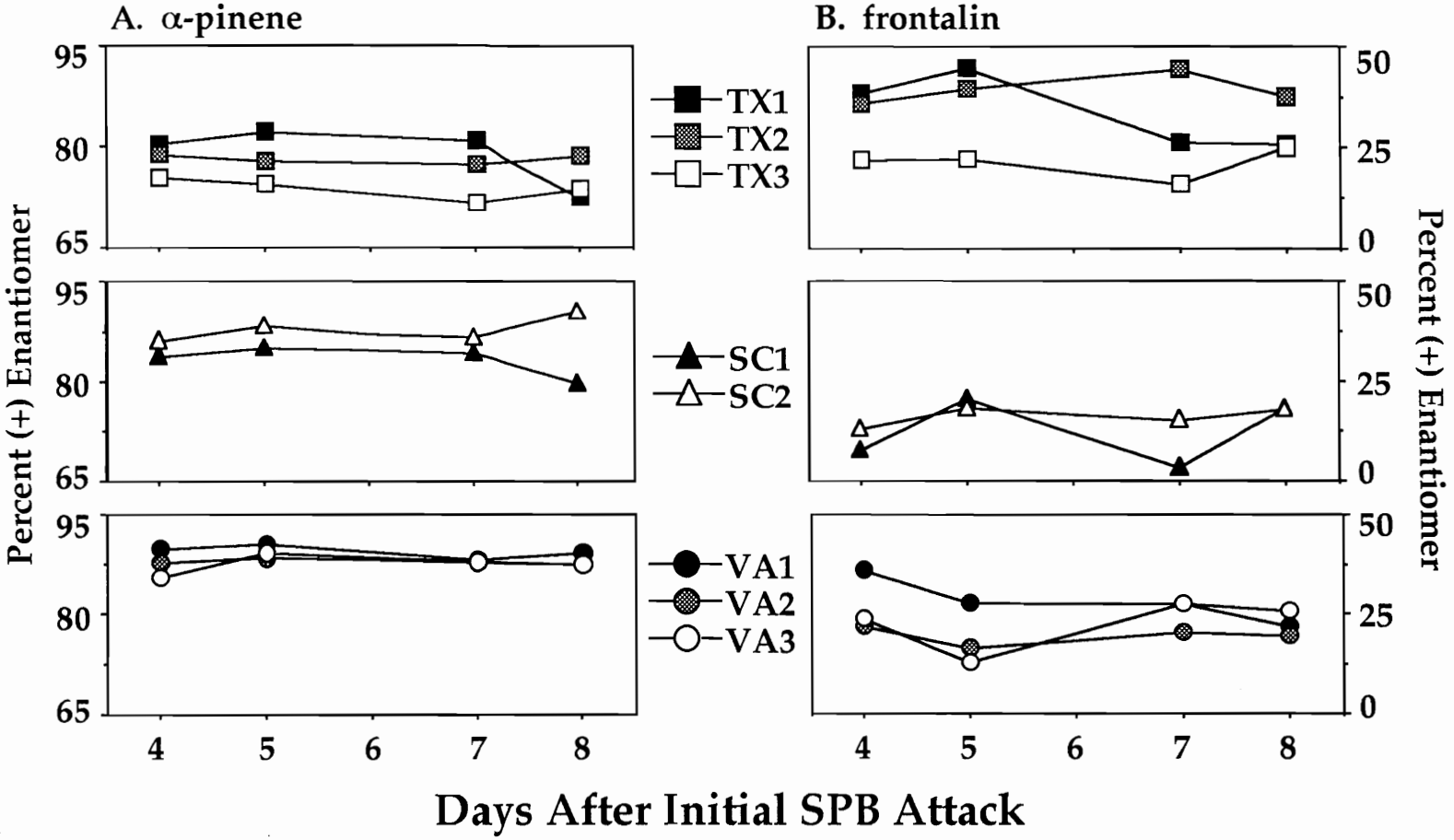

Figure 4.3. Temporal variation in the percent of the (+) enantiomer of (A) α-pinene and (B) frontalin released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC) and Virginia (VA). Chiral range: 65% - 95% (+)-α-pinene, 0% - 50% (+)-frontalin. Standard error bars are omitted for clarity.

TX and VA on days 4 and 7 (Table 4.3). For all sample sites and days, the mean enantiomeric ratio ranged from 3.1%(+) : 96.9%(-) in SC3 to 44.6%(+) : 55.4%(-) in TX2. Variation in F chirality was much greater than for aP with CCV averaging 39%; range 7 to 105%. Although chirality was variable within infestations, there was little change over time (Fig. 4.3B). Thirty-one percent of F chirality was explained by four factors (BP, latitude, aP percent, F amount), but with no variable contributing more than 10% (Table 4.4).

endo-Brevicomin: The mean enantiomeric ratios of eB released from infested logs in each infestation ranged from 48.6%(+) : 51.4%(-) in TX2 to 59.6%(+) : 40.4%(-) in VA3, with CCV averaging 5% (range 0.4 to 15%) (Table 4.2). Table 4.3 shows no geographic area differences in the chirality of eB occurring for all days, however significant differences among infestations within an area were found in VA on days 7 and 8 and in TX on day 8 (Table 4.2). Temporal changes in the chirality of eB were generally the same for infestations within SC and VA, whereas they were more variable in TX (Fig. 4.4A). Very little variability in percentages could be explained by any of the 19 factors (Table 4.4); only day of pheromone collection was significant ($P = 0.05$), but explained less than 4% of the variability.

cis-Verbenol: Considerable local and area variation was observed in cV chirality. Means of enantiomeric ratios of cV ranged from 0.8%(+) : 99.2%(-) collected in TX2 to 38.9%(+): 61.1%(-) collected in VA2, with CCV averaging 49% (range 18 to 114%) (Table 4.2). Differences between infestations within a geographic area occurred in SC only on day 4 (Table 4.2). Significant differences among geographic areas in cV chirality occurred only on day 8 (Table 4.3). At this point in time, the percentage of (+)-cV was less in TX than

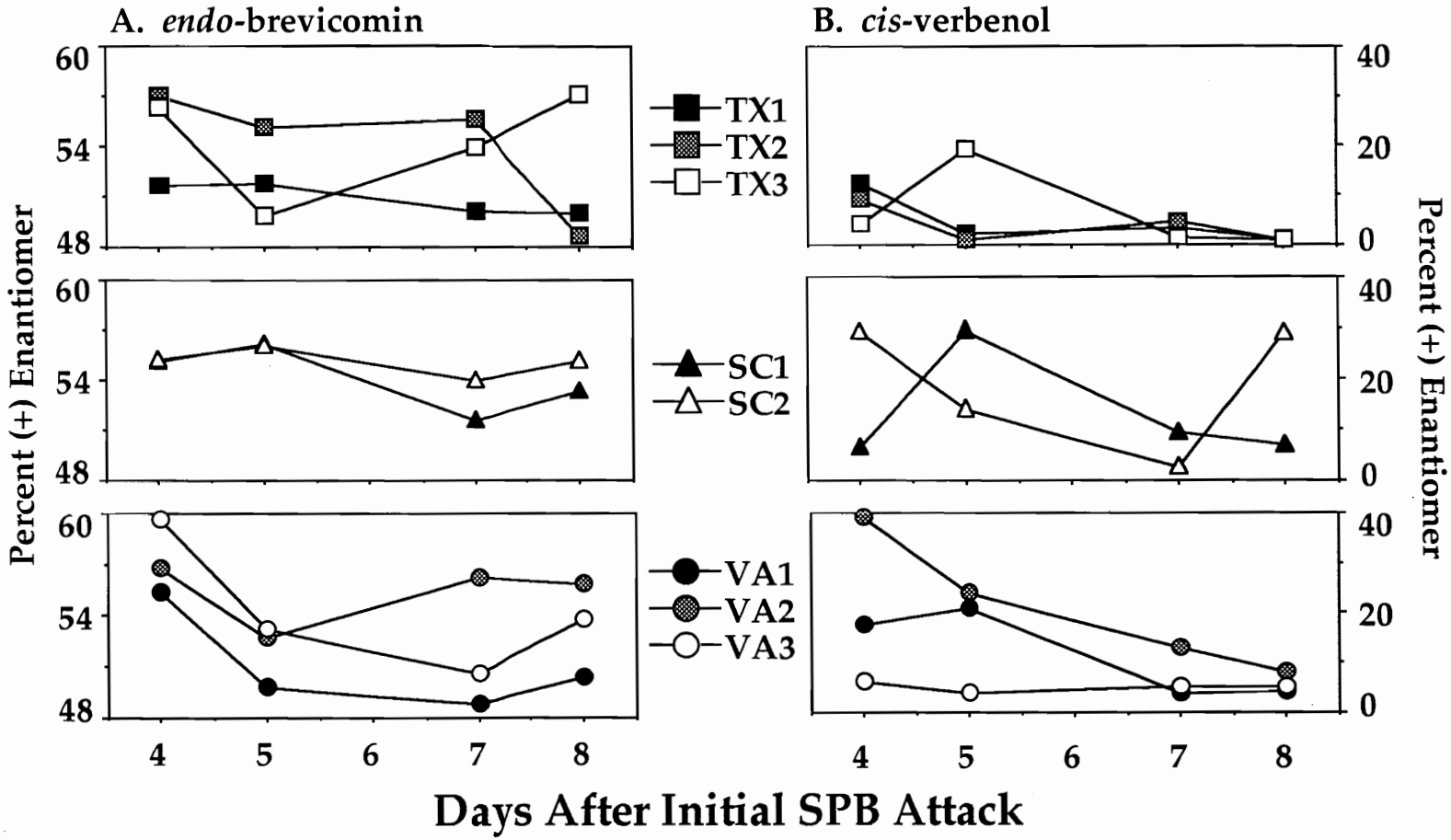

Figure 4.4. Temporal variation in the percent of the (+) enantiomer of (A) *endo*-brevicomin and (B) *cis*-verbenol released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC) and Virginia (VA). Chiral range: 48% - 60% (+)-*endo*-brevicomin, 0% - 40% (+)-*cis*-verbenol. Standard error bars are omitted for clarity.

in SC and VA. Most infestations, except SC-2 and VA-3, experienced a marked decrease in the proportion of the (+) enantiomer by day 8 (Fig. 4.4B). This trend apparently resulted from a substantial increase in the quantity of the (-) enantiomer released from infested logs. Almost 30% the variability in cV chirality could be explained by three variables of which two, day and latitude of infestation location, contributed nearly 25% (Table 4.4).

trans-Verbenol: Mean enantiomeric ratios of tV ranged from 15.5%(+) : 84.5%(-) collected on day 8 in VA1 to 97.9%(+) : 2.1%(-) collected in TX2 on day 5, with CCV averaging 50% (range 8 to 114 %) (Table 4.2). Significant differences among geographic areas occurred on days 5, 7, and 8 with TX logs releasing higher proportions of the (+) enantiomer (Table 4.3). All geographic areas exhibited some differences among infestations within an area although the day(s) in which they occurred varied depending on the area (Table 4.2). These area differences in percentages are believed to result from the fact that all infestations, except TX1, showed dramatic changes over time (Fig. 4.5A). In all VA infestations, the percent (+) of tV peaked on day 5 and then dropped significantly by day 8; whereas, in TX2 and TX3 the percent (+) was significantly higher on days 5 and 7 than on the other days. The two SC infestations showed completely opposite trends; percent (+) in SC1 increased with time; whereas, SC2 showed a decrease with time. Nearly one-third of the variation in the tV enantiomeric ratios observed was explained in part by three factors, two of which included the quantities of two semiochemicals (tV and V) (Table 4.4). Location of the infestation by latitude explained as much as 10% of the chiral tV variability.

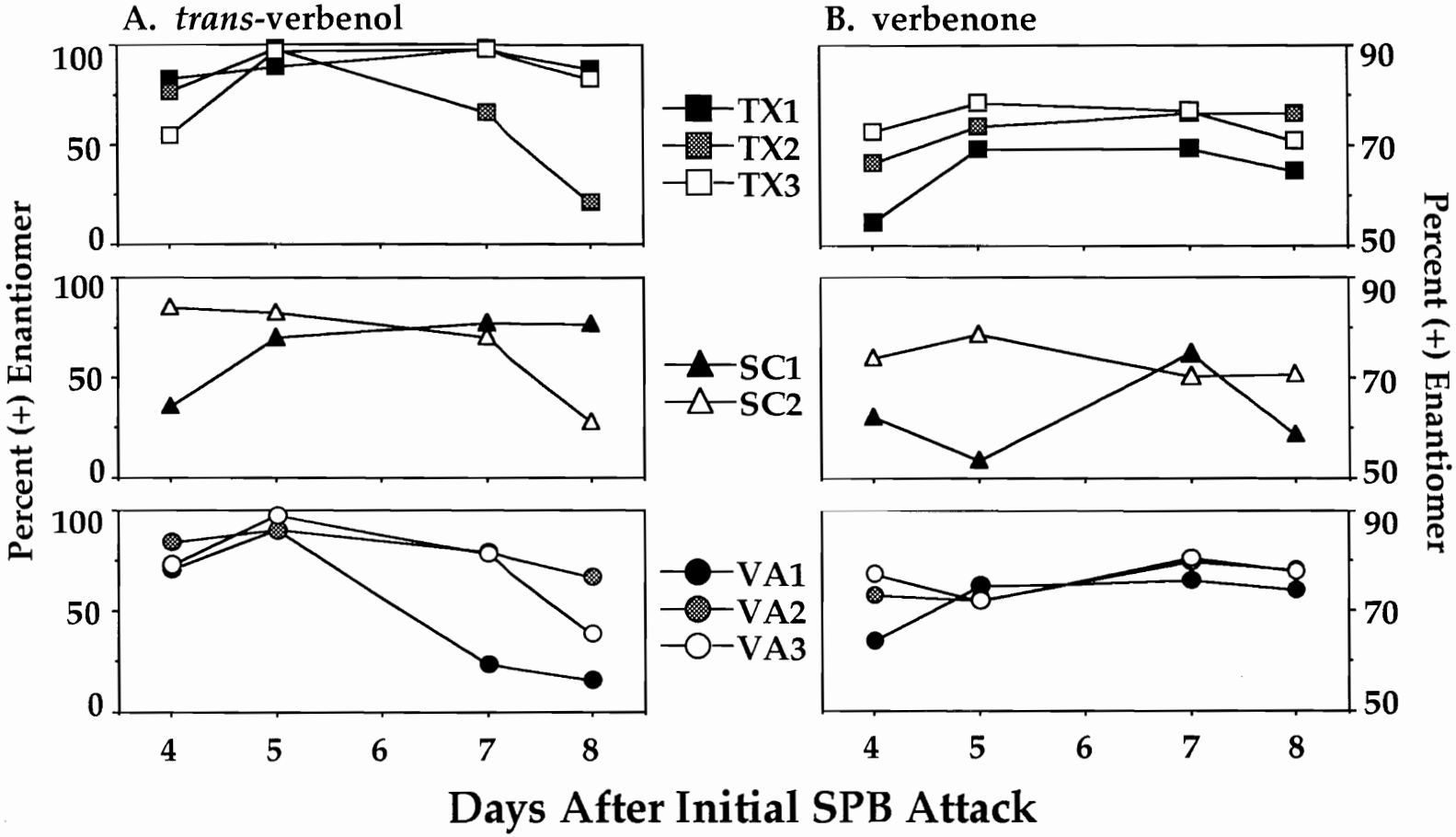

Figure 4.5. Temporal variation in the percent of the (+) enantiomer of (A) *trans*-verbenol and (B) verbenone released from southern pine beetle-infested logs from eight infestations in Texas (TX), South Carolina (SC) and Virginia (VA). Chiral range: 0% - 100% (+)-trans-verbenol, 50% - 90% (+)-verbenone. Standard error bars are omitted for clarity.

