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Southern Pine Beetle Behavior and Semiochemistry

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Keywords

bark beetle
dispersal
host selection
pheromone
semiochemical

Abstract

The southern pine beetle (SPB) feeds both as adults and larvae within the inner bark of pine trees, which invariably die as a result of colonization. Populations of the SPB erupt periodically and produce catastrophic losses of pines, while at other times the beetles persist almost undetectably in the environment. The southern pine beetle has evolved behaviors that maximize its survival and reproduction when local population densities are either high or low. When densities are high, the SPB utilizes pheromones to organize synchronous mass attacks capable of overwhelming the resin defenses of healthy, vigorous trees. They thereby render hosts available to colonization that would not be susceptible to attack by one or a few individuals. When densities are low, the SPB must find and utilize trees that have been previously rendered susceptible by either abiotic factors, particularly lightning strikes, or biotic stressing agents such as attacks by other bark beetle species. This chapter reviews existing knowledge of the behavior and chemical ecology (i.e., use of chemical signals including pheromones) of the SPB and addresses how these aspects of SPB biology may either facilitate or hinder efforts to manage this virulent forest pest.

3.1. INTRODUCTION

Damage from the southern pine beetle (*Dendroctonus frontalis* Zimmermann) (SPB) occurs conspicuously on the landscape as patches of pines that are killed rapidly and sequentially over a period of weeks or months. Each such infestation or SPB “spot” typically consists of a zone of contiguous pines that may be dead and abandoned, currently colonized, and/or undergoing mass attack. This zone is usually surrounded by apparently uninfested trees, and newly infested trees are accumulated typically on just one side of the spot, called the spot’s head. Spot growth (i.e., accumulation of new infested trees in a spot) occurs at a rate that is dependent on the number of currently infested trees, the density of adjacent suitable hosts, the season, and a variety of other factors. Individual spots may accumulate as few as one new tree every 2-3 days (Johnson and Coster 1978, Schowalter and others 1981b) or may engulf dozens of trees per day and grow to encompass hundreds of acres in a matter of weeks. Mass attacks on uninfested trees at the spot head are mediated by pheromones released both by the SPB established in adjacent, recently infested trees within the spot head, and by individuals landing on the uninfested trees.

Less conspicuously, the SPB may attack single, isolated trees in the forest, and during periods of relatively low beetle population numbers (nonoutbreak or latent population phase) this may be the only mortality attributable to this insect. However, with an adequate density of susceptible hosts and/or foraging beetles in the immediate area, spots may develop from such isolated, infested trees (Coulson and others 1985a, Franklin 1970b).

3.2. BEHAVIORAL EVENTS OF THE SPB LIFE CYCLE

The complete life cycle of the SPB can be divided into the following sequence of events: 1. dispersal, 2. host location and selection, 3. host attack and colonization, 4. parent adult reemergence, 5. brood development, and 6. brood emergence.

3.2.1. Dispersal

Upon emergence, the SPB takes flight in search of a host tree. Unlike many other species of aggressive bark beetle (Borden 1982), newly emerged SPB do not require a

period of flight exercise before they respond to attractants (Gara and others 1965, Payne and others 1976), although flight exercise has been shown to enhance responsiveness in walking beetles (Andryszak and others 1982). The SPB apparently does not feed to replenish energy reserves during dispersal, and they can survive merely 1-2 weeks while outside of a host tree and exposed to air temperatures suitable for flight (Wagner and others 1984a). Thus, individuals that are to survive and reproduce must locate a suitable host quickly (Coulson and others 1985a). Tethered SPBs can fly up to 2.6 km and at an average speed 0.77 km/hr (Kinn 1986), and beetles marked with fluorescent powder have been recovered on the day of their release in baited traps located more than 1 km away (Gara 1967). Using concentric rings of baited traps around a central release point, Turchin and Thoeny (1993) estimated the median dispersal distance of the SPB to be 0.69 km. Based upon estimates of the frequency and distribution of lightning-struck pines, these dispersal distances, though not great, are likely adequate to allow SPBs to reach a susceptible host during any season of the year (Coulson and others 1983). Furthermore, genomic studies of SPBs collected from locations separated by dozens or hundreds of kilometers did not reveal a significant correlation between genetic and geographic distance, suggesting that free exchange of genes, and thus dispersal, may occur across substantially greater space than inferred from mark-recapture studies (Allender and others 2008, Schrey and others 2008).

The distance that individual SPBs disperse is influenced by a variety of factors, a key one being the proximity of emerging beetles to sources of attractant. Catch of marked SPBs in baited traps located 1.6 km upwind was much less if the beetle release point was adjacent to trees being mass attacked, whereas captures in unbaited traps adjacent to the release point were much greater (Gara 1967). Similarly, when brood trees in an active SPB spot were coated with fluorescent powder, proportions of marked beetles trapped at artificially induced infestations located 100, 200, and 500 m away were much greater if the spot was subjected to a cut-and-leave treatment that suppressed mass attack of new trees at the spot’s head (Cronin and others 1999). These findings suggest that SPBs emerging within active spots are arrested by semiochemicals released from adjacent, newly infested trees. Consequently, they will

tend to attack trees within or near the spot head rather than disperse to new locations as long as mass-attacked trees persist within the spot (Cronin and others 1999, Gara 1967). Contrariwise, the termination of spot growth and disappearance of newly infested trees from an SPB spot—either due to the artificial removal of these trees or exhaustion of suitable hosts at the location—apparently releases long-range dispersal by beetles emerging from previously infested trees within the spot (Gara 1967). Nonetheless, a significant proportion of emerging beetles do disperse from active, growing infestations (Cronin and others 1999), and these individuals may express different genes than those that remain to attack trees within their natal infestations (Florence and others 1982).

The SPB disperses predominantly during the spring and to a lesser extent in fall, whereas in summer their movements appear to be largely restricted to interiors of established spots. Median dispersal distance by SPBs emerging within concentric rings of baited traps was 1.09 km in the fall but merely 0.53 km in summer (Turchin and Thoeny 1993). Furthermore, the SPB can be captured during the spring in large numbers in traps baited with frontalinal and turpentine and located hundreds of meters from the nearest spot (Billings 1988), whereas in summer such traps appear to be largely ineffective unless placed directly within an active infestation (author's personal observations). Southern pine beetles in a walking olfactometer likewise responded more strongly to an attractant mixture in the spring than in the summer (Roberts and others 1982), suggesting an overall lower responsiveness to attractant semiochemicals during periods of nondispersal. New SPB spots are established predominantly in the spring, with fewer originating in summer and fall (Thatcher and Pickard 1964), and essentially none in winter (Franklin 1970b). Fat content of emergent SPBs is greatest in the spring and fall; hence they have greater energy reserves and flight potential during their major periods of dispersal (Hedden and Billings 1977). Spots commonly stop growing in the late fall and winter, and spring dispersal may be triggered both by the return of average temperatures adequately warm for emergence and flight and the absence of trees releasing attractive semiochemicals within the overwintering spots (Gara 1967). Intense but brief flights of the SPB commonly occur during January and February on days

when the temperatures exceed ~20 °C (Moser and Dell 1979a), although these flights may not generate new mass attacks (Franklin 1970b).

The southern pine beetle terminates or reduces flight activity in winds exceeding about 7 km/hr (Coster and Gara 1968, Coster and others 1978b), or when the maximum daily air temperatures are either below 7 °C or above 37 °C (Moser and Thompson 1986). A much higher air temperature is apparently required for the SPB to initiate flight (i.e., 22 °C) than to sustain it (i.e., 7 °C) (White and Franklin 1976). Solar warming of the bark may permit the SPB to initiate flight on days when the air temperature remains below 22 °C. The optimal flight temperature for the SPB is approximately 27 °C (Moser and Thompson 1986).

Tests with rotary nets indicated that most SPB flight occurs between 1.2 and 5.5 m above the ground, which roughly corresponds to the height of first and most frequent landing on mass-attacked trees during summer [i.e., 3–4 m; (Coster and others 1977a, 1997b)] (Gara and others 1965). Weather influences daily beetle flight activity (inferred from the rate of beetle catch in baited traps) to a greater extent in winter than other seasons, and during this period flight is strongly positively correlated to temperature and amount of sunlight (Geer and others 1981). Heavy rainfall can suppress beetle flight, whereas the onset of summer rainstorms and light rain can increase flight activity somewhat (Coster and others 1978b, Moser and Dell 1979a). Relative humidity greater than approximately 80 percent reduces SPB flight in both fall and winter (Geer and others 1981).