Verbenone: The enantiomeric ratios of verbenone isolated from infested logs ranged from 53.4%(+) : 46.6%(-) in SC1 to 80.3%(+) : 19.7%(-) in VA3, with CCV averaging 9% (range 1 to 25%) (Table 4.2). No differences among geographic areas were observed until day 8 at which time the percent (+) in VA was significantly higher than in SC (Table 4.3). Neither area was significantly different from TX. Differences among infestations within an geographic area were only found on day 4 in VA and on day 5 in SC (Table 4.2). Temporal changes were consistent in VA and TX with the percentage of (+)-V being lowest on day 4, peaking on day 7, and then declining slightly (Fig. 4.5B). However, in SC changes in chirality over time was more variable. Nearly 20% of the variability in V enantiomeric ratios was explained by BP and day after initial SPB attack. None, other than BP, contributed more than 5% (Table 4.4).

Discussion

Semiochemical Chirality. This is the first study that evaluates geographic and temporal variations in the chirality of a blend of bark beetle semiochemicals released from naturally-infested host material. The volatile collection technique used in this study permitted the determination of the enantiomeric ratios of six semiochemicals emitted from SPB-infested log sections four to eight days after initial attack. This is the period of time when the blend of semiochemicals emitted from the host is expected to switch from being attractive to inhibitory to arriving SPB.

In bark beetles, a semiochemical message detected by receptive beetles in flight provides information on the condition and genetic quality of

potential hosts and mates (Miller et al. 1989). Specific information obtained by beetles appears to relay both the quantity and chirality of individual compounds. Understandably, the message is likely to become more complex and provide more information as additional compounds are incorporated into a communication system. Beetle, host, and other environmental factors which may be responsible for variation in chirality of compounds are not necessarily the same as those responsible for the variation in quantity. The following discussion focuses on the extent to which these factors may contribute to temporal and geographic variation in the chirality of the six semiochemicals monitored from infested logs.

Chirality of Log Aerations versus Chirality of SPB Hindguts and Autoxidation. The large chiral range of F (Table 4.2), collected by aeration of infested logs, encompassed the average enantiomeric ratio 29.7%(+) : 70.3%(-) of the compound found predominately in female SPB hindguts (see Chapter 5). The average enantiomeric ratio of tV from female SPB hindguts (26.3%(+) : 73.7%(-) (see Chapter 5) was also encompassed by the chiral range determined from infested logs (Table 4.2); the > 80% range in chirality of tV from logs strengthens the hypothesis that each source of tV produces markedly different ratios. In a similar light, the contrast in enantiomeric ratios of cV (97.4%(+) : 2.6%(-)), V (35.5%(+) : 64.5%(-)) and eB (14%(+) : 86%(-)) (see Chapter 5) produced by SPB as compared to the ratios collected from infested logs (Table 4.2) indicates that potential sources of each compound produce different ratios. It is well known that cV and V are produced as a result of the oxidation of aP by multiple sources, i.e. SPB and autoxidation for cV (Hughes 1973, 1975, Hunt et al. 1989) and also associated microorganisms

for V (Brand et al. 1976). Apparently autoxidation of aP from the host produces very different enantiomeric ratios of cV and V as compared to SPB, with the autoxidation of (+)-aP yielding (-)-cV, but nearly the same percentage of (+)-V (see Chapter 5). The chirality of V produced by microorganism has yet to be determined.

A multiple source argument might also explain the large differences between SPB and log extracts ratios of eB. At present, no source other than bark beetles is known to also produce eB. However, Vanderwel et al. (1992) suggests that eB precursor, (E)-6-nonen-2-one, may originate from the beetle, host, and/or microorganisms. If the latter two sources also biosynthesize eB from the precursor, then it is possible they also contribute different ratios as compared to SPB.

Temporal variation. The enantiomeric ratios of aP exhibited little variability over time (Fig. 4.3A). Because the monoterpene composition of conifers is under strong genetic control (Hanover 1966, 1971), the chirality of these compounds should also be genetically controlled. The result of this genetic control would likely be a stable chirality over time. A slight decline in percent (+) of aP was observed in several infestations, but a relatively large decline occurred in TX1. These declines may indicate differences in the chirality of primary resin versus secondary resin[1]. Alternatively, the observed decline may be due to a higher rate of conversion of the (+) enantiomer of aP to tV by autoxidation in comparison to the rate of

[1] In response to an attack by bark beetles and associated microorganisms, conifers use two types of resin as the first line of defense (Berryman 1972, Reid et al. 1967). The attacking beetles initially encounter primary resin, which is stored preformed in cells and resin-containing cavities. Thereafter, host cells begin production of secondary resin in response to beetle-microorganism activity. (Christiansen and Horntvedt 1983).

conversion of (-)-aP to cV (see Chapter 6). Similarly, enzymes released from damaged plant cells may selectively convert the (+) enantiomer of aP (Leufven and Birgersson 1987).

Chirality of F and eB, exhibited somewhat greater variability over time than did aP (Figs. 4.3B and 4.4A). No common temporal pattern was observed for either compound among infestations. Factors which may influence the chirality of these compounds are purely speculative as the true origin of both compounds and proposed precursors are uncertain. It had been suggested that F and eB are synthesized *de novo* by specific metabolic pathways which are hormonally controlled (White et al. 1980). In addition, these compounds may be directly synthesized by SPB from short-chain dihydroxyketones as precursors (White et al. 1980, citing R.M. Silverstein, personal communication).

In the case of F, the suggested precursor, 6-methyl-6-hepten-2-one, is derived from 6-methyl-5-hepten-2-one, which is reported to be produced by mycangial fungi of SPB (Brand and Barras 1977, Brand et al. 1979). Because females SPB begin releasing F upon contact with a suitable host tree (Renwick and Vité 1969) when associated-fungi are not yet established, the precursor is more likely taken in by developing brood. The act of boring through the bark to emerge may activate hormones in callow adults to begin production of F from the stored precursor. This may explain the presence of small amounts of F in emerged beetles (Coster and Vité 1972, also see Chapter 5). Upon contact with a new host, the act of boring into the host may renew production of F.

Recent evidence supports the idea that eB, at least in *D. brevicomis* LeConte, is directly synthesized from (E)-6-nonen-2-one (Vanderwel et al. 1992). The origin of this precursor is uncertain, but it may be biosynthesized by one or more sources including the beetle itself, the host, and/or beetle-associated microorganisms. The chiral contribution of each precursor source may differ over time. The affects of environmental factors, other than those evaluated in this study including resin composition and flow rates and temperature, on the hormonal control of these pathways may have also resulted in the temporal variations observed.

Because oxygenated monoterpene pheromones, such as cV, tV, and V, are derived from the chiral host monoterpene, aP, it stands to reason that the enantiomeric ratio of aP would dictate the stereoisomer(s) of verbenol and subsequently V produced by bark beetles (Vanderwel and Oehlschlager 1987). This is supported by a study of pheromone production in *I. paraconfusus* in which (-)-aP served as the precursor of (+)-cV and (-)-V, whereas (+)-aP led to (+)-tV and (+)-V (Renwick et al. 1976). Because all aerated logs in the present study contained predominately (+)-aP which exhibited a stable temporal pattern, pheromone production by SPB and other potential pathways should yield predominately (-)-cV, (+)-tV and (+)-V, with all products exhibiting similarly stable patterns over time. Although the overall enantiomeric ratios of each compound released from logs generally conformed to its expected ratio, considerable variation occurred in the chirality of cV, tV, and V over time (Figs. 4.4B, 4.5A, and 4.5B) and is supported in part by the fact that day of pheromone collection explained significant portions of cV and V variability (Table 4.4). The temporal variations observed appear to indicate that the

enantiomeric ratio of each oxygenated monoterpene differs among the potential pathways.

In most infestations, the highest percentages of (+)-cV were released during the first 5 days after initial SPB attack, after which they tended to drop dramatically. Female SPB production of cV yields a high percentage of the (+) enantiomer (97%; range 88 to 100%) (see Chapter 5) and thus the release of this compound by beetles during the first few days of attack likely produces the observed peak at this time. After five days, autoxidation of the (+)-aP in host resin yields predominately (-)-cV; supported by both the increase in percent and quantity of (-)-cV released in later days.

Similarly, changes in tV chirality over time appear to be the result of differences in the enantiomeric ratio of tV produced by SPB and autoxidation. Autoxidation of 73% (+)-aP was shown to yield essentially the same percent (+)-tV (see Chapter 6), whereas tV produced predominately by female SPB from three geographic areas (TX, SC and NC) averaged 26% (range > 0 to 66%) of the (+) enantiomer (see Chapter 5). The increase in percent (+)-tV from day 4 to 5 in most infestations is believed to result from a switching of dominance from one pathway to another. In other words, the influence of female SPB produced tV on the overall ratio is lost by day 4, at which time the influence of tV produced by autoxidation becomes dominant. The decline in percent (+)-tV in later days in most infestations corresponds to a decline in the overall quantity of tV released from infested logs. This indicates that the decline in the production of (+)-tV by autoxidation is much greater than for the (-) enantiomer.

Verbenone chirality also varied with time, though not as dramatically as that observed with the two verbenols (Figure 4.5B). These differences may be explained, in part, by differences in the V chirality produced by SPB and autoxidation. Verbenone produced predominately by male SPB from TX averaging 21% (+) enantiomer, whereas SC and NC SPB averaged 43% (+) (see Chapter 5). In contrast, autoxidation of 73% (+)-aP yielded essentially the same percent (+)-V (70%) (see Chapter 6). As with tV, there is a switching of dominance on day 4 from SPB produced V to autoxidation produced V. A third potential contributor to V chirality are SPB-associated microorganisms. It has been shown that microorganisms, such as fungi, bacteria and yeast are capable of converting cV and tV to V (Brand et al. 1976, Leufven et al. 1984), but it is unknown to what extent the enantiomeric ratio of V produced by this pathway contributes to the overall chirality of V released from infested logs. It is likely that any contribution would not be added until 6 to 8 days after initial beetle attack (Birgersson and Bergstrom 1989).

Geographic variation. α-Pinene produced by host trees, exhibited strong area differences in chirality with the proportion of (+)-aP in TX trees significantly lower than that obtained from both VA and SC trees (Table 4.3). This is supported by the fact that latitude of infestation location explained 50% of aP variability (Table 4.4). As mentioned previously, because terpene composition is under strong genetic control, chirality should also be under genetic control. This implies that regional differences in aP chirality would be one product of natural selection in *P. taeda* upon exposure to different environmental conditions across its range.

Variability of F chirality was much greater within infestations (CCV = 39%) than was eB (CCV = 5%). However, little geographic variation (locally or regionally) occurred in either compound, particularly eB (Tables 4.2 and 4.3). The differences in variability between the two compounds may be linked to the impact of selective pressures on each pheromone. Frontalin serves as the principle aggregation pheromone of SPB, but also as an important kairomone to the dominant predator, *Thanasimus dubius* (F.) (Coleoptera: Cleridae) (Payne et al. 1984). As *T. dubius* is able to discriminate between F blends (Payne et al. 1984), the greater variability in F chirality should allow a greater proportion of a SPB population to escape predation than if chirality was tightly controlled. In addition, the activity of the hormone(s), with regard to enantiospecificity of F production (White et al. 1980), may be in part affected by environmental conditions. Factors such as population density of the host, latitude of infestation location, aP chirality and F quantity explained 27% of the variability in F chirality.

In contrast to F, eB is a multifunctional pheromone with the (+) enantiomer enhancing SPB response to F, whereas the antipode inhibits response (Vité et al. 1985). As mentioned previously, it was suggested that production of eB by SPB is the result of genetically controlled hormone conversions from precursors in separate metabolic pathways. It appears then that eB chirality is tightly controlled by each possible source, which when combined, maintain a near 50% (+) : 50% (-) blend with little variability. Regression analysis indicated that production of eB by a source(s) is not influenced by site or compound characteristics (Table 4.4). No information is

currently available to allow speculation on the possible impact of geographic variations in precursor quantity and/or chirality on chirality of either F or eB.

Both cV and tV chirality exhibited the greatest variability of all compounds evaluated. The large variability within infestations apparently resulted in few differences being among geographic areas and infestations within an area. This variability is likely due to large differences in the enantiomeric ratio contributions of SPB and autoxidation sources at a given time. The chirality of cV and tV produced by autoxidation is dependent on the enantiomeric ratio of the precursor, aP (see Chapter 6). In SPB and other bark beetles, however, detoxification of aP to either terpene alcohol occurs as the result of genetically controlled enzyme reactions (Pierce et al. 1987, Vanderwel and Oehlschlager 1987). The quantity of cV and tV explained a small part of the respective chirality of each compound (Table 4.4). Variation in enzymatic composition due to genetic variation along geographic lines (latitude) (Anderson et al. 1979), however, is most likely the greatest source of variation in chirality in SPB.

V chirality exhibited far less variability (CCV = 9%) than did the precursors, cV and tV. Local differences between SC infestations on day 5 and area differences between SC and VA on day 8 appear to be due to large fluctuations in the chirality of V in one SC infestation (SC1) (Table 4.2 and 4.3 and Fig. 4.5B). All other infestations exhibited similar enantiomeric ratios over time. As with the V precursors, cV and tV, geographic variability is likely due, in part, to differences in the enantiomeric ratio contributions from the different pathways over time. Contributing to this variation appears to be

genetic variability in enzyme production in both SPB (Anderson et al. 1979) and possibly their associated-microorganisms.

Chirality of Semiochemical Blends. The cluster analyses of semiochemical blend chirality for all infestations on each day showed the greatest similarity among infestations on days 5 and 7, with most infestations grouping in their respective geographic area (Fig. 4.1). The increasing and then decreasing similarity of infestations conforms to the proposed response of the host and beetle to each other's activity and to a shifting of dominance in cV, tV and V production from one pathway to another. During the first 3 to 4 days of attack the tree releases large quantities of primary resin. This may result in increased production and changed chirality of F and tV by SPB. During this period, production and release of all three oxygenated monoterpenes is likely made predominately by SPB, than by autoxidation. The multiple sources introduce greater variability to the chiral blend (compared to a single source), thereby reducing similarity of infestations within a geographic area (Fig. 4.1A). The increase in infestation similarity on days 5 to 7 (Figs. 4.1B and C) may be due to reduced primary resin reserves and the likelihood that autoxidation becomes the dominate or even sole source of cV, tV and V. Infestations within geographic areas become less similar by day 8 (Fig. 4.1D). At this time, the host begins production of secondary resin which may contain a different enantiomeric ratio of aP than did the primary resin. Also, production of oxygenated monoterpenes increase as a result of autoxidation of greater quantities of secondary resin and establishment of microorganism. The added variability of the new semiochemical source (i.e., microorganisms) could have caused the reduced similarity of infestations.

Behavioral Implications. The information gained from this study, along with quantitative data from Chapter 3, can be used to elaborate on previously proposed behavioral sequences occurring during the mass attack of host trees by SPB, particularly during the period when the host is most attractive to SPB to the point in time when attack switches to neighboring trees.