In both spring and summer, SPB flight activity is concentrated in the afternoon between 2 pm and 6 pm, with some flight occurring throughout the daylight hours (Vité and others 1964, Vité and Crozier 1968). Hopkins (1909) reported that the SPB flew also at night, but this has not been corroborated by subsequent studies (Bunt and others 1980). The SPB flies upwind in response to sources of attractive odors, and they may land at least briefly on nonbaited, vertical objects downwind from an attractive odor source while orienting upwind (Coster and Gara 1968). Following emergence, they may disperse either in the prevailing upwind or downwind direction, and season appears to influence which is more prevalent (DeMars and Hain 1979, Moore and others 1979).

3.2.2. Host Location and Selection

The SPB must kill their host trees in order to reproduce, whereas it is essentially impossible for a solitary beetle pair to kill a healthy pine. To be successful, an SPB pair must either attack synchronously with sufficient numbers of other pairs to assure capitulation of host defenses, or otherwise select hosts whose defenses have been sufficiently compromised by other agents. Thus, the optimal host location and selection strategies for individual SPB will depend upon the balance between local abundance of conspecifics and the susceptibility of available hosts.

Once SPB attacks have been initiated on an individual tree, the combination of beetle-produced aggregation pheromones and host odors becomes the principle cue used in host location and selection by SPBs that follow. Semiochemicals from these initial attacks attract other host-seeking SPBs to the tree of pheromone origin. The later-arriving beetles, in turn, initiate their own attacks and release pheromones. This self-perpetuating cycle may come to an end once the phloem resource of the host reaches its carrying capacity and no further beetles initiate attacks. However, if the concentrations of semiochemicals reach a high enough level (as occurs if beetles attack rapidly and in large numbers; see below) and suitable pines are adequately close by, arriving beetles may be induced to land on and attack these adjacent pines as well. These adjacent trees may then become new foci of attack for arriving beetles, a process called “host switching,” and semiochemicals from these trees may, in turn, stimulate initial attacks on further adjacent trees. This chain reaction, in which pheromones from trees undergoing mass attack stimulate landings, initial attacks, and consequently pheromone release from adjacent trees, appears to be the underlying mechanism driving the accumulation of new infested trees in SPB spots.

Individuals that first arrive and initiate attacks on a particular tree (so-called “pioneer” beetles) face special risks, since if the tree is vigorous, their survival may depend upon their capacity to release pheromones and attract sufficient numbers of other beetles to join them in a mass attack (Pureswaran and others 2006). Within active infestations, pioneer beetles can rely to a large extent on pheromones released from currently mass-attacked trees both to guide them in selecting a new host (i.e., one adjacent to those already mass-attacked) and to insure this

host’s subsequent mass attack and colonization. In its strictest sense, the term “pioneer” is applied only to those beetles initiating attacks without the benefits of pheromones already being released from either the potential host or adjacent trees. Such conditions exist within the forest outside established infestations or during periods of nonoutbreak population levels. Then pioneer beetles must select a host that is adequately weakened to allow immediate colonization, or else their pheromones must be sufficient for attracting dispersed beetles to overcome host defenses. A separate understanding of host selection processes by either concentrated or dispersed populations is essential to predicting and managing SPB outbreaks, since host selection by dense SPB populations influences infestation persistence and growth rate, whereas host selection by dispersed or dispersing beetles determines when and where infestations become initiated in the first place.

Host Location/Selection within Active Infestations

Within established infestations, beetle populations are normally high enough to permit colonization of vigorous pines through mass attack. New hosts are selected for attack in response to at least four factors: 1. their proximity to recently attacked trees, 2. the density and rate of attack on such adjacent trees, 3. wind direction, and 4. appropriate visual cues from the prospective host.

Proximity to recently attacked trees

Within growing infestations, adjacent pines are typically attacked sequentially, such that the trees infested next are typically those located nearest ones currently undergoing mass attack (Vité and Crozier 1968). This effect is apparently due to the ability of semiochemicals from mass attacked trees to concentrate beetle landings in the immediate area, as suggested by the fact that both natural and artificial sources of SPB attractants (particularly the female-produced pheromone frontalin combined with host terpenes) are likewise capable of stimulating landings and attacks on all trees within a limited radius of their release point (Coster and Gara 1968, Vité 1970). In a small infestation where only one tree was being mass attacked (and thus serving as an attractant source) at any single time, Johnson and Coster (1978) determined that the probability of attack on any tree declined as the inverse square of its distance from the most recently infested

tree. However, mass-attacked trees are never observed to induce new attacks more than 6-7.5 m away (Gara and Coster 1968). Consequently infestations will normally collapse (i.e., cease growing) if the distance between the most recently infested trees and uninfested pines exceeds this minimum distance (Schowalter and others 1981b), a circumstance that often occurs when the advancing heads of infestations encounter stand boundaries, power line right-of-ways, roads, sites of previous infestations, and other natural and man-made landscape discontinuities in pine abundance.

Density, age, and rate of attacks on adjacent, infested trees

The capacity of SPB-infested trees to induce attack on adjacent trees is limited to those that have been freshly infested and are still attractive to flying beetles (Johnson and Coster 1978). The attractiveness of infested trees to flying beetles (and, likewise, attractant production by attacking beetles) varies over time and is typically short-lived: approximately 99 percent of beetles are trapped on infested trees within the 7 days following attack initiation, with a distinct peak in response occurring at day 3 (Coster and others 1977a). Removal of recently infested trees from a growing SPB infestation terminated both beetle aggregation and attacks on uninfested hosts (Gara 1967). The tendency for SPB colonization to be induced on trees adjacent to an attractant source appears to be dependent on the intensity of the attractant stimulus. In an experiment conducted in an epidemic SPB area (i.e., within 1 mile of active SPB spots), the number of SPB attacks on a pine post necessary to induce mass attack on adjacent uninfested posts was approximately linearly related to the distance between the posts. In addition, such baited pine posts induced attacks on adjacent, unbaited posts only when these were located in epidemic stands, but failed to do so in stands with low populations (Gara and Coster 1968). The accumulation of fresh attacks on the baited post was much slower in the endemic stand than in the epidemic stand, and these authors speculated that the absence of spillover attacks in the former was due to the insufficient concentrations of attractants produced by the poorly synchronized attack on the baited post. Production of attractant by unpaired female SPBs occurs for only a few days (Coster and Vité 1972); hence, a host that accumulates attacks over many weeks cannot form as intense a pheromone plume as one receiving the same number of attacks

synchronously. Studies conducted using synthetic attractants confirm the importance of attractant dose to the likelihood of attacks on adjacent trees: low doses of frontalin/*alpha*-pinene (0.3 mg frontalin/hour) concentrated beetle landings near the point of bait release; rates 3-10 times this induced attacks on adjacent trees, while extremely high rates (1 g frontalin/hour) caused attacks on trees up to 40 m away (Vité 1970).

Wind direction

Within infestations, the SPB tends to select host trees immediately downwind from those most recently infested, and for this reason SPB infestations tend to grow in the prevailing downwind direction (Coster and others 1978a). Flying SPBs respond to contact with an attractant odor plume by flying upwind (upwind anemotaxis), and thus they are more likely to land on objects located downwind of an attractive source than other directions (Coster and Gara 1968). Boles of uninfested trees no doubt intercept the semiochemical plumes arising from infested ones located upwind, resulting in exceptionally high concentrations of secondhand aggregation stimulants occurring at the bark surface of such downwind trees. A stream of air containing concentrated volatiles from attractive SPB-infested bolts can stimulate SPB attacks on any tree where it is directed (Gara and others 1965); hence, the wind passing through recently infested trees may produce a similar effect in trees downwind.

Visual cues from the prospective host

In the presence of high concentrations of semiochemical attractants, the SPB will tend to alight on and may attack any dark object possessing a strongly vertical silhouette similar to the bole of a standing host tree (Gara and others 1965). Within an active SPB infestation, baited pine posts were much more attractive to the SPB when placed in a vertical rather than horizontal orientation (Gara and others 1965). This presumably visually mediated preference for vertical hosts agrees well with the long-standing observation that SPB infrequently infest downed timber (Dixon and Osgood 1961; however see Moser and others 1987), and this preference may help the SPB to discriminate against nonliving hosts (Payne and Coulson 1985). In addition, this visual response appears to be only to objects with a low degree of light reflectance (i.e., dark in appearance), since multiple funnel traps are far less attractive to the SPB if painted white or yellow as opposed

to black or other dark colors possessing a low average reflectance value (Strom and Goyer 2001). Furthermore, white-painted lower boles of uninfested pines received many fewer SPB landings and attacks than the unpainted portions of the same trees or adjacent, black-painted pines (Strom and others 1999).

Host selection by SPB within infestations: Other factors

Host Susceptibility. As SPB infestations expand, generally all pines above ~10 cm in diameter in the path of the moving head of the infestation are colonized and killed, resulting in an uninterrupted zone of dead pines (Dixon and Osgood 1961). However, susceptibility to bark beetle attack, as assessed by measuring resin flow and other indicators of vigor and defensive capacity, typically varies greatly among pines within stands (Cook and Hain 1987a, Martinson and others 2007). Hence the characteristic scorched earth nature of SPB infestations suggests that, within infestations at least, the SPB largely does not discriminate hosts according to their relative susceptibility (Gara and others 1965, Thatcher 1960). However, experimentally grown progeny of infrequent escape trees (pines that survive an infestation that kills all surrounding trees) produce above-average resin flow, suggesting that survival of their parents was related to an inherited capacity to resist attack or avoid selection by mass-attacking beetles (Strom and others 2002).

Host Species. At the head of an active infestation, the SPB lands on uninfested pines, as well as entirely unsuitable hardwood species, with equal frequency. In fact, the SPB can be induced to mine into hardwood trees if a stream of concentrated volatiles from logs of mass-attacked pines is directed at their bole (Gara and others 1965). Such attacks on nonhosts are ultimately abortive, but the phenomenon demonstrates that, with very high local concentrations of volatiles from mass-attacked trees, the SPB cannot distinguish host from nonhost trees prior to landing or even penetrating into the bark.

Acceptable pine host species for the SPB differ in the relative frequency of SPB-inflicted mortality, and pines with higher average levels of mortality (e.g., loblolly and shortleaf) are described as being more susceptible and/or preferred relative to those with lower mortality (e.g., longleaf and slash) (Hicks 1980, Blanche and others 1983). However, as already implied,

the SPB may not discriminate pine species of differing susceptibilities when these are intermixed within active infestations. For example, SPBs are equally likely to land on, attack, and kill either loblolly or longleaf pine when these species co-occur along the front of a growing infestation (Martinson and others 2007), and other evidence suggests that SPBs may discriminate between these host species only in the absence of a pheromone plume (Friedenberg and others 2007b). However, when Virginia pine and loblolly were intermixed in a growing SPB infestation, Virginia pines received significantly higher rates of landings, attacks, and mortality than loblolly (Veysey and others 2003).

Inhibitory Pheromones. Some evidence suggests that SPB-produced olfactory repellants or attraction inhibitors may play a role in host selection. Devices releasing certain male-produced volatile compounds (e.g., verbenone and *endo*-brevicomin) can reduce SPB responses to attractant-baited traps and/or reduce landings and attacks on pines within active infestations (Payne and others 1978, Richerson and Payne 1979, Salom and others 1992). Since males are the secondarily arriving sex, these male-produced attraction inhibitors have been thought to play a role in both terminating attraction to fully colonized trees (thus serving as antiaggregation pheromones) and inducing switching in attack focus from colonized trees to adjacent, uninfested trees (Payne 1980, Renwick and Vité 1969, Smith and others 1993). However, female SPBs likewise produce compounds that can inhibit conspecific attraction to baited traps, and during attack they may actually release these compounds earlier than or simultaneously with their major attractant frontalinal (Sullivan 2005, Sullivan and others 2007a). In addition, there is no *in situ* evidence that arriving males and their associated semiochemicals cause female attacks to become significantly less attractive to flying SPBs. Pine bolts and posts infested with beetle pairs are reported to be similarly attractive to conspecifics as ones infested with solitary females (Coster and Vité 1972, Franklin 1970b, Svihra 1982), and in a location with low beetle densities, standing trees infested with both sexes were more attractive than trees infested with females alone (Sullivan and others 2007b). Furthermore, the rapid loss in attractiveness of SPB-colonized trees to conspecifics (Coster and others 1977a) can be explained readily from the decline in concentration of beetle- and

host-produced attractants (Vité and Crozier 1968, author's unpublished data) rather than the release of inhibitor pheromones. It is also likely that the inhibition observed to male volatiles in trapping and tree protection tests involved unnaturally high concentrations of the semiochemicals, and thus may not have reflected normal SPB behavior. Hence, the evidence is equivocal regarding whether the SPB utilize antiaggregation pheromones during host selection and colonization.

Host Location/Selection Outside of Active Infestations

Pioneer SPBs that initiate colonization of hosts located substantial distances from existing infestations cannot rely on large numbers of conspecific attacks to assist them in overcoming host defenses, and thus they must locate hosts whose defenses have been weakened prior to their arrival (Flamm and Coulson 1988). Two alternative hypotheses have been proposed for how such pioneer bark beetles locate hosts: either flying beetles orient to primary attractants arising from the uninfested host, or alternatively, they land on trees at random and evaluate the suitability of a host based on gustatory and other close-range cues (Byers 1989b, Raffa and Berryman 1979, Wood 1982b). After some unknown threshold number of beetles has become established on a host and release pheromones (perhaps as few as one), secondary attraction presumably becomes the predominant means by which subsequently arriving beetles locate the isolated host.

Host-produced odors, specifically the major resin monoterpenes, are potent synergists of the SPB's aggregation pheromones, and thereby play an important role as secondary attractants (Smith and others 1993). However, resin monoterpenes do not attract SPB to traps in the absence of pheromones (Billings 1985, Payne and others 1978). Healthy pines subjected to certain stressing treatments will become attacked by the SPB very rapidly, suggesting that host stress induces the production of attractive olfactory cues (Coulson and others 1985a, Heikkinen 1977). Lightning strikes are the single most important agent for rendering trees susceptible to the SPB in nature (Hodges and Pickard 1971, Lovelady and others 1991), and authors have speculated on the possible production of primary attractants by these trees. Two artificial procedures that duplicated the bole-length bark wound typical of a lightning strike (one employing blast cord, the other a

sickle) induced SPB landing and attacks on treated pines situated far from natural sources of beetles (Coulson and others 1983, Payne 1986). Southern pine beetles responding to lightning-struck pines may be attracted by massive quantities of host volatiles released precisely within the height range of typical SPB flight, with the vertical silhouette of the tree bole providing an appropriate visual stimulus (Payne 1986). However, the aforementioned wounding/stressing experiments did not screen the trees from attacks by bark beetles; hence, it is not possible to conclude that SPB responses were due to host-produced, primary attractants alone. Under the alternative hypothesis that the SPB lands randomly on potential hosts, there is some evidence to suggest that the massive quantities of resin volatiles released by the lightning wound could mediate host selection by arresting SPB flight on the struck trees (Payne and Coulson 1985).

In the Southern United States, trees infested by the SPB are typically attacked simultaneously and/or in rapid succession by several additional bark beetle species, specifically, *Ips calligraphus* (Germar), *I. avulsus* (Eichhoff), *I. grandicollis* (Eichhoff), and the black turpentine beetle (BTB), *Dendroctonus terebrans* (Olivier) (Coulson and others 1986, Flamm and others 1993). These five species, collectively called the "southern pine bark beetle guild," each produce their own, specific attractant pheromone blends that nonetheless share many components (Smith and others 1993). Since the SPB may not be the first species to arrive at isolated, susceptible trees (Hodges and Pickard 1971, Coulson and others 1985a), it is possible that pheromones of first-arriving species may serve as host location cues (i.e., kairomones) to subsequently arriving SPBs (Svihra and others 1980). However, the SPB does not appear to be attracted to logs infested with any of the three *Ips* species or to synthetic blends of *Ips* spp. pheromones (Birch and others 1980, Smith and others 1990). On the other hand, the BTB produces frontalin, the major component of the aggregation pheromone of SPB, as well as *exo*-brevicomin, *endo*-brevicomin, and *trans*-verbenol—compounds that can synergize frontalin's attractiveness to the SPB (Payne and others 1987, Pureswaran and others 2008a, Sullivan and others 2007b). Synthetic blends that mimic the natural pheromone blend of the BTB are also significantly attractive to the SPB in field-trapping trials (Smith and others 1990). Unlike the SPB, the BTB responds