Upon landing on a host and initiating attack, pioneering females release F (Kinzer et al. 1969; Renwick and Vité 1969, 1970) containing predominately the (-) enantiomer (Stewart et al. 1977, also see Chapter 5). This enantiomer elicits significantly greater response than the (+) antipode (Payne et al. 1982). Frontalin, along with (+)-aP released in defensive host resin, attract large numbers of SPB, predominantly males (Payne 1980). At the same time, females release large amounts of (-)-tV and small quantities of (+)-V and eB (see Chapter 5; T.L. Payne, unpublished data), all of which enhance beetle response to (-)-F (Payne et al. 1978, Renwick and Vité 1969, Rudinsky et al 1974, Salom et al 1992, Vité et al. 1985,). Although, F quantities in SPB hindguts were found to decrease significantly within 48 h after feeding (Coster and Vité 1972), our observations indicate that large quantities of F continue to be released by SPB, their frass, and/or from gallery walls even longer than 8 days after initial attack.

After locating the entrance holes of females, males begin releasing (-)-V (see chapter 3). At low male densities, this compound enhances female response thereby balancing the sex ratio of arriving beetles. Dixon and Payne (1979) reported the number of SPB landing on a host is greatest (i.e., the host is most attractive) 3 to 5 days after initial attack. After this period beetle

response to aggregation pheromones begins to be inhibited resulting in a significant drop in the number of arriving beetles.

Because F quantities do not decline, the switching of mass attack to neighboring trees appears to be the result of several events. First, reduction of primary resin reserves in the host reduces the quantity of aP available as a kairomone. Second, there is shifting of tV chirality from the predominately (-)-tV produced by SPB to almost pure (+)-tV produced through autoxidation. Although, the behavioral response of SPB to different enantiomers of tV has not been investigated, the peaking of (+)-tV generally occurs after the point when peak attraction to the host is occurring. This suggests that perhaps the (-) enantiomer of tV produced by SPB is a synergist of F, whereas (+)-tV is inactive or even inhibitory. Third, it appears that racemic eB and (-)-V quantities must each reach levels of > 15% of F quantities before SPB response is inhibited. This situation differs, however, from *D. ponderosae* where V levels must be > 30% of tV levels (the primary aggregation pheromone of this species) before it would exert its antiaggregative properties (Miller et al. 1995). Although a recent study by Salom et al. (1992) evaluated the effects of (-)-V and racemic-eB on response of SPB to attractant-baited traps and showed no additional inhibitory effects of eB when included in traps already containing V, it should be noted that the concentration of V released in this study was almost 40 times higher than that of eB. It is likely that the inhibitory effect of V would be enhanced by the release of greater quantities of eB as was found by Payne et al (1978).

Chapter 5

Geographic and Gender Differences in the Quantity and Chirality of Semiochemicals Present in Southern Pine Beetle, *Dendroctonus frontalis* Zimm. (Coleoptera: Scolytidae) Upon Emergence

Introduction

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Scolytidae: Coleoptera) is considered to be the most important cause of damage and mortality in the southeastern pine forests of the United States (Drooz 1985). This bark beetle has a complex semiochemical communication system by which it is able to find, attack and colonize its primary hosts, loblolly pine, *Pinus taeda* (L.), and shortleaf pine, *P. echinata* (Mill.).

The presence of female-produced attractants in SPB was first shown in isolation experiments (Pitman et al. 1969, Renwick and Vité 1968, Tsao and Yu 1967). Gas-liquid chromatographic (GLC) analyses reported frontalin (F) (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) (Kinzer et al. 1969, Vité and Pitman 1969) and *trans*-verbenol (tV) (*trans*-2,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-ol) (Renwick 1967) in female hindguts. Frontalin together with tV and the host volatile, α-pinene (aP) (2,6,6-trimethylbicyclo [3.1.1] hept-2-ene) (Mirov 1961), was the most attractive blend of semiochemicals in field trapping experiments (Payne et al. 1978). Male SPB, in turn, are reported to be the primary producers of verbenone (V) (4,6,6-trimethylbicyclo [3.1.1] hept-3-en-2-one) (Renwick 1967) and *endo*-brevicomin (eB) (*endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-octane) (Pitman et al. 1969, Silverstein et al. 1968). These two compounds inhibited response of beetles to attractant-baited traps

in field trials (Payne et al. 1978, Salom et al. 1992). Most pheromones mentioned above are present in the opposite sex, but usually in much smaller quantities (Rudinsky et al. 1974).

Frontalin and eB are thought to be synthesized *de novo* by specific metabolic pathways which are hormonally controlled (White et al. 1980). In addition, these compounds may be directly synthesized by SPB from short-chain dihydroxy ketones as precursors (White et al. 1980, citing R.M. Silverstein, personal communication). In contrast, *cis*-verbenol (cV), tV and V are produced by SPB as a result of the oxidation of aP (Hughes 1973, 1975). Byers (1983), Klimetzek and Francke (1980), Lindstrom et al. (1989), and Renwick et al. (1976) demonstrated that production of cV and tV are dependent on the enantiomeric ratio of aP.

Several studies have identified the enantiomeric ratio of semiochemicals present in SPB or hosts: 15%(+):85%(-) F (Stewart et al. 1977) and 60%(+):40%(-) tV (Plummer et al. 1976) from female SPB; 15%(+):85%(-) V (Payne and Billings 1989, citing J.P. Vité, personal communication) and 93%(+):3%(-) eB (Redlich et al. 1987) from male SPB; and 69%(+):31%(-) aP from loblolly pine (Mirov 1961). Beetles used in all these studies were collected in eastern Texas or Louisiana.

Other studies have discovered variations in isozyme (Anderson et al. 1978) and pheromone response (Berisford et al. 1990) in beetles from Texas, Georgia and Virginia. Berisford et al. (1990) suggest regional differences in behavioral response may be due, in part, to quantitative and/or qualitative differences in semiochemicals released by SPB.

Previous studies, particularly during the period from 1966 to 1985, which attempted to isolate, identify, and quantify semiochemicals present in bark beetles often required the pooled samples of several hundred beetles to obtain sufficient quantities of semiochemicals for these determinations. Recent advances in GLC and capillary column technology now permit the separation and quantification of semiochemical enantiomers extracted from individual insect specimens. The objective of this study was to determine if the quantity and chirality of semiochemicals present in individual SPB hindguts varied with geographic location and gender.

Methods

Biological material. Bark containing SPB pupae and/or callow adults was collected from infested loblolly pine in July, 1994 from Texas (TX) (Angelina Co.) and in August, 1994 from South Carolina (SC) (Union Co.) and North Carolina (NC) (Chatham Co.). Bark from each geographic area was placed in separate emergence chambers (Browne 1972) with the temperature and relative humidity maintained at 27 °C and 85%, respectively, or stored at 10 °C until needed. Emerging beetles were collected daily, sexed, and placed individually in vials and stored at -60 °C.

The hindgut extraction technique was modified from those described by Madden et al. (1988) and Miller et al. (1989). A standard solvent solution was prepared by adding 10 ug of 2-octanol (internal standard) per ml of 'Baker Analyzed'® pentane (Baxter Diagnostics Inc., McGaw Park, IL). The hindguts from 10 to 15 male and female SPB were pulled and each placed seperately in a chilled 1.8-ml vial containing 100 ul of the standard solution. Each hindgut

was macerated with a microspatula for 15 sec and then the vial was shaken to promote solvent penetration. The resulting extract was drawn off and transferred to a clean vial. An additional 50 ul of chilled standard solution was added to the vial containing the abdominal remains, shaken, and the resulting extract transferred to the clean vial. Hindgut extracts were stored at -60°C. Just prior to GLC analysis, hindgut extracts were concentrated under nitrogen gas to 50 ul.

GLC Analysis: Individual extracts were analyzed by splitless injection of 1 uL of extract into a Shimadzu GC-14A (FID detector equipped) using a Chiraldex G-TA capillary column (40 m X 0.25 mm ID) (Advanced Separation Technologies Inc., Whippany, NJ) connected to a methyl phenyl guard column (5 m X 0.53 mm ID, Restek, Inc.). High purity helium was used as a carrier gas (160 kPa) and the temperature was programmed for 32 °C for 1 min., 2 °C/min. to 68 °C for 4 min., 5 °C/min. to 115 °C for 5 min., 8 °C/min. to 140 °C for 7 min. A dilution series with concentrations of 0.5, 1.0, 5.0, 10.0, 50.0 and 100 ug/ml/enantiomer was made from a stock solution containing equal amounts of racemic aP, F, eB, cV, tV, and V. To reduce column contamination and check for accuracy, pentane and a standard (10 ug/ml/enantiomer), respectively, were injected (1 ul/injection) before and after a series of four extract injections. Identification of beetle- and host-produced volatile peaks was made through comparisons with standard peak retention times. The identities of compounds of interest were verified by chromatography-mass spectrometry (GC-MS) analysis. Quantification of semiochemical enantiomers was made by internal standards and the equation:

$$\frac{\text{Area of extract peak} \times \text{Conc. of std.}}{\text{Area of the std. peak}} = \text{ug/ml} \times \text{extract vol.} = \text{ug/extract}$$

Statistical Analysis: The quantitative and qualitative data obtained from beetles of different gender and geographic origin were found to be nonnormal. Subsequently, quantity data were transformed by the equation $y = \log_{10} (x + 1)$ and proportions of (+) enantiomers were arcsine transformed. Differences among geographic areas and sexes were determined using Tukey's Compromise (P = 0.05). Corrected coefficients of variation (CCV= SD/mean) (Sokal and Rohlf 1981) were calculated for the quantity and chirality of each semiochemical using transformed data. Correlations between quantity and chirality of each semiochemical and between characteristics of different compounds were determined for each sex. The SuperANOVA statistical program (Abacus Concepts Inc., Berkley, CA, 1989) was used for the Tukey's Compromise test and correlation.

Results

Semiochemical Quantity: The quantities of all semiochemicals evaluated had nonnormal and highly skewed distributions. With regard to differences between sexes, most SPB males had lower quantities of aP, F, cV, and tV, whereas females often had lower quantities of eB and V. These frequency distributions are similar to those observed for pheromones produced by *D. ponderosae* Hopkins (Borden et al. 1986), *Ips pini* (Say) (Miller et al. 1989) and *I. typographus* (L.) (Birgersson et al. 1984). Most semiochemicals evaluated in this study also had CCVs, often greater than 100% (Table 5.1). These high CCVs may be due to the fact that SPB from each geographic area were reared from bark collected from two or more trees.

Individuals from different trees are more likely to be exposed to different host volatiles and environmental conditions (Schlyter and Birgersson 1989).

Male and female SPB contained relatively similar amounts of aP in their hindguts (Table 5.1). Statistical analysis of each sex seperately revealed that only males exhibited differences among geographic areas. Significantly higher amounts of aP were present in SPB from SC than in those from TX. Amounts present in SPB from NC were not significantly different from beetles from the other geographic areas. With sexes pooled SPB from SC again contained higher amounts of aP than did TX beetles.

Frontalin was isolated from both male and female hindguts. Contrary to an initial analysis of the data which showed no geographic area by sex interaction ($F_{2,59} = 1.448$; $P \geq 0.24$) nor differences among areas ($F_{2,59} = 2.949$; $P \geq 0.0602$) or sexes ($F_{1,59} = 1.397$; $P \geq 0.242$), subsequent comparisons of sexes within an area confirmed reports that females from TX produce significantly higher amounts than do males from the same area (T.L. Payne, personal communication) (Table 5.1). In contrast, both sexes from NC and SC contained essentially the same amounts of F. Analyses of sexes separately revealed that males from SC contained significantly higher amounts of F than did those from TX.

In contrast to a previous report by Rudinsky et al. (1974) which indicate that only male SPB produce eB, small amounts also were found in females (Table 5.1). Significant geographic area by sex interaction ($F_{2,59} = 5.51$; $P \leq 0.01$) dictated that the data be analyzed by sex and area separately. Amounts did not differ among areas for each sex; however, only males from SC contained significantly higher amounts of eB than did females.

Table 5.1. Quantities of semiochemicals present in male and female southern pine beetle from Texas (TX), South Carolina (SC), and North Carolina (NC).

		Quantity of Semiochemicals (ug)								Differences
		Male				Female				Between
Semiochemical	State	N	Mean[a]	SE	CCV[b]	N	Mean	SE	CCV	Sexes[c]
α-Pinene	TX	10	0.059 **b**	0.038	192%	15	0.183 **a**	0.091	158%	ns
	SC	10	0.741 **a**	0.205	77	10	1.342 **a**	0.749	139	ns
	NC	10	0.321 **ab**	0.161	133	10	0.557 **a**	0.419	188	ns
Frontalin	TX	10	0.011 **b**	0.008	225	15	0.124 **a**	0.034	100	*
	SC	10	0.158 **a**	0.045	87	10	0.169 **a**	0.070	119	ns
	NC	10	0.082 **ab**	0.027	99	10	0.088 **a**	0.056	189	ns
endo -Brevicomin	TX	10	0.011 **a**	0.002	62	15	0.019 **a**	0.004	89	ns
	SC	10	0.032 **a**	0.010	95	10	0.008 **a**	0.003	137	*
	NC	10	0.013 **a**	0.003	68	10	0.009 **a**	0.002	77	ns
cis -Verbenol	TX	10	0.006 **a**	0.006	273	15	0.144 **a**	0.068	165	*
	SC	10	0.036 **a**	0.020	171	10	0.270 **a**	0.069	77	*
	NC	10	0.007 **a**	0.003	137	10	0.113 **a**	0.032	89	*
trans -Verbenol	TX	10	0.078 **b**	0.006	106	15	6.042 **ab**	1.615	56	*
	SC	10	0.160 **ab**	0.020	70	10	9.984 **a**	2.395	51	*
	NC	10	0.239 **a**	0.003	56	10	2.693 **b**	0.716	56	*
Verbenone	TX	10	3.259 **b**	1.189	68	15	0.026 **b**	0.006	92	*
	SC	10	7.949 **a**	1.533	32	10	0.116 **a**	0.028	76	*
	NC	10	3.624 **ab**	0.591	27	10	0.036 **b**	0.013	116	*

[a] For each column, within each semiochemical, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

[b] Corrected coefficient of variation from data transformed by arcsine sqrt X

[c] Significant differences are represented by * for $P \leq 0.05$, Tukey's Compromise; ns = not significant.

This study confirms past reports that females produce large amounts of terpene alcohols (Renwick 1967, Hughes 1973, 1975, Rudinsky et al. 1974, Silverstein, unpublished data); however, males also contained these compounds, but in much smaller quantities (Table 5.1). Females contained 10 and 39 times the amounts of cV and tV, respectively, than did males. Significant differences among geographic areas were detected only for tV with NC males containing larger amounts than TX males and SC females having larger amounts than NC females. Although not significant, both sexes of SPB from SC generally contained larger amounts of cV than did beetles from the other areas.

Significant area by sex interaction for verbenone quantities ($F_{2,59} = 5.423$; $P \leq 0.01$) required analysis of sexes and areas separately. This study confirmed previous reports that quantities of verbenone in males are substantially higher (68 to 125 times in this study) than amounts found in females (Table 5.1) (Hughes 1973, 1975). However, for both sexes, SC beetles again produced greater amounts of pheromones than did beetles from the other geographic areas.