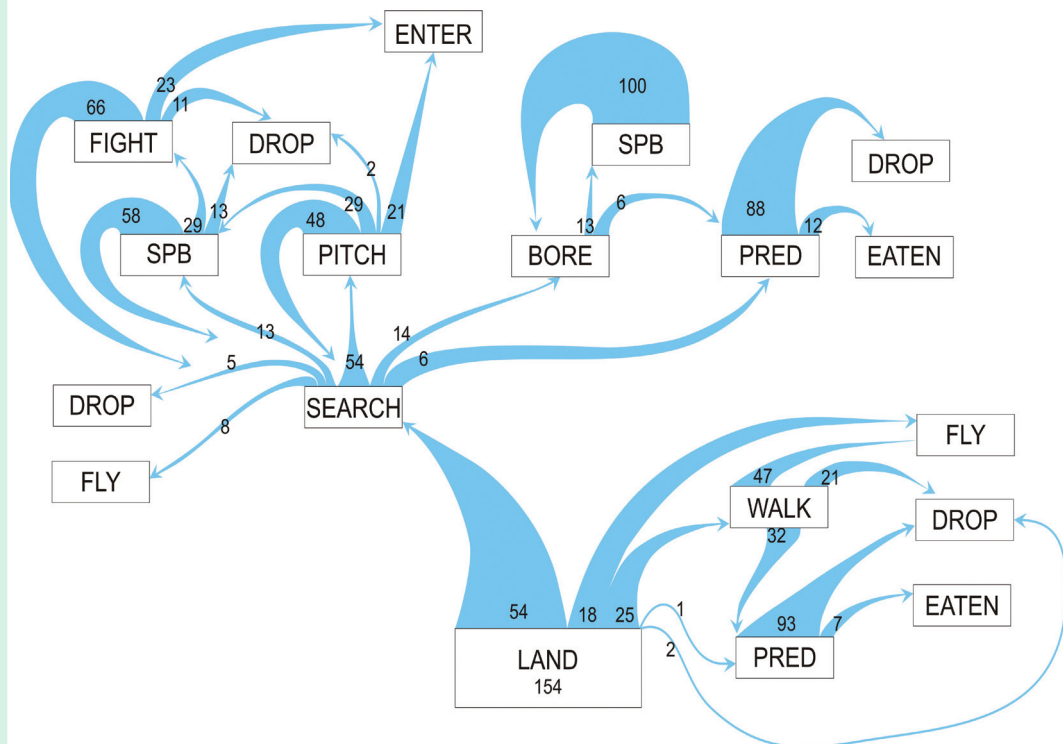
strongly to identified, primary attractants released by physically wounded pines such as lightning strikes, namely, resin monoterpenes (Fatzinger 1985, Siegfried and others 1986), and this capacity may assist them in being almost invariably the first species to arrive at lightning-struck pines (Hodges and Pickard 1971). Hence the SPB may exploit the capacity of BTB to utilize primary host attractants by responding to BTB pheromones as secondary host attractants.

3.2.3. Host Attack and Colonization

Females are the sex that initiates gallery construction, and the beetles that first arrive on mass-attacked trees are predominantly females (Coster and others 1977a). Attacks are initiated near mid-bole (3-4 m height) and spread upward and downward along the bole with decreasing attack densities (Fargo and others 1978). Bunt and others (1980, data reanalyzed by Bishir and others 2004) published the only quantitative description of on-bark SPB behavior during mass attack (overview shown in Figure 3.1), and the following narrative is based on their studies unless noted otherwise. Following landing on the bark, a female searches an average of 10.2 minutes and travels 22 cm of bark surface before initiating a gallery entrance, usually within a crevice in the bark. If the female encounters an established beetle entrance, she may investigate it and afterward initiate her own entrance less than 2 cm away.

Extractable chemical cues in the outer bark of preferred pine species can stimulate biting by female SPB; hence, such bark compounds may stimulate gallery initiation (Thomas and others 1981). She must chew into the bark for more than 1 hour before she is completely inside, and during this time she is highly vulnerable to predation by the clerid predator *Thanasimus dubius* (F.). She typically does not chew directly to the phloem, but rather mines the entrance at a shallow angle into the corky bark such that it penetrates the phloem tissue only gradually (Payne 1980). This behavior likely assists her in slowing the defensive response of the tree. The female uses her legs and elytra to push resin exuded by the damaged phloem tissue to the entrance where, apparently by working her back legs in the liquid, she forms it into a pitch tube of semicrystallized resin surrounding the entrance. Pitch tubes are formed only if the host is releasing resin; attacks occurring after host resistance has been reduced by previous beetle attacks or other causes may produce small or no pitch tubes (Payne 1980). If unimpeded by resin, the female then widens the tunnel where it penetrates the phloem to produce a “nuptial chamber”; this expansion provides the male adequate space to reorient his body for mating (Fronk 1947, Wagner and others 1981a). Until joined by a male, the female continues to mine in the phloem, constructing short (2-3 cm) galleries that radiate from the nuptial chamber (author’s personal observations).

Figure 3.1—Behavioral sequences for individual SPB during mass attack on a host pine. Abbreviations of on-bark behaviors: LAND=landing; WALK=undirected walking; SEARCH=active searching of the bark surface; PITCH=investigating an entrance hole; SPB=encountering another SPB; FIGHT=fighting with another SPB; PRED=encountering a predator; EATEN=captured by a predator; FLY=flying from the tree; DROP=dropping from the tree; BORE=boring a gallery; ENTER=entering an existing gallery. Arrows are labeled with the numbers of individuals observed (out of a total of 154) to proceed from one behavior to the next. (reprinted from Bunt and others)



While forming the gallery, she occasionally stridulates, producing a series of staccato chirps (Figure 3.2A) that may signal her presence to females in adjacent galleries (Rudinsky and Michael 1973, Ryker 1988).

When the female lands on the host, her hindgut contains small amounts (tens of nanograms) of the attractant pheromone frontalin, along with approximately 100 fold greater quantities of the frontalin synergist *trans*-verbenol (Pureswaran and others 2006). Females release these compounds continuously at a slow rate while outside of a host, and they no doubt do so also while on the bark surface searching for a suitable boring site or initiating a gallery. Upon entering the bark, the female rapidly releases most of this cache of pheromone in her hindgut, presumably as a consequence of a renewal of eating and defecation. She then begins to release frontalin at a much higher rate than she did prior to entering the host (Pureswaran and others 2008b, Pureswaran and Sullivan unpublished data). Frontalin combined with either *trans*-verbenol or *alpha*-pinene from the host resin attracts SPBs of both sexes to the attacked tree, and these compounds appear to be the key stimuli for initiating and then sustaining mass attack on the host. *alpha*-Pinene and *trans*-verbenol are apparently interchangeable and

redundant as attractive synergists for frontalin (Payne and others 1978); hence, *trans*-verbenol may be critical for stimulating mass attack prior to large-scale penetration of the phloem and resin release (Renwick and Vité 1969).

Both sexes search in a predominantly upward direction from their landing point (Gara and others 1965). Forty-six percent of landing beetles leave without initiating searching behavior; they either drop or fly off the bark surface. These beetles likely renew searching flight, as suggested by evidence that the SPB may land and take flight again multiple times in close proximity to an attractive source, and presumably they may even land again on the same host (Coster and Gara 1968). Encounters between either males or females, particularly when near the entrances to galleries, often result in battles in which the two insects butt heads and push against one another. If the male encounters another male in an entrance, he may attempt to dislodge the first by pushing and biting, and if larger than the first, he often succeeds. If the battle occurs within the gallery, the male may kill and dismember his rival (Yu and Tsao 1967). Whenever males encounter each other on the bark or inside a gallery, they stridulate vigorously with a rapidly pulsed “rivalry chirp” (Figure 3.2B; Ryker 1988).