Semiochemical Chirality: Intrapopulation variation (CCV) in the chirality of most semiochemicals were markedly lower than what had been observed for quantities (Table 5.2). Corrected coefficients of variation were generally highest in eB and cV from males and in F and tV from females.

No significant differences in aP chirality occurred between sexes ($F_{1,53} = 0.076$; $P \geq 0.8085$) (Table 5.2); as a result, data were pooled within each geographic area. Chirality was significantly different in all areas, with enantiomeric ratios ranging from 41.8%(+) : 58.2%(-) in SC to 74.6%(+) :

Table 5.2. Chirality of semiochemicals present in male and female southern pine beetle from Texas (TX), South Carolina (SC), and North Carolina (NC).

		Chirality of Semiochemicals [(+) : (-)]								Differences
		Male				Female				Between
Semiochemical	State	N	Mean ratio[a]	SE	CCV[b]	N	Mean ratio	SE	CCV	Sexes[c]
α-Pinene	TX	8	81.7 : 18.3 **a**	4.8	14%	14	70.6 : 29.4 **a**	6.0	26%	ns
	SC	10	41.1 : 58.9 **c**	6.2	35	7	51.6 : 48.4 **a**	2.0	7	ns
	NC	10	64.1 : 35.9 **b**	4.4	17	10	59.7 : 40.3 **a**	3.6	14	ns
Frontalin	TX	2	71.3 : 28.7 **a**	24.1	45	13	29.2 : 70.8 **a**	6.4	56	*
	SC	8	67.8 : 32.2 **a**	7.1	27	8	34.9 : 65.1 **a**	5.5	28	*
	NC	9	62.8 : 37.2 **a**	4.5	42	7	25.1 : 74.9 **a**	6.9	47	*
endo -Brevicomin	TX	9	8.7 : 91.3 **a**	3.7	59	11	8.2 : 91.8 **b**	2.2	55	ns
	SC	9	12.1 : 87.9 **a**	4.6	62	6	24.5 : 75.5 **a**	4.2	24	*
	NC	10	21.1 : 78.9 **a**	5.5	48	10	0.0 : 100 **c**	0.0		*
cis -Verbenol	TX	2	1.7 : 98.3 **a**	1.7	159	13	97.2 : 2.8 **a**	1.1	10	*
	SC	8	27.8 : 72.2 **a**	9.8	66	9	96.1 : 3.9 **a**	1.0	5	*
	NC	5	13.6 : 86.4 **a**	3.6	59	10	99.0 : 1.0 **a**	0.4	5	*
trans -Verbenol	TX	9	60.9 : 39.1 **a**	8.7	33	15	18.1 : 81.9 **a**	4.0	56	*
	SC	9	25.8 : 74.2 **b**	6.4	48	9	28.4 : 71.6 **a**	3.1	20	ns
	NC	10	13.3 : 86.7 **b**	1.0	13	10	31.1 : 68.9 **a**	5.5	37	*
Verbenone	TX	10	20.7 : 79.3 **b**	6.4	70	11	58.1 : 41.9 **a**	8.4	37	*
	SC	10	44.2 : 55.8 **a**	3.8	18	9	82.3 : 17.7 **a**	5.0	17	*
	NC	10	41.7 : 58.3 **a**	3.1	15	9	57.5 : 42.5 **a**	9.3	37	ns

[a] For each column, within each semiochemical, means followed by the same letter are not significantly different (Tukey's Compromise, $P > 0.05$).

[b] Corrected coefficient of variation from data transformed by arcsine sqrt X

[c] Significant differences are represented by * for $P \leq 0.05$, Tukey's Compromise; ns = not significant

25.4%(-) in TX. The ratio from SPB from TX was slightly lower than the ratio of 77%(+) : 23%(-) identified from SPB-infested logs from the same geographic area (see Chapter 4). In contrast, the percent (+)-aP from SC beetles was 45% lower than aP identified from infested logs from the same area [86%(+) : 14% (-)].

No differences amoung geographic areas were found in the chirality of F for both sexes ($F_{2,41} = 1.357$; $P \geq 0.2687$); however, significant differences were found between sexes in each area ($F_{1,41} = 54.319$; $P \leq 0.05$) (Table 5.2). When data from areas were pooled, the enantiomeric ratio averaged 65.9%(+) : 34.1%(-) for males and 29.8%(+) : 70.2%(-) for females, which is nearly twice the percentage of (+) previously reported for females [15%(+) : 85%(-)] (Stewart et al. 1977).

Analysis of eB chiral data revealed significant area by sex interaction ($F_{2,49} = 20.364$; $P \leq 0.0001$). Separate analysis by sex showed no differences among geographic areas in male eB chirality (Table 5.2). With data from the three areas averaged together, the mean enantiomeric ratio was 14.3%(+) : 85.7%(-). This is in stark contrast to the 97%(+) : 3%(-) previously isolated from Texas male SPB (Redlich et al. 1987). This large difference may be due, in part, to the fact that our beetles originated from loblolly pine, whereas Redlich's beetles had emerged from shortleaf pine. The two pine species are known to each have different proportions and chiralities of resin components (Mirov 1961). The eB chirality from female SPB was significantly different for all geographic areas, with ratios ranging from 0%(+) : 100%(-) in NC to 24.5%(+) : 75.5%(-) in SC. Evaluation of sexes within each area showed

significant differences only in SC and NC. Whereas, the percent (+) was higher in SC females than in SC males, the reverse was true in NC.

Significant geographic area by sex interactions were found in cV chirality ($F_{2,41} = 6.09$; $P \leq 0.005$). However, it was determined that there were no differences in chirality for either sex (Table 5.2). Therefore, area data were pooled for each sex. Females contained a significantly higher percent of the (+) enantiomer (97.4%) than did males (19.6%).

Significant area by sex interactions also were found in tV chirality ($F_{2,56} = 17.98$; $P \leq 0.0001$). Geographic area comparisons for each sex showed no differences among females (Table 5.2). The pooled enantiomeric ratio was 24.6%(+) : 75.4%(-). This ratio is in contrast to the 60%(+) : 40%(-) collected from female SPB by Plummer et al. (1976). Again, the difference may be due to the fact that Plummer's beetles were reared from shortleaf pine; whereas, all beetles in this study were reared from loblolly pine. These two pine species exhibit marked differences in proportion and chirality of aP; the tV precursor (Mirov 1961). Texas males, however, contained significantly higher proportions of the (+) enantiomer of tV than did males from other geographic areas or females from the same area. The opposite was found in the chirality of tV from male and female SPB collected in NC.

Analysis of V chirality from SPB hindguts showed significant differences between sexes. Whereas males averaged about 36%(+) : 64%(-), females contained 65%(+) : 35%(-). Due to the fact that males contain and likely release much greater quantities of verbenone than do females, it was deemed important to evaluate geographic area differences in chirality with sexes separated. Texas males produced a significantly lower proportion of the

(+) enantiomer than did SC and NC populations (Table 5.2). The enantiomer ratio [20.7%(+) : 79.3%(-)] isolated from TX males was similar to those previously reported for TX males [15%(+) : 85%(-)] (Payne and Billings 1989, citing J.P. Vité, personal communication). In contrast, females did not exhibit any differences among geographic areas.

Correlation Between Semiochemicals and Their Characteristics: Data were examined for each sex separately to determine if any significant correlations existed between quantity and chirality of each semiochemical and among compounds. In males, quantities of aP and tV were significantly correlated to chirality; whereas, in females respective characteristics of aP and V were correlated (Table 5.3). No relationships were found between characteristics of F, eB, and cV in either sex. Most oxygenated monoterpene quantities were significantly correlated with aP quantities in both sexes, but generally were not correlated to aP chirality. On the other hand, only in males did aP chirality show significant correlation with tV and V chiralities and the percentage of *trans*-isomer of the verbenols [% tV = tV/(cV + tV) X 100]. Comparisons of cV and tV quantities, singularly and combined (*cis*- plus *trans*-), with V amounts were all significant in both sexes. Whereas, the correlation between percent tV and V amounts was significant only in males. A significant correlation was found in females between cV and V chiralities, while a correlation of similar magnitude between tV and V chiralities was found only in males.

Table 5.3. Correlations between quantity and chirality of individual semiochemicals and between characteristics of different semiochemicals present in male and female southern pine beetle.

		Male		Female	
Y[a]	$\log_{10}$ X[a]	r	r^2	r	r^2
aP Quant.	Pct. (+)-aP	0.597 **[b]	0.356	0.398 *	0.159
F Quant.	Pct. (+)-F				
eB Quant.	Pct. (+)-eB				
cV Quant.	Pct. (+)-cV				
tV Quant.	Pct. (+)-tV	0.575 **	0.331		
V Quant.	Pct. (+)-V			0.529 **	0.280
aP Quant.	cV Quant.	0.364 *	0.133	0.431 **	0.185
	tV Quant.			0.356 *	0.127
	V Quant.	0.514 **	0.264	0.712 ***	0.507
Pct. (+)-aP	cV Quant.	0.374 *	0.141		
	tV Quant.				
	V Quant.				
	Pct. (+)-cV				
	Pct. (+)-tV	0.471 *	0.221		
	Pct. (+)-V	0.503 *	0.253		
cV Quant.	tV Quant.			0.641 ***	0.411
	V Quant.	0.557 **	0.310	0.646 ***	0.417
	Pct. (+)-V				
Pct. (+)-cV	Pct. (+)-V			0.650 ***	0.422
tV Quant.	V Quant.	0.458 *	0.210	0.643 ***	0.417
	Pct. (+)-V				
Pct (+)-tV	Pct. (+)-V	0.631 ***	0.398		
Pct (+)-aP	Pct. tV[c]	0.414 *	0.172		
aP Quant.	Total Verbenol[d]			0.357 *	0.127
Total Verbenol	V Quant.	0.551 **	0.303	0.645 ***	0.416
Pct. tV	V Quant.	0.617 ***	0.380		

[a] aP, α-pinene; F, frontalin; eB, *endo* -brevicomin; cV, *cis* -verbenol; tV, *trans* -verbenol; V, verbenone

[b] Significant correlations are represented by * for $P < 0.05$, ** for $P < 0.005$, and *** for $P < 0.0005$.

[c] Pct. tV = tV/(cV + tV) X 100

[d] Total Verbenol = cV + tV

Discussion

Semiochemical Quantity: In general, larger quantities of most pheromones were present in both male and female SPB from SC than in beetles from other geographic areas. It appears that these larger quantities reflect the beetle's response to the better health (e.g., greater quantities of host compounds present) of trees in this area (see Chapter 3). Birgersson et al. (1984) similarly found larger quantities of pheromones in the hindguts of beetles attacking trees with high resin flow than in beetles from trees of poor health. This hypothesis is also supported by higher release rates of pheromones from trees which produce resin pitch tubes upon attack by bark beetles versus those that do not (Birgersson and Bergstrom 1989, see Chapter 3).

The skewed quantitative distributions and high variability of all semiochemicals evaluated were very similar to those observed in compounds from other scolytid species which are capable of killing trees (Birgersson et al. 1988). Such distributions may indicate that at high population (epidemic) levels, as observed in this study, there is a weak selection pressure for high pheromone production (Birgersson et al. 1988). As a result, there would be many low pheromone producing individual SPB which may "hitch-hike" on the signal of a large group. Conversely, at low population levels (endemic), there is a stronger selection pressure to produce large quantities of pheromones because a larger proportion of individuals would be pioneers or members of small groups. The latter portion of the hypothesis, however, has yet to be confirmed.

This study confirmed previous reports indicating significant sex dominance in the production of cV, tV, and V (Renwick 1967, Rudinsky et al. 1974, Silverstein, unpublished data). However, it contrasts with their earlier findings in that my data indicate that male and female SPB from two out of three geographic areas produced nearly equal quantities of F and eB. These discrepancies may be due in part to the fact that F and eB are present in very low quantities to begin with and that GLC techniques used during the late 1960's and early 1970's were not as sensitive as equipment available today. Even when an earlier study used pooled samples from several hundred beetles, eB in females and F and cV in males was not detected (Silverstein, unpublished data); whereas, my analysis of individual beetles consistently detected these compounds in both sexes.

The presence of aP in the hindguts of bark beetles upon emergence has been previously reported (Madden et al. 1988) and is likely the remnants of phloem material consumed as larvae. However, this compound may also be absorbed as vapors or upon contact with the cuticle (Hughes 1973). Although, monoterpenes absorbed in this manner are reported to be oxidized in the hemolymph to verbenols and V, it may be possible that some of the aP remains unconverted and is secreted into the hindgut by the Malpighian tubules.

Semiochemical Chirality: It appears that analysis of aP present in SPB hindguts may not provide an accurate means of measuring the enantiomeric ratio of this compound present in the host in which the beetle developed. Although the chirality of aP was not determined directly from each host tissue, the percentages of (+)-aP found in SPB from TX and SC were markedly

lower and the variability greater than those detected from infested logs from the same areas (see Chapter 4). One reason for the discrepancy may be linked to the toxicity or abundance of one enantiomer of aP compared to the antipode. Smith (1963, 1965) and Coyne and Lott (1976) determined that host monoterpenes, such as aP, are toxic to varying degrees to several *Dendroctonus* species. As a result, bark beetle species, including SPB, have evolved general metabolic pathways which utilize microsomal mixed-function oxidases (e.g. cytochrome P-450) to detoxify aP by converting it first to aP oxide and then to one of several alcohols which have pheromonal properties (White et al. 1980). No study has yet been conducted to determine if, one enantiomer of aP is more toxic than the antipode. If one enantiomer is more toxic and/or more abundant than the other then it is advantageous for SPB to selectively convert the enantiomer of greater toxicity or abundance to the corresponding enantiomers of verbenols and V. As a result, the proportion of unconverted (+)-aP remaining in the hindguts would decline as this enantiomer is converted to verbenols. This hypothesis is supported by the very weak correlation between the chirality of aP and the percentage of the *trans*-isomer of the verbenols [%trans = tV/(trans + cis) X 100] (Table 5.3). On the other hand, two studies evaluating pheromone production by *I. typographus* did show that the enatiomeric ratio of aP (determined from the host) dictates the isomeric composition of the verbenol product (Klimetzek and Francke 1980, Lindstrom et al. 1989). In each study, the correlations between the chirality of aP and the *cis-/trans-* ratios of verbenol present in the beetle's hindguts were highly significant.

All three oxygenated monoterpenes (cV, tV, and V) exhibited chiral and quantitative differences between male and female SPB from most geographic areas (Tables 5.1 and 5.2). Relationships among the characteristics of these compounds also differed markedly between the sexes. This suggests that radical differences occur in metabolic pathways used by each sex in the production of oxygenated monoterpenes. Gender differences in these pathways may reflect differences in hormonal, neural, microsomal and excretory processes in each sex (White et al. 1980). In addition, bacterial and fungal symbionts which contribute to the production of these pheromones (Brand et al. 1977) may also differ between sexes. The differences among geographic areas observed in the chirality of tV and V in male SPB (Table 5.2) also are likely due to genetic variation in these pathways or contributions made by symbionts.

The metabolic pathways involved in the production of the bicyclic ketals, F and eB, differ from those involved in oxygenated monoterpene production. It has been suggested that ketal production likely involves steroid or hormone metabolism (White et al. 1980). However, similar factors such as genetic differences in hormonal and neural processes, could account for gender and, to a lesser extent, geographic area differences in enantiomeric ratios of each compound (Table 5.2).

Behavioral Implications with Regards to Pheromone Quantity and Chirality: This study has shown that male and female SPB from two of three geographic areas contain nearly equal quantities of F upon emergence from the host tree. Rudinsky et al. (1974) showed that males do produce F when confined with other males, however, the GLC traces provided by Rudinsky

appear to indicate that males produce little or no F when confined with females, although no quantitative data were reported. The lack of F production by males in the presence of females appears to be supported indirectly by volatile collections from infested logs (see Chapter 4). Frontalin released from TX and SC logs averaged 31% and 14% of the (+) enantiomer, respectively; yet, F present in males from TX and SC averaged 71% and 68% of the (+) enantiomer, respectively. If males did release F at the same rates as females (29 to 35%(+)), the pooled ratio would average approximately 50%(+) : 50%(-). The cumulative data from this and previous studies indicate that F may actually serve as a multifunctional pheromone. The (-)-F released by females upon contact with the host is very attractive in combination with host volatiles (Payne et al. 1982). Males, on the other hand, may only release their (+)-F during rivalry fighting in or near a female's entrance hole. Yu and Tsao (1967) reported that males could identify other males nonvisually, and they attributed this ability to male pheromone(s). These pheromones may include (+)-F and/or V.

Both male and female SPB produced low, but nearly equal quantities of eB upon emergence in TX and NC. In addition, both sexes produced predominately the (-) enantiomer. Vité et al. (1985) showed that even at low concentrations this enantiomer inhibits response of SPB to attractant-baited traps. The chirality of eB released from infested logs was nearly 50%(+) : 50%(-) (see Chapter 4). Although racemic eB also inhibits SPB response to attractants, but to a lesser extent than the (-) enantiomer alone, the differences in chirality suggest that either the chirality produced by SPB shifts after contact with a new host or that there is an additional source of eB that

produces predominately the (+) enantiomer. Vanderwel et al. (1992) suggested that the eB precursor, (E)-6-nonen-2-one, may originate from the beetle, host, and/or microorganisms. If the latter two sources also biosynthesize eB from the precursor, then they may also contribute different ratios as compared to SPB.

Although both sexes of SPB were found to produce cV, with quantities much greater in females than in males, SPB have not been found to respond to this compound (T.L. Payne, personal communication). The importance of this compound maybe as a intermediate precursor in V production.

Similarly, tV is produced by both sexes, but with females containing substantially greater quantities than males and predominately the (-) enantiomer. Laboratory and field bioassays using racemic tV, however, have found this compound to be very important in the communication system of SPB by synergizing the effects of F and may substitute for aP when quantities of this host terpene decline (McCarty et al. 1980, Payne 1979, Payne et al. 1978). Response of SPB to enantiomers of this compound have not been evaluated. The fact that predominately (+)-tV is released from SPB-infested logs suggests that contributions made by autoxidation likely shift the enantiomeric ratio of this compound 3 to 4 days after initial attack (see Chapters 3, 4 and 6). The shift in chirality further suggests that the (-) enantiomer is the active synergist, whereas the antipode is either inactive or inhibitory. It is apparent that further research is needed on roles of enantiomers of this important pheromone.

Verbenone differed from the other oxygenated monoterpenes in that male SPB contained substantially greater quantities and higher percentages of

the (-) enantiomer than did females. Laboratory walking bioassays have shown that concentration of V (chirality not reported) had a marked affect on SPB behavior, with low concentrations arresting males at the pheromone source, whereas, high concentrations inhibited response to an attractant mixture (Rudinsky 1973, Rudinsky et al. 1974). Similarly, dose-dependent inhibition of response by V was shown in field trials with SPB to an unreported chirality (Payne et al. 1978) and to 34%(+) : 66%(-) (Salom et al. 1992), and *D. brevicomis, I. pini* and *I. latidens* (LeConte) to 17%(+) : 83%(-) (Miller et al. 1995). At moderately high concentrations (2.9 mg/h), V containing 34% to 50% of the (+) enantiomer was significantly more effective at inhibiting male SPB response to attractant-baited traps than was the 69%(+) treatment. However, at even higher concentrations (10.4 mg/h) all enantiomeric ratios [17%(+), 34%(+), and 67(+)] showed equal inhibitory effectiveness (D.M. Grosman, unpublished data). Additional research is needed to assess the effects of low concentrations of V containing predominately the (+) enantiomer (as found in female SPB) on male and female behavioral response.

Chapter 6

Variation in the Quantity and Chirality of Semiochemicals Produced by Autoxidation of the Plant Terpene α-Pinene

Introduction

The monoterpene α-pinene (aP) is the major component in the resin of most *Pinus* spp. (Mirov 1961), as well as in that of many other conifers. The resin produced by these trees serves as both a physical and chemical barrier to macro- and microorganism invasion (Smith 1965). The viscous substance produced from a healthy tree can "pitch-out" invading bark beetles (Payne 1980), is toxic to both beetles and associated microorganisms (Berryman 1972), and ultimately seals the wounds created by invaders.

Bark beetles may use aP as a primary attractant to locate host trees or as a synergist of pheromones to mass aggregate beetles on individual trees (Borden 1982, Payne et al. 1978). This terpene component also has been found to serve as a precursor in the biosynthesis of pheromones (i.e., *cis*- (cV) and *trans*-verbenol (tV), verbenone (V), and myrtenol (M)) by *Dendroctonus* and *Ips* species (Francke and Vité 1983; Hughes 1973, 1975; Klimetzek and Francke 1980, Renwick et al. 1973, 1976) and associated microorganisms (Bhattacharyya et al. 1960; Brand et al. 1975, 1976; Hunt and Borden 1990; Leufven et al. 1984, 1988). In addition, aP oxidizes in the presence of air (autoxidation) to produce tV, cV, M, V, and other compounds at elevated (Moore et al. 1956) and ambient temperatures (Hunt et al. 1989). The authors of the latter study demonstrated that large quantities of oxygenated monoterpenes are produced as a result of this process. They also suggested that these products play a role in attracting bark beetles to lightning-struck and wounded trees during the

initial stage of attack and during the mass attack stage (Coulson et al. 1983, 1986), or in inhibiting response to attractants once a host is fully colonized.

The enantiomeric composition or chirality of aP can differ among conifer species (Klimetzek and Francke 1980, Mirov 1961). Within the genus *Pinus*, the composition can range from almost pure (+), as in *P. halipenis* $[\alpha]_D$ = +48.53°, to almost pure (-), as in *P. pinaster* $[\alpha]_D$ = -43.4° (Mirov 1961). Comyns and Lucas (1957) reported that pure (+)-aP had $[\alpha]_D$ = +52.4°. The enantiomeric composition of aP can also differ within conifer species of different genetic origin as was found in loblolly pine, *P. taeda* L. (see Chapter 4), ponderosa pine, *P. ponderosae* Laws. (Mirov 1961) and Norway spruce, *Picea abies* (L.) Karst (Lindstrom et al. 1989). The chirality of the aP within a conifer has been found to be important with regard to the stereoselective production of oxygenated pheromones by bark beetles. Renwick et al. (1976) showed that *Ips paraconfusus* Lanier produces (+)-cV from (-)-aP; whereas (+)-aP yields (+)-tV. The chiral relationship between the oxygenated monoterpenes (cV, tV, M, and V) produced by autoxidation and the aP present in conifers is unknown.

The objectives of this study were to determine the quantities and chiralities of oxygenated monoterpenes present 1) in solution and 2) in the headspace above solution resulting from the autoxidation of different enantiomeric ratios of aP.

Material & Methods

Experiment 1 - Liquid Extraction. Evaluation of autoxidation of aP generally followed procedures described by Hunt et al. (1989). Aliquots of aP

(0.2 ml ≈ 160 mg each), having an enantiomeric ratio of 73.2%(+) : 26.8%(-) (ICN Biomedicals Inc., Irvine, CA), 51.1%(+) : 48.9%(-) (> 99% pure, Aldrich Chemical Co., Milwaukee, WI), or 10.6%(+) : 89.4%(-) (> 99% pure, Aldrich Chemical Co.), were transferred to three separate 1.8-ml vials. Each treatment (ratio) was replicated three times. The uncapped vials, containing the aP, were exposed to air at 23-24°C. At 24 - 48 h intervals, 1 mg samples of the contents of each vial were extracted in pentane for gas-liquid chromatographic (GLC) analysis.

Experiment 2 - Headspace Extraction. An uncapped vial containing 0.2 ml of an aP ratio was placed upright in 500-ml Mason jar and the jar was tightly sealed. Each treatment (ratio) was repeated three times in separate jars. Headspace samples of the atmosphere within each jar were taken at 24 h intervals by inserting the needle of a 10-ml, gas-tight syringe (Hamilton Co., Reno, NA) through a rubber septum in the lid. For each sample, the syringe was pumped four times before injecting 3-ml directly into the GLC.

GLC Analysis: Both extract and headspace samples were analyzed on a Shimadzu GC-14A (split/splitless injector and FID detector equipped) using a Chiraldex G-TA capillary column (40 m X 0.25 mm ID) (Advanced Separation Technologies Inc., Whippany, NJ) connected to a methyl phenyl guard column (5 m X 0.53 mm ID, Restek, Inc.). High purity helium was used as a carrier gas (160 kPa) and the temperature program was 32°C for 1 min., 2°C/min. to 68°C for 4 min., 5°C/min. to 115°C for 5 min., 8°C/min. to 140°C for 7 min. A dilution series with concentrations of 0.5, 1.0, 5.0, 10.0, 50.0 and 100 μg/ml/enantiomer was made from a stock solution containing equal amounts of racemic aP, cV, tV, M and V. To reduce column contamination

and check for accuracy, pentane and a standard (10 μg/ml per enantiomer), respectively, were injected (1 μl per injection) before and after a series of four extract injections (1 μl/injection). Identification of autoxidation-produced volatile peaks was made through comparisons with standard peak retention times and gas chromatography-mass spectrometry (GC-MS) analysis. Quantification of semiochemical enantiomers from extracts were made by internal standards and the equation:

$$\frac{\text{Area of extract peak X conc. of std.}}{\text{Area the std. peak}} = \text{ng}/\mu\text{l X extract vol.} = \text{ng prod./mg aP}$$

Concentrations of each semiochemical enantiomer (ng of product/mg of aP) in a jar at a given time were quantified from standard curves of each enantiomer using the equation:

$$\frac{d \text{ X } e/f \text{ X } g}{h}$$; where $d = \frac{\text{(peak area - Y intercept)}}{\text{slope}}$ = the standard curve value;

e = volume of the jar (500 ml); f = headspace injection volume (3 ml); g = injection volume of standards (1 μl); and h = initial weight of aP in the vial.

Statistical analyses: The experiment was run as a repeated measures design with percentage of (+)-aP treatment as the between subject effect and time (hours) as the within subject effect. The data were analyzed using analysis of variance [Abacus Concepts, SuperANOVA. (Abacus Concepts Inc., Berkley, CA]. Variances tended to increase with means of both the quantity and percent (+) enantiomer of each oxidation product. Subsequently, quantity data were transformed by the equation $y = \log_{10}(x + 1)$ and proportions of (+) enantiomers were arcsine transformed. Because some compounds were not detected at early sample intervals, differences among product quantities and

among percentages of the (+) enantiomers from different aP ratios were determined only for data obtained at 144 h using Tukey's Compromise (P = 0.05). However, because in a repeated measures design, the within subject variables (i.e., times) are correlated with each other, quantitative and qualitative comparisons of temporal means for each compound could not be made using the standard post-hoc tests. Therefore, trends in temporal data are discussed.

Regression analysis (SAS Institute Inc. 1985) was used to estimate the quantities and chirality of oxygenated monoterpenes produced as the result of autoxidation of aP containing different enantiomeric ratios.

Results

Quantity of Oxygenated Monoterpenes: Preliminary GLC analysis of each aP ratio detected no oxidation product contaminants prior to the initiation of each experiment. Both experiments confirmed the report by Hunt et al. (1989) that aP oxidizes in the presence of air at ambient temperatures to produce at least four oxygenated monoterpenes (cV, tV, M, and V) and that these products are released into the atmosphere.

Experiment 1 - Liquid Extraction. Quantities of tV, cV, M, and V present in each aP solution were dependent on the enantiomeric ratio of the aP. Significantly greater quantities of each product were produced with increasing percentage of the (+) enantiomer of aP (Fig. 6.1). Detectable quantities of all products were found at the first sample interval (24 h) in treatments containing 73%(+) and 51%(+) aP (hereafter referred to as the (+)- and (±)-aP treatments, respectively) (Fig. 6.1). In the 11%(+)-aP treatment

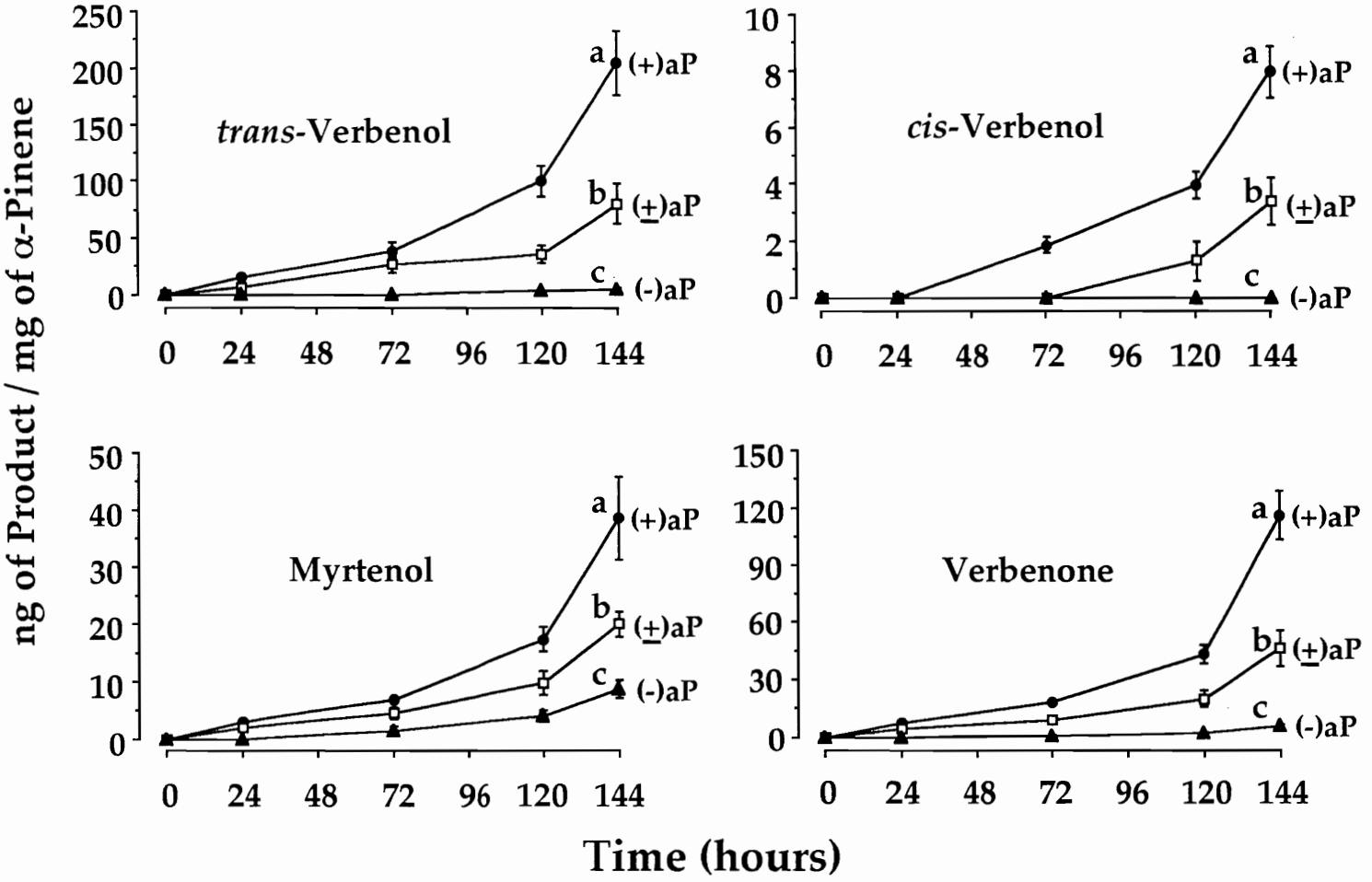

Figure 6.1. Mean quantities (± SE) of *trans*-verbenol, *cis*-verbenol, myrtenol, and verbenone produced by autoxidation of (+), (±), and (-) α-pinene (aP). Treatment means at the 144 h sample interval having the same letter are not significantly different ($P > 0.05$; Tukey's Compromise). $N = 3$ for each treatment at each sample interval.