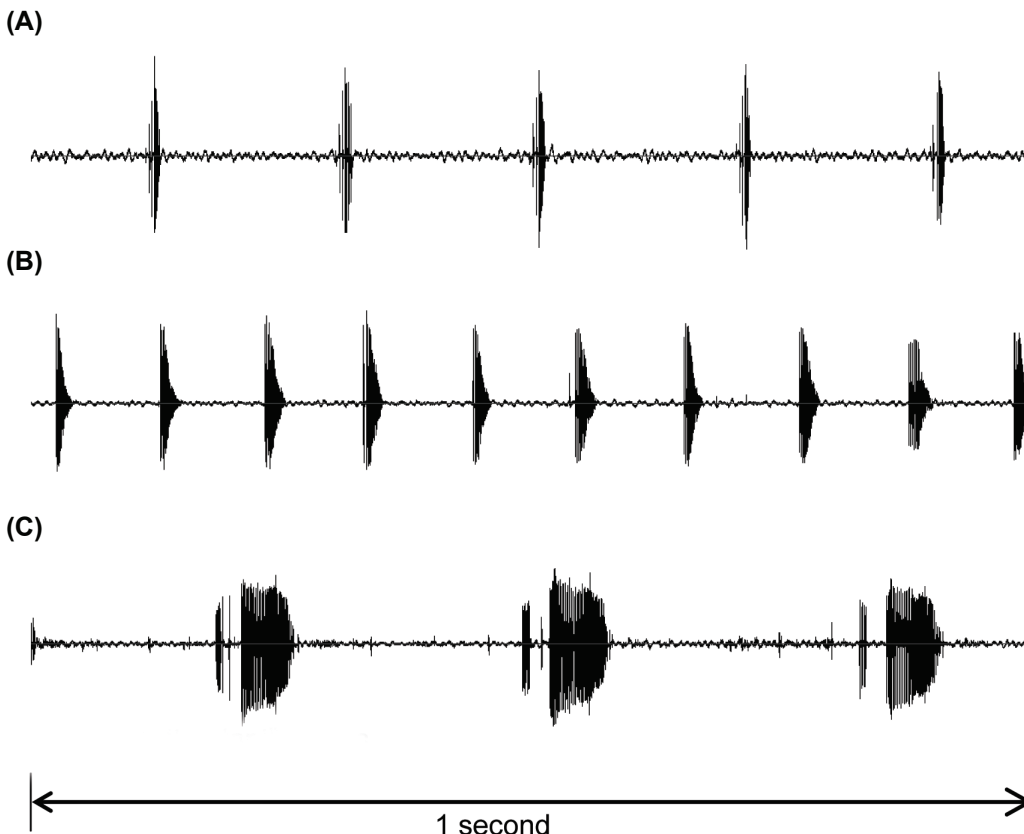


Figure 3.2—Oscillograms from sound recordings of SPBs inside a newly initiated gallery system. (A) Female chirps, (B) male “rivalry chirps”, and (C) male “attractant chirps”. (author’s unpublished data)

Landing males search an average of 6.6 minutes and may investigate several entrances along a mean 25 cm path before stopping at one and entering. Whereas it appears that some males merely happen upon entrances at random on the bark, search by others appears more directed, possibly in response to pheromones emitted from the female entrances. This is implied by laboratory olfactometer studies in which male SPBs walked upwind to point sources of female odors (McCarty and others 1980), and their walking could be arrested by female odors emitted from artificial entrance holes (Rudinsky 1973). If the male selects a female entrance filled with liquid resin, he wades in and joins the female in clearing the gallery (Figure 3.3). Otherwise, the male first circles the entrance, digs through any frass blocking the tunnel, and then proceeds into the gallery

to locate the female, who likely is extending the gallery and facing away from the entrance (Wagner and others 1981a). Upon reaching the female, he initiates courtship by jostling her elytra, while emitting a distinctive “attractant chirp” (Figure 3.2C; Yu and Tsao 1967, Ryker 1988). After possibly receiving some signal of acquiescence from the female, the male backs into the nuptial chamber or a gallery branch, reverses direction, and then backs toward the female to make end-to-end contact with her. The pair makes genital contact with the longitudinal axes of their bodies turned 90-180 degrees, and copulate for about 2 minutes (Yu and Tsao 1967). The pair mates repeatedly during gallery construction. The SPB is monogamous, and gallery systems are normally occupied solely by a single male and female. Nonetheless, if the male is removed and repeatedly replaced with another, the female will mate successively with these other males (Yu and Tsao 1967).

Similar to females, males arrive with accumulations of at least two sex-specific pheromones in their hindgut: minute quantities of (+)-*endo*-brevicomin and large quantities of verbenone (Pureswaran and others 2006). The males likewise release these compounds gradually while outside the host, and then rapidly empty their hindgut during or shortly following pairing with a female. Once paired, the male begins releasing elevated levels of (+)-*endo*-brevicomin and continues to produce verbenone (Pureswaran and others 2008b, Sullivan and others 2007b). Both compounds have been shown to have complex effects on the behavior of SPB, and hypotheses concerning the biological function of both (+)-*endo*-brevicomin and verbenone for SPB are discussed elsewhere in this review.

Attacking SPB adults can survive extensive periods completely immersed in liquid resin—a remarkable feat given the demonstrated toxicity of pine resin constituents to insects including the SPB (Byers 1989b, Coyne and Lott 1976, Smith 1963). However, this capacity is not unlimited, and the beetles can be “pitched out” by an overwhelming flow of resin or “pitched in” when crystallizing resin traps them inside the gallery. The pair cannot extend their gallery or begin laying eggs until resin flow has largely ceased. Despite the risk that resin flow poses to SPB survival, SPB-attacked pine is more attractive to conspecifics if it generates a strong defensive response and produces copious resin. Resin expelled from gallery entrances releases enormous quantities of *alpha*-pinene, which



Figure 3.3—A pair of SPB “working” a pitch tube on a newly attacked pine.

at high doses has been shown to dramatically increase SPB responses to frontalin (Billings 1985), and a vigorous host response may stimulate or sustain attractant pheromone production by attacking beetles (Vité and Crozier 1968).

Following cessation of resin flow and mating, the pair proceeds to extend their gallery into the phloem with the female in front, chewing into the tissue, and the male behind, assisting the female in clearing the front portion of the gallery of frass. Initially the frass is expelled from the entrance, but once the pair has penetrated a few centimeters into the phloem, the male packs the frass tightly behind him, thereby sealing off the entrance. Thereafter, he continues to pack frass behind him while maintaining an open space of 1.5-2.5 cm at the distal end of the gallery (Fronk 1947). The pair cuts a narrow (average 3.4 mm wide), 10-35 cm long, broadly S-shaped gallery into the phloem at a rate of 2-4 cm per day at 20-25 °C (Fronk 1947, Wagner and others 1980). They mine 1.5-2 days and extend the gallery 2.8-3.8 cm before the first egg is laid. This period of feeding presumably allows time for the enlargement of the female's reproductive organs, autolysis of flight muscles, and other physiological changes crucial to egg production. Since the first few centimeters of the gallery are more prone to be soaked with resin, the behavior may also assure greater egg survival. The length of this preliminary gallery may more than double at low temperatures, but it is unaffected by the density of attacking beetles in the surrounding bark (Wagner and others 1981a).

The female then begins to chew egg niches, semicircular pits 1-2 mm deep, into the phloem on alternating sides of the gallery (Fronk 1947). She lays a single egg into each niche after it is completed and then packs frass into the niche, covering the egg and securing it in place. The frass covering is pressed and smoothed to match the original contour of the gallery (Wood 1982a). The female lays an egg every 0.4-2.0 cm (mean 1.59 eggs/cm gallery; Foltz and others 1976). The spacing of eggs remains constant during egg laying, and is unaffected by the density of attacking beetles in the bark, but is greatest at 20-25 °C. Generally, it is reported that fewer than 3-4.5 percent of niches are left empty (Clarke and others 1979, Wagner and others 1981a), but high rates of empty niches (38 percent) have also been observed (Lashomb and Nebeker 1979). The length of this egg-bearing portion of the gallery and

the total number of eggs laid per female is inversely related to density of attacking beetles in the surrounding bark, and consequently, pairs that attack relatively late will produce less egg-bearing gallery and fewer eggs than those that attack earlier. Thus mining females reduce the length of the egg gallery and the number of eggs they lay in response to greater numbers of ovipositing females around them, and this results in a final egg density within the bark that is independent of the density of attacking adults. This compensatory negative-feedback process assures a uniform amount of food is available to all larvae (Coulson and others 1976b). The females may curtail oviposition after sensing the stridulation by beetles in adjacent galleries, or they may detect chemical changes in the phloem tissue resulting from degradation and damage caused by the beetles and their associated microbes (Wagner and others 1982). These same cues may cause females to divert their galleries away from other galleries rather than cross them (Grosman and others 1992), a behavior which is responsible for much of the sinuousness typical of SPB galleries. Females mine through (i.e., cross) existing galleries frequently only when beetles are at high densities.