(= (-)-aP treatment), however, M and V were not detected until 72 h, tV not until 120 h; cV was not detected, even after 144 h. The order of abundance of products from both (+) and (±)-aP treatments at all sample intervals was tV > V > M > cV. In contrast, (-)-aP produced M > V > tV. The orders found in this study differ from those reported by Hunt et al. (1989); they found tV > V > cV (M was not reported) for the first 48 h after which verbenone was produced in the greatest quantities followed by tV and cV. In my study, highly significant relationships were found between the chirality of aP treatments and quantities of tV, cV, M, and V after 144 h of exposure to air (all with $P \leq 0.0001$) (Fig. 6.2).

Experiment 2 - Headspace Extraction. Quantities of tV, M, and V present in the atmosphere above an aP source were dependent on the enantiomeric ratio of the aP precursor. *cis*-Verbenol levels were too low to be quantified at all sample intervals from all treatments. All treatments were significantly different with regards to the quantities of tV, M, and V present in the atmosphere above the precursor after 144 h (Fig. 6.3). The greatest quantities of all products were detected in the atmosphere above the (+)-aP treatment, whereas little or no oxygenated monoterpenes were detected in the atmosphere above the (-)-aP treatment.

Chirality of Oxygenated Monoterpenes

Experiment 1 - Liquid Extraction. The chiralities of tV, cV, M, and V also were dependent on the chirality of the aP treatments. All treatments differed significantly from each other, with higher proportions of the (+) enantiomer of all products being produced from treatments with high percentages of the (+) enantiomer than from treatments with low percentages

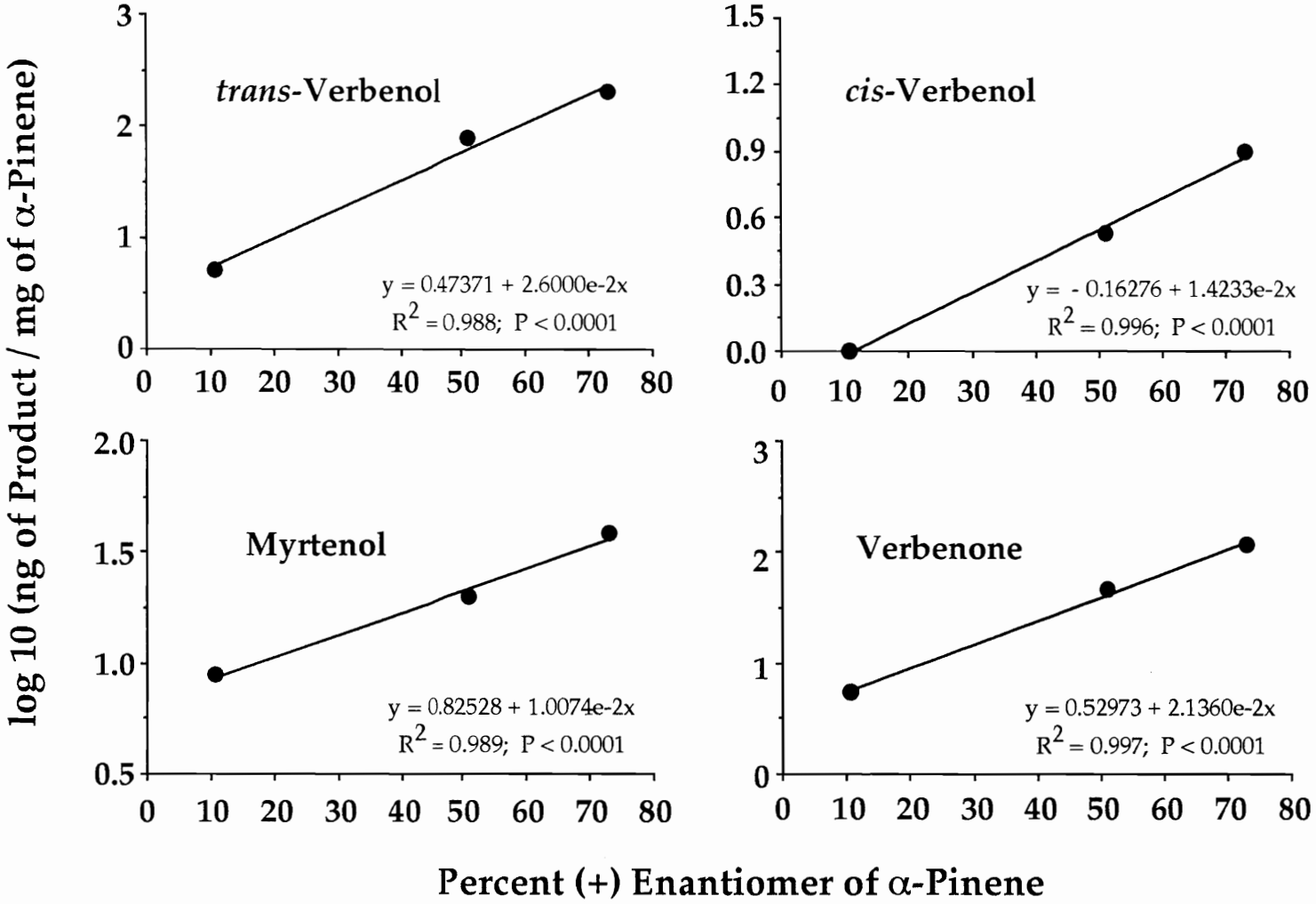

Figure 6.2. Relationships between α-pinene chirality and *trans*-verbenol, *cis*-verbenol, myrtenol, and verbenone quantities produced by autoxidation of α-pinene after 144 hrs.

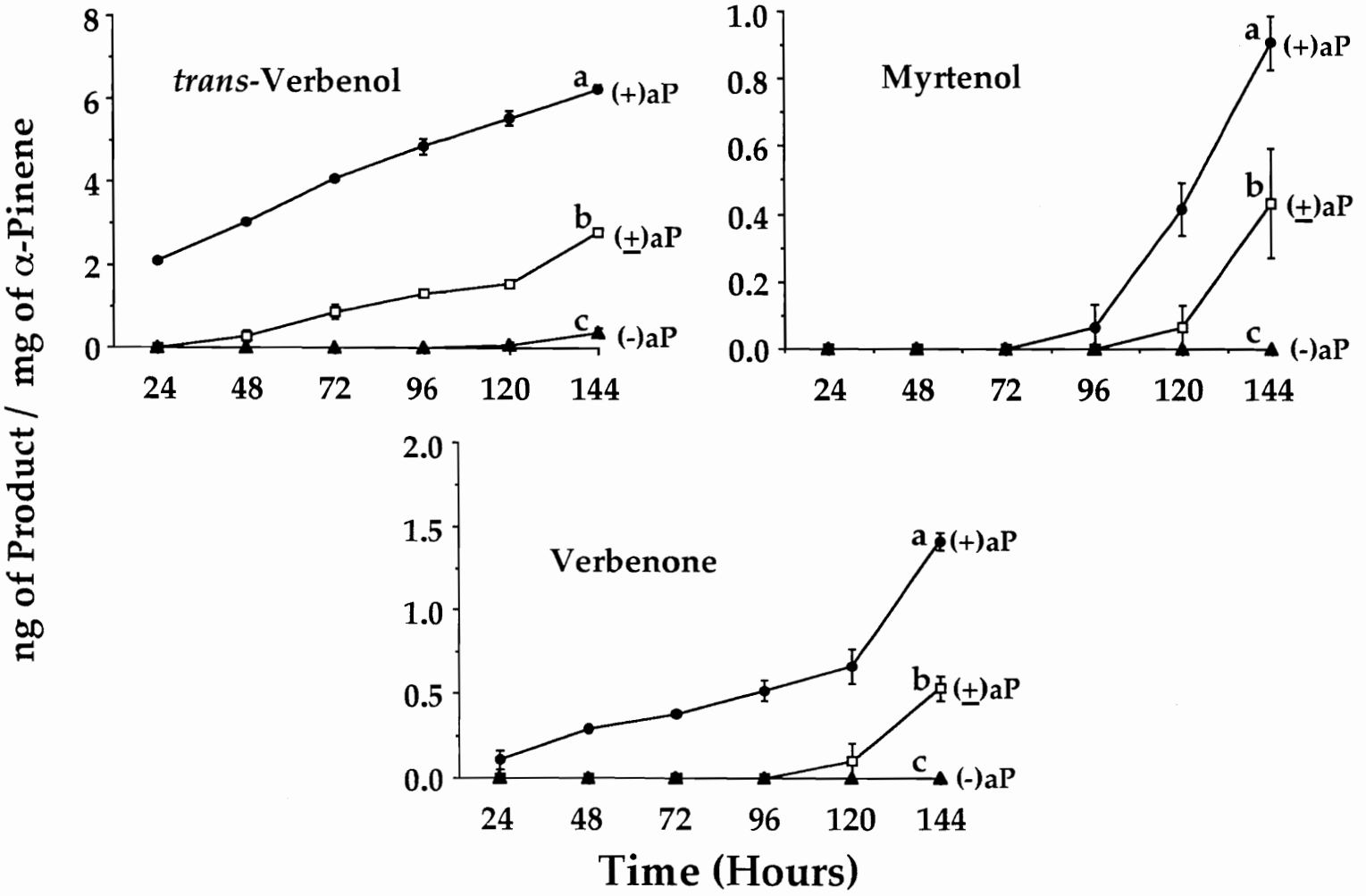

Figure 6.3. Mean quantities (± SE) of *trans*-verbenol, myrtenol, and verbenone present in the atmosphere within a 500 ml jar containing 0.2 ml of (+), (±), or (-) α-pinene (aP). Treatment means at the 144 h sample interval having the same letter are not significantly different ($P > 0.05$; Tukey's Compromise). $N = 3$ for each treatment at each sample interval.

(Table 6.1). The percent (+)-V and tV produced from each treatment closely reflected the precursor's ratio. In contrast, the percent (+)-M, although significantly different among treatments, appeared to approach a racemic ratio despite the chirality of its precursor. Somewhat surprisingly, cV produced from the (+)-aP treatment was nearly racemic. This contrasts with previous reports that indicated that the oxidation of (+)-aP yields the (-) enantiomer of cV (Vanderwel and Oehlschlager 1987). The differences between the chirality of cV and its precursor's chirality tended to increase with the percentage of (+)-aP. Highly significant relationships were found between the chiralities of aP treatments and the chiralities of tV, M, and V after 144 h (all with $P < 0.0001$) (Fig. 6.4).

Experiment 2 - Headspace Extraction. Significantly higher proportions of the (+) enantiomer of tV and V were produced with increasing percent of (+)-aP treatment (Table 6.1). The chirality of V could not be determined from the (-)-aP treatment. There were no significant differences in M ratios detected in the (+)-aP and (±)-aP treatments. Chirality of cV could not be determined from any of the treatments. The mean enantiomeric ratios of tV present in the atmosphere above each aP treatment were essentially the same as those present in solution. In contrast, atmospheric proportions of (+)-M and (+)-V were markedly higher than those found in solution.

Discussion

My experiments confirmed reports by Hunt et al. (1989) that the accumulations of oxygenated monoterpenes cV, tV, M, and V in and above aP exposed to air are the result of the autoxidation of aP at room temperature

Table 6.1 Chirality of oxygenated monoterpenes in and above samples of (+), (±), and (-) α-pinene exposed to air for 144 hours.

α-Pinene Treatment	Chirality of Oxidation Products [(+) : (-)]							
	trans -Verbenol		*cis* -Verbenol		Myrtenol		Verbenone	
	Mean Ratio	±SE	Mean Ratio	±SE	Mean Ratio	±SE	Mean Ratio	±SE
Exp. 1 - Liquid Extraction								
73 % (+)	69.8 : 30.2 a[a]	0.5	54.3 : 45.7 a	0.1	61.1 : 38.9 a	0.4	65.4 : 34.6 a	0.6
51 % (+)	48.6 : 51.4 b	0.4	40.3 : 59.7 b	0.1	57.2 : 42.8 b	0.3	42.5 : 57.5 b	0.8
11 % (+)	4.3 : 95.6 c	4.3	----[b]	--	54.4 : 45.6 c	0.5	22.9 : 77.1 c	0.9
Exp. 2 - Headspace Extraction								
73 % (+)	70.6 : 29.4 a	0.1	----	--	70.8 : 29.3 a	0.1	69.5 : 30.5 a	0.1
51 % (+)	49.0 : 51.0 b	0.8	----	--	82.6 : 17.4 a	17.4	49.5 : 50.5 b	0.7
11 % (+)	7.8 : 92.2 c	7.8	----	--	----	--	----	--

[a] Means in a column, for each experiment, followed by the same letter are not significantly different ($P > 0.05$, Tukey's Compromise).

[b] Compound not detected from this treatment.

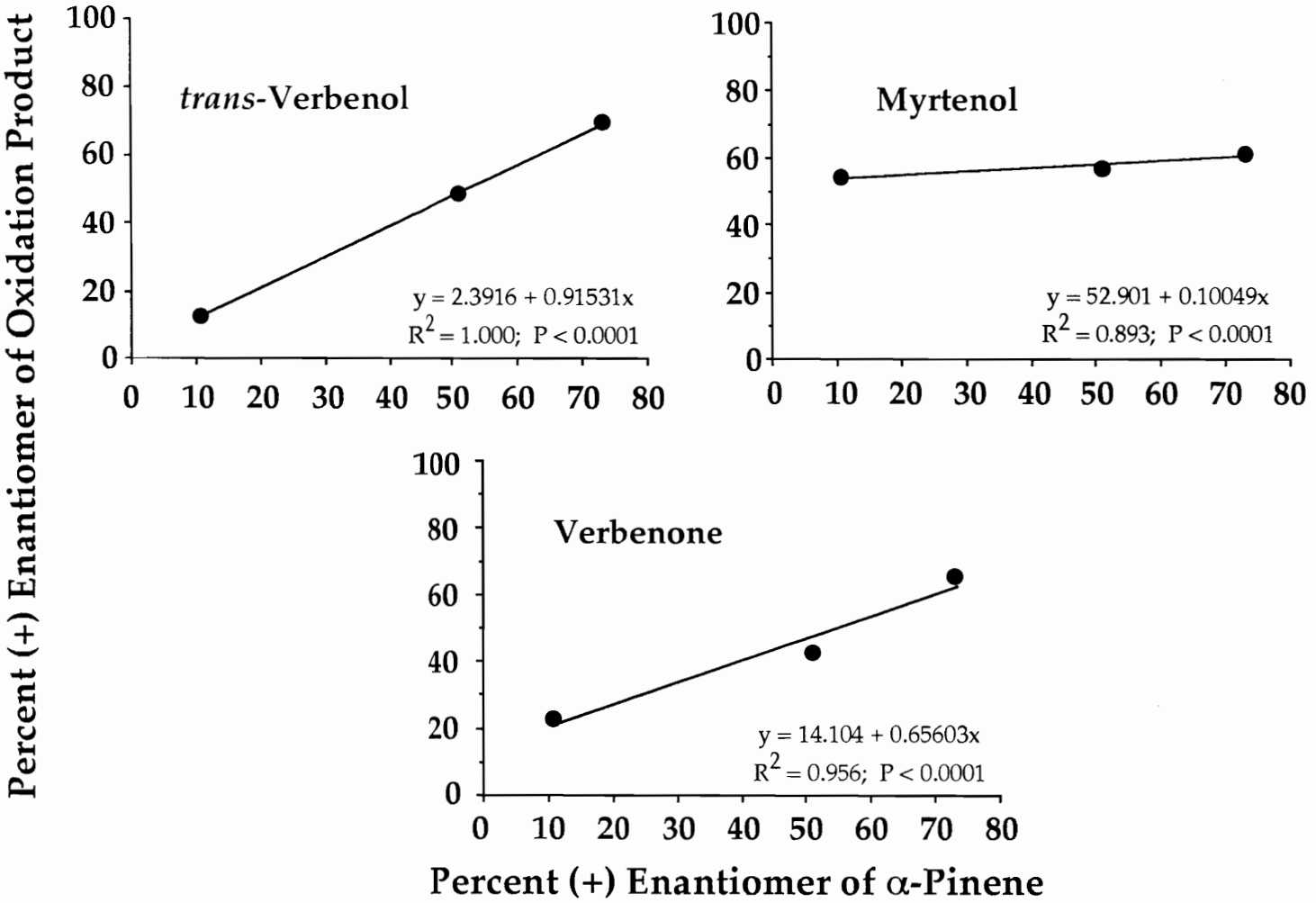

Figure 6.4. Relationships between α-pinene chirality and the chirality of *trans*-verbenol, myrtenol, and verbenone produced by autoxidation of α-pinene after 144 hrs.

(Figs. 6.1 and 6.3). In addition, this study demonstrated that both the quantity and chirality of all oxidation products produced were dependent on the enantiomeric ratio of the aP precursor (Figs. 6.2 and 6.4). Amounts and proportions of the (+) enantiomer of each oxygenated compound increased with the proportion of (+)-aP (Figs. 6.1 & 6.3 and Table 6.1). The much higher quantities of tV, cV, and V (approximately 140, 270, and 390 ng/mg of aP, respectively) reported by Hunt et al. (1989) after 96 h suggests that they experimented with nearly 100%(+)-aP, although the true ratio was not known (D.W.A. Hunt, personal communication). If they had used 100%(+)-aP, the relationship between aP chirality and M quantity (Figure 6.3) suggests that significantly greater amounts of M would have been produced at this higher ratio. However, unlike my study which showed that significant quantities of M were present in the atmosphere above 73%(+)-aP after 144 h (Figure 6.2), Hunt et al. (1989) were not able to detect this compound even after 192 h. I have no explanation for this discrepancy.

Verbenone is not produced directly from aP, but rather is a result of a secondary oxidation reaction involving cV and/or tV (Hunt et al. 1989, Vanderwel and Oehlschlager 1987). Myrtenol has not been suggested as a potential contributor in V production. However, in this study, the rate of V released into the atmosphere paralleled that of tV, but then increased dramatically after 120 hrs and paralleled that of M (Fig. 6.3). In addition, the regression line between chiralities of aP and V lies midway between the regressions of tV and M (Figure 6.4). These patterns provide some evidence to support the hypothesis that M contributes to V production. However, it remains to be determined if pure M would autoxidize to V.

The oxygenated compounds produced by autoxidation in this study also are produced by several scolytid species, particularly those which colonize conifer host species (Borden 1982) and are important with regard to their effects on bark beetle behavior. Data from this study along with those reported by Hunt et al. (1989), indicate that large quantities of tV, M, V, and cV are produced by autoxidation of aP and may constitute a significant proportion of the overall amount of each compound released from bark beetle-infested hosts during the later stages of attack. However, the quantity and chirality of these products released from tree wounds would likely differ among conifer species due to variability in the proportion of turpentine in the oleoresin, the proportion of aP in the turpentine, the chirality of aP, and rate of resin flow from wounds. Using these parameters and Figures 6.5A & B, it was possible to estimate the quantity and chirality of tV released into the atmosphere from aP in the jars and from wounds as might be caused by bark beetles attacking three different host species (Table 6.2). For example, aP from loblolly pine, an important host of *D. frontalis*, has an enantiomeric ratio of approximately 73%(+) : 27%(-) in Texas (see Chapter 4). Such a ratio would yield approximately 68%(+)-tV (Table 6.2). Resin flow from *P. taeda*, resistant to attack by *D. frontalis*, averaged 1.02 ml/h (Hodges et al. 1979). As 71% of *P. taeda* resin is composed of turpentine and aP constitutes about 19% of monoterpenes (Mirov 1961), then 0.141 ml of aP would be released per h or approximately 113 mg/h. At 73%(+)-aP, tV production in the atmosphere above aP in the jar was 0.043 ng/mg of aP/h; however, if aP is released from a wound on a resistant tree at the rate of 113 mg/h, than nearly 5 ng of tV would be produced per hour (Table 6.2). In comparison, *D. frontalis* females

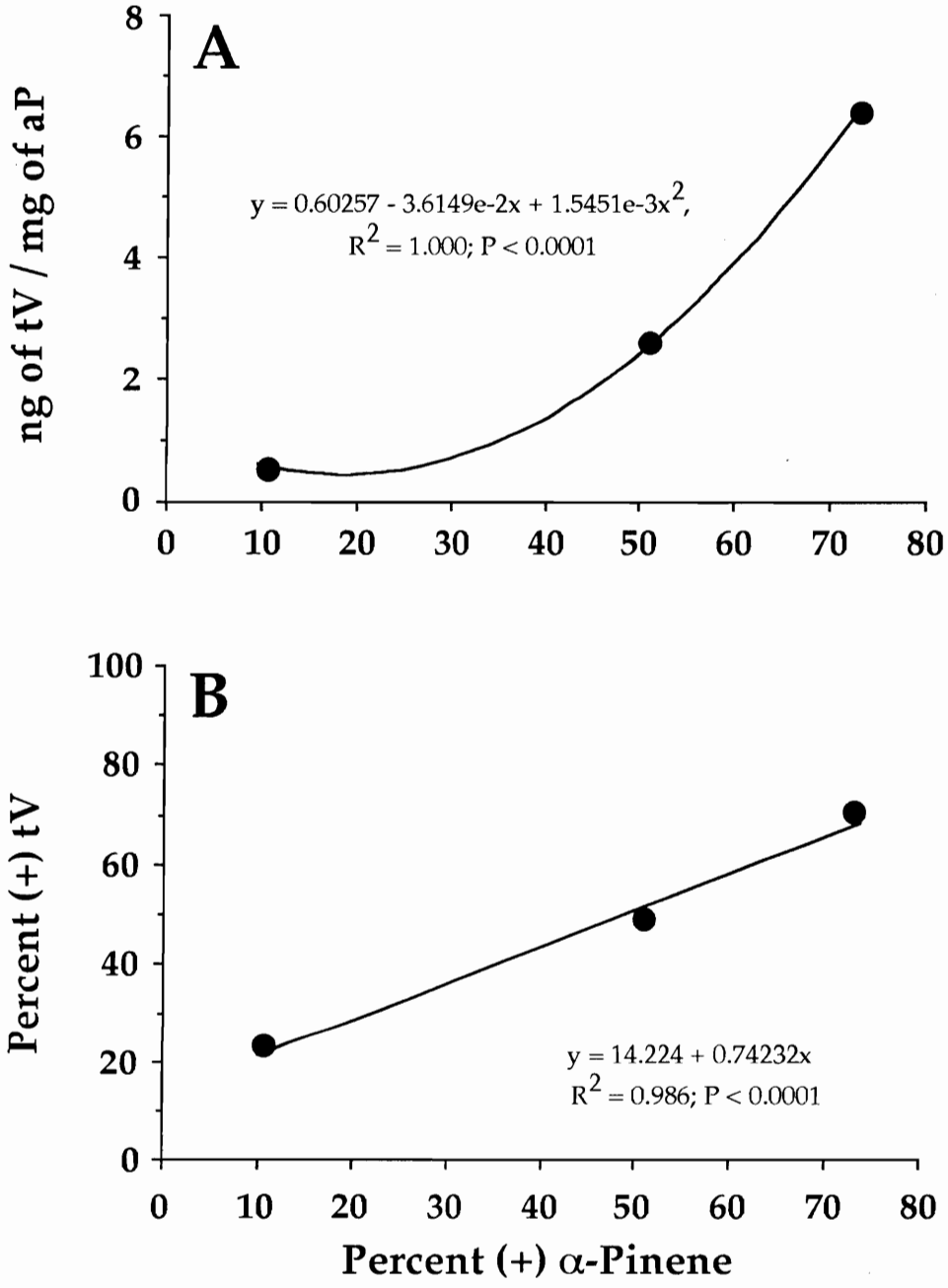

Figure 6.5. Relationships between α-pinene chirality and *trans*-verbenol (A) quantity and (B) chirality produce by autoxidation of 0.2 mL of α-pinene and released into the atmosphere in a 500 ml jar after 144 h.

Table 6.2 Estimated quantity and chirality of *trans*-verbenol produced by autoxidation of α-pinene from three conifer species.[a]

Tree Species	Classification[b]	Chirality [(+) : (-)] α-Pinene (known)[c]	Chirality [(+) : (-)] *trans*-Verbenol (estimated)[d]	% Turpentine in Resin[c]	% α-Pinene in turpentine[c]	Flow Rate (ml/h)[b] Resin	Flow Rate (ml/h)[b] α-Pinene	Estimated Quantity of tV[d] (ng/mg of aP/h) In jars	Estimated Quantity of tV[d] (ng/mg of aP/h) Tree wounds
Pinus taeda	Resistant	73:27	68:32	71	19.3	1.02	0.141	0.04	4.85
	Susceptible					0.53	0.073		2.51
Pinus echinata	Resistant	59:41	58:42	85	21.4	1.26	0.229	0.03	4.90
	Susceptible					0.12	0.042		0.90
Pinus contorta	Resistant	35:65	40:60	6	14	0.72	0.006	0.01	0.05

[a] Ref. Loblolly pine, *Pinus taeda* - see Chapter 4; Shortleaf pine, *P. echinata* and Lodgepole pine, *P. contorta* - Mirov 1961.

[b] Resistant = resistant to bark beetle attack; Susceptible = susceptible to bark beetle attack. Ref. Hodges et al. 1979.

[c] Ref. Mirov 1961

[d] Estimated from Figs. 6.5A and B.

apparently release about 125 ng/h for the first 48 h after feeding (Coster and Vité 1972, see Chapter 5). Trees susceptible to *D. frontalis* attack released about half the amount of aP produced by resistant trees (c.f. Hodges et al. 1979) and would subsequently produce about half the amount of tV (Table 6.2).

Shortleaf pine, *P. echinata* Mill., another important host of *D. frontalis*, produces lower proportions of (+)-aP than does *P. taeda*, but has higher proportions of monoterpenes and aP (Mirov 1961) and greater resin flow in resistant trees (Table 6.2). The combination of these characteristics results in nearly equal estimated tV production rates from the two tree species.

Lodgepole pine, *P. contorta* var. *latifolia* Engelm., is an important host of *D. ponderosae* and other scolytids. In contrast to *P. taeda* and *P. echinata*, the proportions of (+)-aP, turpentine in resin, and aP in turpentine are low in *P. contorta* var. *latifolia*. Therefore, very little tV would be produced as a result of autoxidation of aP (Table 6.2). This contrasts markedly with projections made by Hunt et al. (1989), however, as mentioned above, the authors apparently did not take into account the chirality of aP found in *P. contorta* and likely used aP consisting of nearly 100%(+) in their experiment.

The quantitative and chiral effects of oxygenated monoterpenes produced by autoxidation of aP on bark beetle behavior are essentially unknown. Most semiochemicals produced by bark beetles are released within the first few days upon following initial attack of the host (Birgersson and Bergstrom 1989, Coster and Vité 1972). However, my study indicates that five or more days are required before appreciable amounts of most oxygenated monoterpenes are present in the atmosphere above an aP source. In addition, the chirality of autoxidation products can differ significantly from that

produced by bark beetles. For example, female *D. frontalis* emerging from *P. taeda* were found to produce predominately (-)- tV, (+)cV, and (+)-V, whereas males contain (-)-cV and (-)-V, and variable tV ratios depending on the geographical location (see Chapter 5). In contrast, based on the fact that the chirality of aP present in *P. taeda* ranges from 69%(+) : 31%(-) to 94%(+) : 6%(-) (Mirov 1961, see Chapter 4), it can be expected that the (+) enantiomer will predominate in all oxygenated monoterpenes produced by autoxidation (Figures 6.4 and 6.5).

As Hunt et al. (1989) suggested, there is a need to re-evaluate several bark beetle behavioral experiments involving aP because the activity attributed to aP may be partly or entirely due to the effects of oxygenated monoterpenes produced by autoxidation. It is apparent from my study that the need is greatest with regard to experiments which examine conifer species containing predominately (+)-aP or large proportions of aP in the resin. Of the 94 *Pinus* species evaluated by Mirov (1961), 56 produce racemic or predominately (+)-aP. Of these, aP constitutes > 60% of the turpentine component of resin in 40 species. Many of these tree species are attacked by bark beetle species which utilize tV, cV, M and/or V as pheromones (Borden 1982, Wood 1982).

Chapter 7

Summary and Recommendations

The preceding chapters described aspects of the semiochemical communication system of the southern pine beetle (SPB), *Dendroctonus frontalis*. Each chapter describes the quantitative and chiral component contributions of semiochemicals that have been made to the system by several sources. In addition, three chapters describe geographic and temporal differences in the components of the system. I will briefly summarize each chapter and describe the possible implications with regard to behavioral responses of SPB to the quantities and chiralities of the six semiochemicals evaluated. Finally, I will suggested areas of further research.

Past research focused to a large extent on the semiochemicals produced by bark beetles and released from infested materials during the initial stages of attack. The goal of the research described herein was to provide a better understanding of the role of semiochemicals during the middle to later stages of host colonization by SPB and to provide insight into geographic and temporal variability of olfactory cues produced and released by this beetle alone and in combination with its host.