Throughout the gallery but predominantly in the egg-bearing portions, the pair mines "turn-arounds"—short side branches extending either a variable distance outward into the corky bark but not to the surface, or <2 mm sideways into the phloem (Wagner and others 1981a). The frequency of turn-arounds mined into the corky bark increases with temperature, and these diverticula possibly play a role in improving ventilation within the gallery.

The final 2-3 cm of the parental gallery is typically free of eggs, and its length is independent of attacking beetle density (Wagner and others 1981b). Males and females generally mine separate exit galleries into the outer bark, and the time between the initiation of these galleries and reemergence occupies from one-third to one-half of the beetles' time within the host. This prolonged period of relative inactivity may coincide with internal physiological changes required for reemergence and flight. While constructing the exit galleries, the beetles periodically return to the phloem and may construct short (≤ 1 cm) side galleries, presumably for nutritional reasons (Wagner and others 1981a). On average, males reemerge before females (Yu and Tsao 1967).

3.2.4. Parent Adult Reemergence

Following establishment of a brood population in one host, SPB reemerge and may attack and establish broods in two or perhaps three additional trees (Clark and Osgood 1964). Field studies have reported that 65-97 percent of attacking adults reemerge (Cooper and Stephen 1978, Coulson and others 1978). Reemergence begins as early as 4-8 days following initiation of mass attack and lasts approximately 10 days during typical summer temperatures (Coulson and others 1978, Franklin 1970b, Thatcher and Pickard 1964). At temperatures $<20^{\circ}\text{C}$ or $>30^{\circ}\text{C}$, reemergence is delayed 4-17 days and lasts much longer (Gagne and others 1982). Relative to SPBs establishing an initial brood, SPBs establishing second broods produce longer egg galleries, produce more eggs, lay eggs closer together, and spend greater time in the bark before initiating an exit gallery (Wagner and others 1981b). Reattacking females are apparently capable of tunneling and laying viable eggs without a male; nonetheless, they

typically pair with a male in the new host. However, when artificially deprived of males, reattacking females establish fewer galleries with egg niches, produce shorter galleries, and lay fewer eggs per day than pairs making either a first or second attack (Van Sambeek and Kile 1981).

Reemergence behavior may be critical to the SPB's capacity to establish infestations from single, infested trees (Franklin 1970b). Reemerged SPBs are capable of producing aggregation pheromones (Coster 1970); hence, reemerging beetles attacking a second or possibly third adjacent tree could help maintain an attractive center at the site until brood from the original tree have completed development (Coulson and others 1978, Franklin 1970b). In addition, reemergence and reattack of adjacent trees rapidly generates overlapping generations of beetles, a condition that is key to sustaining a continuous emergence of beetles and recurrence of fresh attacks.

3.2.5. Brood Development

The eggs hatch in 4-12 days at a temperature of 30°C - 15°C , but they fail to develop at temperatures $\leq 5^{\circ}\text{C}$ or $\geq 35^{\circ}\text{C}$ (Gagne 1980). After hatching, the ~ 2 mm-long larva spends its first two instars mining a threadlike gallery 3-60 mm into the cambium at approximately right angles to the parental gallery (Thatcher and Pickard 1967). This gallery may exit the cambium and disappear into the phloem tissue for the remainder of larval development, particularly in thick-barked trees (Payne 1980). Both bark beetle adults and larvae feed by cutting pieces of phloem tissue from the end of the gallery with their mandibles, and then crushing this material with their mouthparts to extract liquids. Very little solid material is ingested, and the frass consists largely of undigested phloem tissue. Nonetheless, the larvae consume their exuviae after each molt (Fronk 1947). The larva regularly pushes accumulated frass to the back of the gallery, thereby maintaining an open space of a few millimeters at the terminus. In the third instar, the larva widens the end of its mine into an ovoid or irregularly spherical feeding chamber (~ 3 - 5 mm long/wide) that gradually fills with frass as the larva develops (Figure 3.4). The total length of the final gallery is typically <2 cm. However, if the early larva encounters a zone of phloem colonized by the blue stain fungus *Ophiostoma minus*, it does not form a feeding chamber but rather extends its narrow winding gallery many



Figure 3.4—Later-instar SPB larvae mining within feeding chambers in host phloem.

centimeters through the stained tissue and may die before completing development (Franklin 1970a). In the fourth instar, the larva mines into the outer, corky bark and constructs an ovoid pupal chamber approximately 1.5-4.5 mm wide by 4-5 mm long, and there molts into a pupa (Figure 3.5; Fronk 1947). In very thin-barked trees, the pupal chamber may be formed at the interface between the corky bark and phloem. The entire larval period lasts a mean 17-45 days at constant temperatures in the laboratory (25°-15 °C) (Fargo and others 1979). It was found to range more widely (10-62 days) in a field study in Mississippi (Mizell and Nebeker 1978). The time spent in each of the four instars is roughly equal (Goldman and Franklin 1977). Larvae held at 10 °C die before pupating (Gagne 1980). At constant temperatures, the pupal stage lasts an average of 5-17 days at 30° to 15 °C (Payne 1980), although field studies have reported pupal development as long as 26 and as short as 3 days (Mizell and Nebeker 1978, Thatcher and Pickard 1967). Under controlled conditions, a small fraction of beetles complete pupal development in merely 1-2 days (Gagne 1980). The cuticle of the newly eclosed callow adult darkens in approximately 1 week (Figure 3.6), and then the beetle begins mining through the corky bark to produce an exit hole. The interval between eclosion of the adult and emergence lasts 6-14 days at 30° to 15 °C (Gagne 1980).

3.2.6. Brood Emergence

During typical spring and summer temperatures in the Southeast, peak emergence occurs in the midafternoon, and this timing appears to obey an endogenously controlled rhythm rather than daily cycles in temperature, humidity, or light intensity (Kinn 1978). However, the rate of emergence increases at elevated barometric pressures (Kinn 1978). The lowest temperature at which emergence has been recorded is 12.5 °C (Gagne 1980), and since adults are rendered immobile at temperatures below 9 °C (White and Franklin 1976), the minimum temperature threshold for emergence presumably falls between these two values. During summer, brood emergence begins approximately 40 days after the initiation of mass attack (Franklin 1970b) and lasts an average of 28 days. Both sexes emerge at approximately the same time (Coulson and others 1979b).



Figure 3.5—Outer bark flakes peeled away to reveal SPB pupae within pupal chambers in the corky bark of a host.



Figure 3.6—Bark sliced perpendicularly to reveal a callow adult SPB maturing within a pupal chamber in the corky bark. Dotted lines demarcate the frass-packed gallery that the fourth instar larva mined from the phloem tissue.

3.3. OLFACTION IN SPB BEHAVIOR

The organs of olfaction in bark beetles consist of hair-like sensilla located on the antennae (Payne and others 1973). In the SPB these sensilla are located almost exclusively on the circular, laterally flattened antennal club and consist of two distinct morphological types (Figure 3.7): sensilla basiconica—cylindrical