Chapter 2 provided a detailed review of current knowledge relating to the biology, life history, semiochemical communication system, associated microorganisms, and pest management strategies of SPB. The complexity of semiochemical structures, blends, and concentrations along with selection pressures extending over a large geographic range were provided as a basis for

evaluating the geographic and temporal variations in quantity and chirality of semiochemicals produced and/or utilized by SPB.

Chapters 3 described the extent of geographic and temporal variation in the quantitative component of semiochemicals released from SPB-infested logs from eight infestations in Texas, South Carolina, and Virginia and collected four to eight days after initial attack. The quantities of most semiochemicals showed variations among and within geographic areas as well as temporal variation from the same logs. One group of semiochemicals, including α-pinene (aP), frontalin (F), and *endo*-brevicomin (eB), apparently originate from a single source; either the host (aP) or SPB (F and eB). Changes in the quantities of these compounds probably result from the responses of the host and the beetle to each other's activity at a given time and differences in their health within a geographic area. The second group included *cis*-verbenol (cV), *trans*-verbenol (tV) and verbenone (V). Variability in the quantity of these compounds was much greater than that exhibited by the first group, presumably due to the fact that all three compounds originate from multiple pathways (SPB, autoxidation, and microorganisms).

The chiral component of the SPB semiochemical system is described in Chapter 4 and also discusses the extent of geographic and temporal variation in the semiochemicals released from the same logs. The chirality of most semiochemicals showed variations within and among geographic areas and differed markedly from chiralities previously identified from SPB hindguts in Texas. The (+) enantiomer predominated for aP, tV, and V, the antipode predominated for F and cV, while eB was racemic (Table 7.1). The chiralities of aP, F, and eB generally remained stable over time; whereas, significant variation among geographic areas, particularly for aP and F, is believed to be due to

Table 7.1. Chiralities of southern pine beetle semiochemicals released from different sources.

Source	Range of Mean Percentages of (+) Enantiomer						
	aP[a]	F	eB	cV	tV	M	V
SPB-Infested Logs:	74 - 89	13 - 39	51 - 55	4 - 21	50 - 90	- - - -	62 - 76
SPB Hindguts:							
Male	41 - 82	63 -71	9 - 21	2 - 28	13 - 61	- - - -	21 - 42
Female	52 - 71	25 -35	0 - 24	96 - 99	18 - 31	- - - -	58 - 82
Autoxidation of:							
73% (+)-aP	- - - -	- - - -	- - - -	54	69 - 71	61 - 71	65 - 70
51% (+)-aP	- - - -	- - - -	- - - -	40	46 - 49	57 - 83	43 - 50
11% (+)-aP	- - - -	- - - -	- - - -	- - - -	4 - 8	54	23

[a] aP, α-pinene; F, frontalin; eB, *endo* -brevicomin; cV, *cis* -verbenol; tV, *trans* -verbenol; V, verbenone

genetic variation among individual beetles and hosts. Large differences between the chirality of eB released from logs and eB previously identified from SPB hindguts indicate that additional sources are involved in the production of this compound (Table 7.1). As with quantities of oxygenated monoterpenes, both temporal and geographic variation in the chiralities of cV, tV and V were much greater than those exhibited by aP, F, and eB and are likely due to the fact that they originate from multiple pathways.

Chapter 5 described the chiral and quantitative component contributions of semiochemicals made by both sexes of SPB to the overall semiochemical blend released from SPB-infested host material. The same six semiochemicals (F, eB, cV, tV, V, and aP) were isolated from hindguts of individual beetles from Texas, South Carolina, and North Carolina. Quantities of cV and tV were substantially greater in females than in males; whereas, males contained much greater amounts of V. Quantities of F, eB, and aP were generally the same in both sexes. Geographic differences were found in quantities of tV and V in both sexes and in aP and F in males only. The chiralities of most semiochemicals present in SPB hindguts differed markedly from those released from SPB-infested logs (Table 7.1). Males produced predominantly the (+) enantiomer of F and the (-) enantiomer of eB, cV, and V. The chirality of tV produced varied considerably among the geographic areas. In contrast, females produced predominantly the (+) enantiomer of cV and V and the antipode of F, eB, and V. The (+) enantiomer of aP predominated in both sexes, however, the percentage of (+)-aP was generally lower than that determined from host trees from the same geographic area. Differences in the chirality of tV and V among geographic areas were significant in males as were differences in the chirality of eB in females.

Covariation between quantity and chirality of a semiochemical or between characteristics of different compounds differed between the sexes.

Chapter 6 described the potential chiral and quantitative component contribution of semiochemicals produced by autoxidation of aP to the overall semiochemical blend released from SPB-infested host material. The monoterpene aP autoxidizes under ambient temperatures to form tV, myrtenol, V and to a lesser extent cV. Both the quantities and chiralities of these oxygenated monoterpenes were dependent on the chirality of the aP precursor. Significantly greater amounts and proportions of the (+) enantiomer of each compound were produced when (+)-aP was predominant than when the antipode of the precursor was predominant (Table 7.1). *Pinus* spp., such as *P. taeda* and *P. echinata*, whose monoterpene component contains a large proportion of (+)-aP, likely produce large quantities of autoxidation products five days after they are wounded by SPB. On the other hand, pine species with relatively small amounts of (+)-aP in their resin (e.g., lodgepole pine, *P. contorta* var. *latifolia* Engelm.) would yield very little autoxidation products after the same period time.

Data from the preceding chapters indicate that the geographic and temporal variability in semiochemical quantities and chiralities may be directly or indirectly attributed to several factors, including: 1) beetle population levels, 2) genetic and phenotypic differences among individual beetles, 3) genetic and phenotypic differences among individual hosts trees, 4) asynchronous attack by SPB, 5) differences in species of microorganisms and their establishment rate in SPB galleries, and/or 6) differences in the contributions of two or more pathways

to the overall quantity of oxygenated monoterpene pheromones(s) emitted from a colonized host.

Behavioral Implications for Host Colonization by SPB

The information gained from these experiments (Chapters 3 - 6) can be used to elaborate on previously proposed behavioral sequences occurring during the mass attack of host trees by SPB, particularly during the period from when the host is most attractive to SPB to the point in time when attack switches to neighboring trees.

Upon landing on a host and initiating attack, pioneering females release (-)F which, along with (+)-aP released in loblolly pine resin, attracts large numbers of SPB, predominantly males (Fig. 7.1). At the same time, females release large amounts of (-)-tV and small quantities of (+)-V and eB, all of which are believed to act as synergists to enhance beetle response to (-)-F. Although, F quantities in SPB hindguts were found to decrease significantly within 48 h after feeding (Coster and Vité 1972), our observations indicate that large quantities of F continue to be released by SPB, their frass, and/or from gallery walls even longer than 8 days after initial attack. The extended release of F probably ensures the successful colonization of host trees; even those which are resistant to beetle attack.

After male SPB land on the host, they locate the entrance hole of single females and begin to release (+)-F. This compound may be used in short range communication to reduce rivalry fighting and competition with other males. In addition, (-)-V and (-)-eB are released, but apparently function in long range communication as population regulators. At low male densities and V concentrations, female response is enhanced thereby balancing the sex ratio of

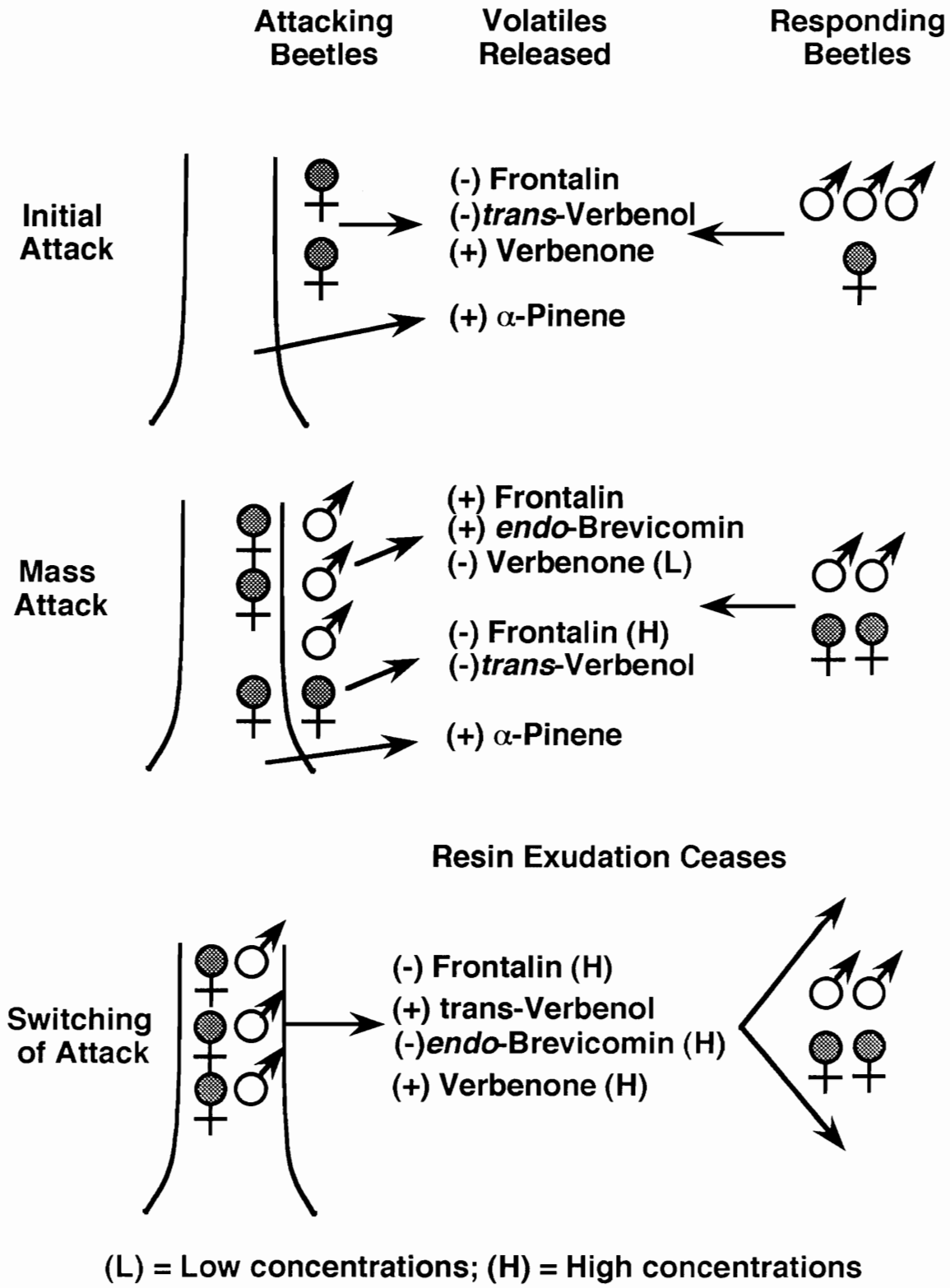

Figure 7.1 Revised mechanism of host tree colonization by the southern pine beetle, *Dendroctonus frontalis*.

arriving beetles. At the height of mass attack (3 to 5 days after initial attack) large numbers of males release high concentrations of V and eB which inhibit the response of both sexes to F and causes a significant drop in the number of arriving beetles.

Because F quantities do not decline after the mass attack stage, the switching of attack to neighboring trees appears to be the result of several events. First, reduction of primary resin reserves in the host reduce the quantity of aP available as a kairomone. Second, there is shifting of tV chirality from the predominately (-)-tV produced by SPB to almost pure (+)-tV produced through autoxidation. Although, the behavioral response of SPB to different enantiomers of tV has not been investigated, (+)-tV quantities generally peak after the point when peak attraction to the host occurs. This suggests that the (-)-tV produced by SPB is the synergist of F, whereas (+)-tV is inactive or even inhibitory. Third, it appears that racemic eB and (-)-V quantities must each reach levels of > 15% of F quantities before they inhibit the beetle's response to F. Data from this and previous studies indicate that the inhibitory effect of V would be enhanced by nearly equal quantities of eB.

Several aspects of the SPB semiochemical communication system remain to be studied. This information would support the long-term goal of building our understanding of semiochemical-based communication in bark beetles and for improving the use of semiochemicals in pest management efforts. Future areas of research should include:

1. *Evaluate, via field bioassays, the extent of geographic variation in the response of SPB to different chiralities of F and V at levels comparable to those currently used in prediction surveys and suppression tactics,*

respectively. The discovery of significant geographic variation in response to chiral F and V would require that the chiralities of these compounds be modified depending on the geographic area. Such modifications would likely increase the accuracy of survey results and the effectiveness of control measures. On the other hand, if SPB response to F and V is similar throughout its range, a single enantiomeric ratio of a pheromone could be used at reduced cost to forest managers.

2. *Confirm or identify the roles of the enantiomers of F, eB, cV, tV, M, and V in the system through the use of dose-dependent response in laboratory and field bioassays.* Some compound enantiomers may serve to enhance the activity of other compounds. These enantiomers could then be incorporated into pest management strategies to increase their effectiveness.
3. *Determine the quantitative and chiral contributions of V made by microorganisms to the system and the roles these compounds play in influencing SPB behavioral response.* The discovery that significant quantities of V are produced by microorganisms could possible lead to more economically viable methods of V production in the laboratory. In addition, it may be the found that the enantiomeric ratio of V produced by microorganisms is important with regard to the switching of SPB attack from one tree to another. This information could then be used to improve upon current control measures.
4. *Evaluate the roles of other compounds isolated from SPB hindguts and/or infested logs (Table 2.1) with regard to SPB behavioral response.* Several of these compounds may be found to significantly enhance the attractiveness

or inhibitory property of F or V, respectively, and could ultimately be incorporated into SPB pest management strategies.

5. *Identify all potential sources of F and eB and their chemical precursors and their role as part of the SPB communication system.*. The information gained would improve our understanding of semiochemical biosynthesis which, in turn, may lead to more economically viable methods of chemical production in the laboratory.

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Vitae

Donald Michael Grosman was born in San Bernardino, California on March 23, 1963. He attended the public schools in Oakland, New Jersey and graduated from Indian Hills High School in 1981.

He entered Ramapo College in 1981, and after completing two years, transferred to the State University of New York - College of Environmental Sciences and Forestry, Syracuse, to pursue studies in Forest Biology. He received his Bachelor of Science degree in December, 1985.

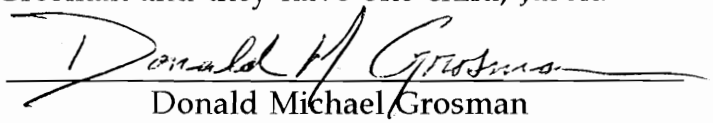
During the period from 1986 to 1988 he attended the University of Maine at Orono and served as a research assistant under Dr. Fred B. Knight in the College of Forest Resources. He received his Master of Science degree in Forestry in December, 1988.

He worked for one year with the University of Maine Cooperative Extension Service before accepting a research position under Dr. Thomas L. Payne in the Department of Entomology at Virginia Polytechnic Institute & State University.

In January, 1992 he was enrolled for graduate study in the Department of Entomology at VPI & SU and served as a graduate research assistant under Drs. Scott M. Salom and F. William Ravlin.

In August, 1995 he accepted a position with the Forest Pest Control Section of the Texas Forest Service as Entomologist II.

He is married to Rachael Grosman and they have one child, Jason.



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