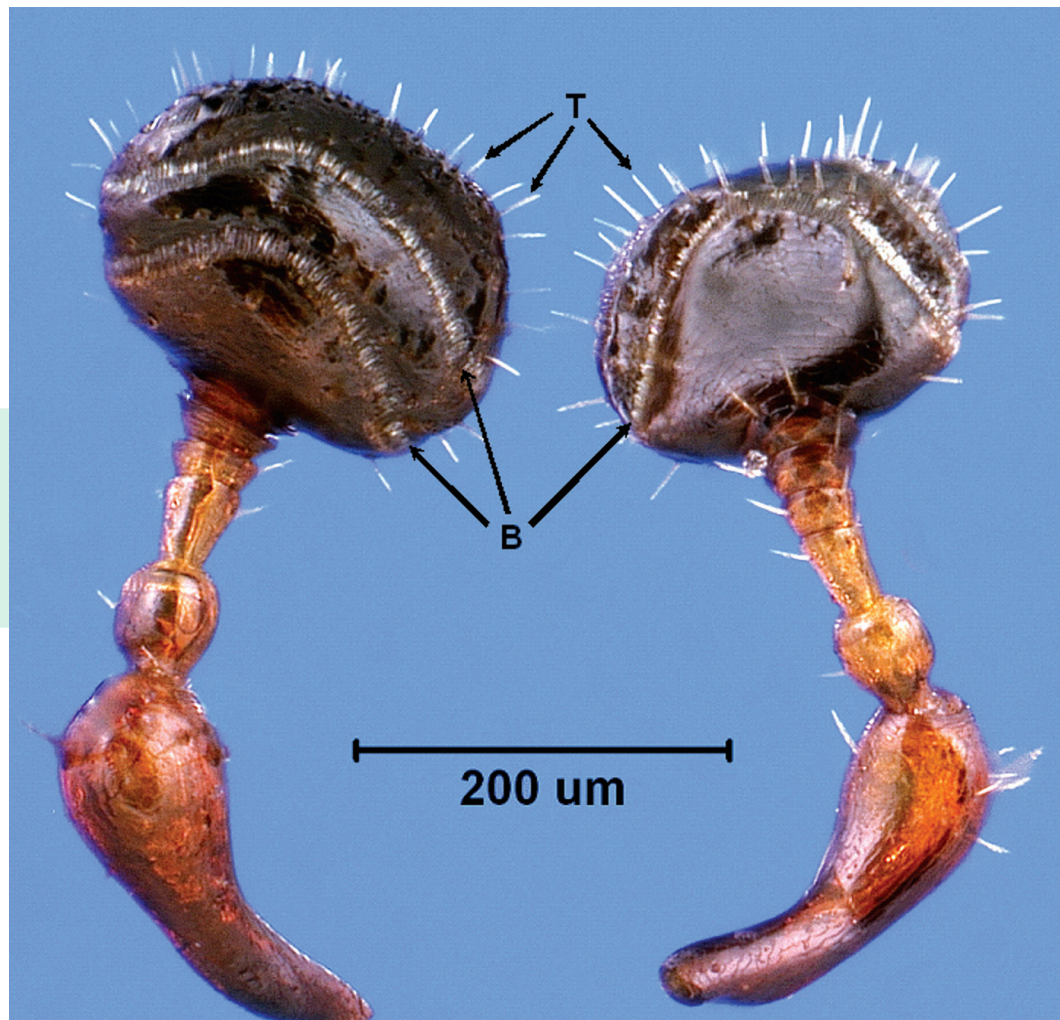
hairs that lie nearly flat against the cuticle, point distally, and are concentrated in two distinct bands that encircle the club; and sensilla trichodea II—tapering, curved hairs that protrude from the surface of the cuticle at a roughly 45-degree angle and are scattered over the more distal portions of the club (Payne and others 1973). The antennal club of both sexes has approximately 700 sensilla basiconica and 80 sensilla trichodea II. Preliminary electrophysiological studies demonstrated that both types of sensilla could respond to pheromones as well as host tree compounds (Dickens and Payne 1978b).

3.3.1. Methods of Investigating Olfaction in SPB

Olfactory sensitivities of the SPB to known or suspected semiochemicals have been studied by at least three methods: electroantennogram (EAG), coupled gas chromatography-electroantennographic detection (GC-EAD), and single sensillum recording (SSR) (Bjostad

1998, Payne 1974b). In EAG, changes in DC voltage across an antenna (in the specific case of SPB, the voltage between the antennal club and the head) are measured while individual test compounds or combinations are introduced into a stream of purified air blowing over the antenna. In GC-EAD, the effluent of a gas chromatograph (GC) is introduced into this air stream, and the antenna is exposed to compounds as they elute from the GC. GC-EAD possesses several advantages over EAG; namely, that unknown components within crude, natural extracts can be separated and assayed without prior purification steps, and test compounds can be delivered to the antenna in precisely known quantities and in greater purity than they are typically available commercially. In SSR, action potentials arising from sensory neurons within a single sensillum are recorded by electrodes measuring the voltage difference between the receptor lymph within the sensillum and the hemolymph of the antenna. Whereas EAG and GC-EAD presumably measure a summed response of many sensilla

Figure 3.7—Anterior and posterior views of an SPB antenna, showing the encircling bands of sensilla basiconica (B) as well as individual sensilla trichodea (T).



at once and thus provide a picture of average responsiveness of sensilla, SSR measures responses of only the sensory neurons within the single sensillum to which the electrode is attached. Thus only SSR can identify chemical specificities of individual sensilla and olfactory neurons and precisely determine the capacity of an insect to discriminate among individual olfactory stimulants.

The most uncontroversial use of EAG and GC-EAD data is qualitative; that is, the determination of whether an insect can sense a particular airborne compound. However, at least three kinds of quantitative EAG/GC-EAD data appear to have some predictive value: 1. the response threshold; that is, the smallest concentration of an olfactory stimulant capable of eliciting a detectable voltage change in the antenna, 2. saturation voltage; that is, the maximum response amplitude that can be elicited by a single compound, and 3. the difference in concentration between threshold and saturation or the dose range of EAG response (Dickens 1979). Presumably, a relatively lower response threshold to a compound indicates greater sensitivity, a relatively higher saturation voltage indicates a greater abundance of olfactory receptors that can be stimulated by a compound, and a wider EAG response range suggests a

greater capacity to distinguish concentrations of a stimulant (Dickens 1979, Dickens and Payne 1977). In addition, adaptation (desensitization to a single olfactory stimulant after prolonged exposure) can be used with EAG to estimate the capacity of antennae to discriminate among stimulants (Payne and Dickens 1976).

3.3.2. Olfactory Responses in SPB

Dozens of either insect- or host-produced compounds are capable of inducing electrophysiological responses in the antennae of the SPB (Figure 3.8; Smith and others 1993, Sullivan 2005). Southern pine beetles have a lower threshold of antennogram response and broader dose-response range for the bicyclic ketals frontalinal and brevicomin than host monoterpenes (Dickens 1979, Sullivan and others 2007b, author's unpublished data). The saturation voltage produced by the female-produced attractant frontalinal is greater than for any other SPB semiochemical, and a mixture of frontalinal with its behavioral synergist *alpha*-pinene does not produce a greater electrophysiological response than frontalinal alone (Payne 1975). Habituation of SPB antennae to frontalinal blocked olfactory responses to the SPB pheromones *endo*-brevicomin, *trans*-verbenol, and verbenone, as well as to the host compounds *alpha*-pinene

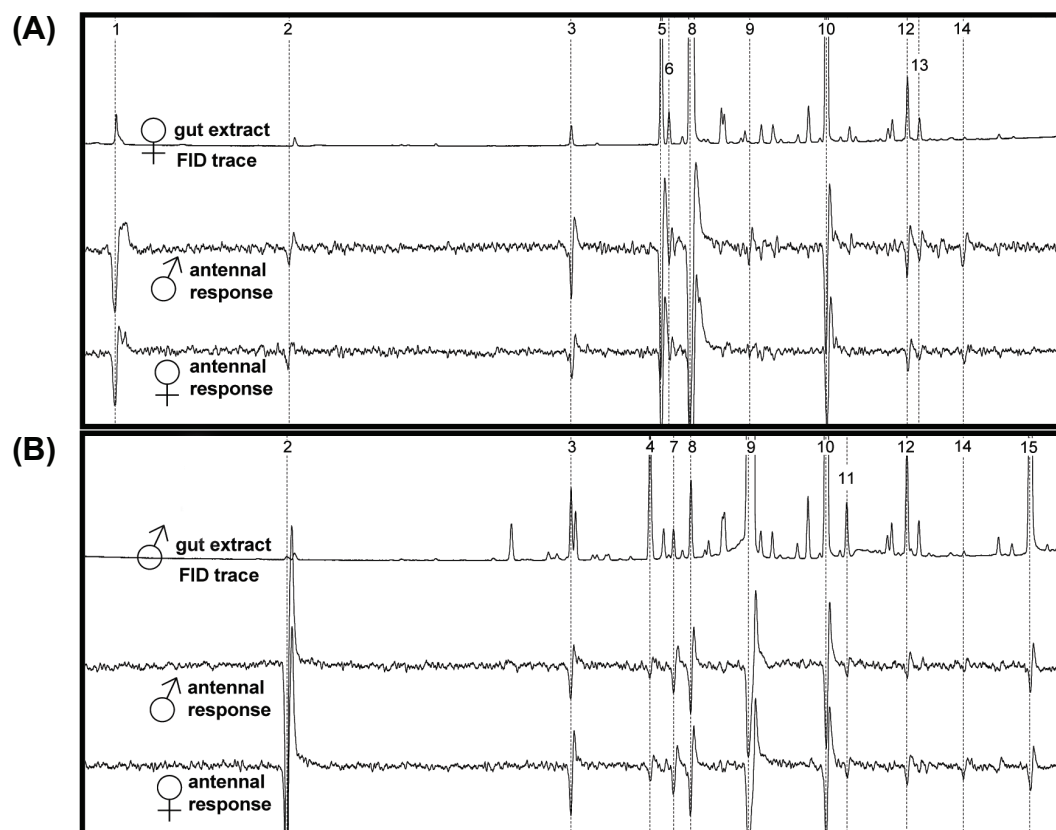


Figure 3.8—Electrophysiological responses of SPB antennae to volatile compounds extracted from the hindguts of newly emerged female (A) or male (B) adults as measured with a GC-EAD. Active compounds: (1)frontalinal, (2)*endo*-brevicomin, (3)fenchyl alcohol, (4)myrtenal, (5)*cis*-verbenol, (6)*trans*-pinocarveol, (7)acetophenone, (8)*trans*-verbenol, (9)verbenone, (10)myrtenol, (11)unknown, (12)*trans*-myrtenol, (13)*cis*-myrtenol, (14)2-phenylethanol, and (15)unknown. (reprinted from Sullivan 2005)

and 3-carene. Conversely, habituation of SPB antennae to any of these latter compounds did not terminate responses to frontalin. These authors concluded that frontalin could interact with all acceptors for the other compounds but that none of these latter compounds could interact with all acceptors for frontalin. These data suggest that, in the presence of high concentrations of frontalin, all olfactory receptor neurons on the antennae may become habituated, rendering the SPB incapable of sensing or responding to semiochemicals. It is not known whether under natural conditions SPBs are exposed to concentrations of frontalin adequate to habituate all olfactory neurons simultaneously; however, if so, it may explain why SPBs land apparently indiscriminately in the presence of high concentrations of their pheromones (Gara and others 1965). Comparison of the EAG saturation voltages for these different compounds suggests that the pheromones *endo*-brevicomin, verbenone, and *trans*-verbenol interact with a higher percentage of olfactory acceptors on the antennae than the host odors *alpha*-pinene and 3-carene, and that SPB possess somewhat greater numbers of olfactory acceptors to the pheromones of the opposite sex (Dickens and Payne 1977).

During EAG recordings from SPBs with the indifferent electrode inserted into the base of the antenna, Payne (1974a) observed an alternating current signal that was superimposed on the slow voltage deflections induced by pheromone exposure, and he concluded that these were potentials arising from antennal muscle movement. The amplitude of the muscle potentials induced by frontalin were substantially reduced if antennae were exposed simultaneously to either *trans*-verbenol or verbenone (Dickens and Payne 1978a), suggesting that these two oxygenated monoterpenes may act, at least in part, by inhibiting frontalin's capacity to stimulate specific movements in the SPB.

3.3.3. Identified Semiochemicals of SPB

Host colonization by the SPB is mediated by airborne behavioral chemicals (semiochemicals) released by the beetles, their host trees, and also possibly their microbial and arthropod associates and nonhost tree species (Table 3.1). Due to the importance of these compounds to the SPB's capacity to initiate and sustain infestations, much effort has been invested in developing synthetic

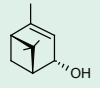
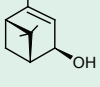
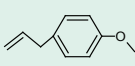
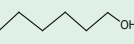
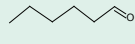
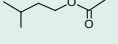
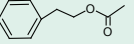
semiochemicals as management tools. The semiochemicals of aggressive bark beetles, including the SPB, are believed to function in at least four important ways (Borden 1996): 1. As aggregation pheromones—beetle-produced attractants of conspecifics that concentrate beetle arrivals on one or a few trees and insure attack densities adequate to overcome host defenses. Aggregation pheromones may also function as sex pheromones that bring the sexes together for mating. 2. As antiaggregation pheromones—beetle-produced inhibitors of conspecific responses to attractants. These compounds may be produced by beetles once they are successfully established on a host and terminate mass attack. This function presumably benefits both producing and responding beetles by preventing excessive densities of conspecifics on any single host, thereby lessening the deleterious effects of intraspecific competition (Byers 1989a). 3. As multifunctional pheromones—beetle-produced compounds that may either enhance or inhibit attraction of conspecifics depending upon their rate of release and possibly other factors. 4. As host selection kairomones—compounds produced by host trees, nonhost trees, or organisms associated with the beetle or its habitat that may be used by flying individuals to locate hosts of a suitable species and condition for colonization and to avoid unsuitable hosts. These compounds may either enhance attraction (presumably, if they indicate a suitable host) or inhibit attraction (if indicators of an unsuitable host).

SPB-Produced Compounds

Numerous volatile organic compounds have been identified from the SPB, typically by means of solvent extraction of tissues and frass followed by analyses of these extracts with gas chromatography or coupled gas chromatography-mass spectrometry. The SPB apparently resemble other bark beetles in that they lack specialized glands for the production and secretion of pheromones; rather these compounds are produced within the tissues of the alimentary canal or in the hemolymph, transferred to the lumen of the gut, accumulated in the hindgut, and released from the anus during defecation (Borden 1982).

The quantities of pheromones produced by SPBs from outbreak populations vary greatly among individuals (coefficient of variation, 60-182 percent) (Pureswaran and others 2006). Due to the large aggregation sizes and

Table 3.1—Volatile compounds known to influence movement of southern pine beetle

		Class ^b	Source ^c	Behavioral Activity ^a		References ^d
				Walking	Flight	
Frontalin		BK	F	A	A	1,2,3,4
<i>endo</i> -Brevicommin		BK	M	I	I,S,S/I	2,5,6,7,8,9
<i>exo</i> -Brevicommin		BK	M	--	I,S	5,10
<i>trans</i> -Verbenol			F	--	S	2,11,12
Verbenone			M	A,S/I	I,S/I	2,4,6,8,11,13
Myrtenol			F, M	S/I	I	6,14
Fenchyl Alcohol			F, M	--	I	15
Myrtenal			M	--	I	15
<i>cis</i> -Myrtenol			F, M	--	I	15
<i>trans</i> -Myrtenol			F, M	--	I	15
<i>trans</i> -Pinocarveol			F	--	I	15
<i>cis</i> -Verbenol			F	--	S	15
2-Phenylethanol		NMA	F, M, YA	I	I	14,15,16
Acetophenone		NMA	M	--	I	15
<i>alpha</i> -Pinene		HM	H	A	S	2,4,11,12
4-Allylanisole		PP	H	R	I	17,18
Hexanal		--	NH	--	I	19
1-Hexanol		--	NH	--	I	19
Isoamyl Acetate		--	YA	S	--	16
2-Phenylethyl Acetate		NMA	YA	S	--	16

^a Affect on SPB movement: A = attractive; I = inhibits attraction or arrestment; S = synergizes or enhances activity of attractant; S/I = alternately synergistic or inhibitory depending upon concentration, chirality, or other factors; R = repels.

^b Chemical Class: BK = bicyclic ketal; OM = oxygenated monoterpene; NMA = non-monoterpene aromatic; HM = hydrocarbon monoterpene; PP = phenylpropanoid

^c Biological origin(s) of compound: F = female SPB; M = male SPB; YA = yeast associate of SPB galleries; H = host tree; NH = non-host plant species (note: The list gives the predominant source of the compound identified in the normal environment of SPB; hence, only one sex of SPB is listed if the opposite sex produces far smaller quantities.)

^d ¹Kinzer and others 1969, ²Payne and others 1978, ³Payne and others 1988, ⁴McCarty and others 1980, ⁵Vité and Renwick 1971, ⁶Rudinsky and others 1974, ⁷Vité and others 1985, ⁸Salom and others 1992, ⁹Sullivan and others 2007a, ¹⁰Pureswaran and others 2008a, ¹¹Renwick and Vité 1969, ¹²Renwick and Vité 1970, ¹³Rudinsky 1973, ¹⁴Sullivan and others 2007b, ¹⁵Sullivan 2005, ¹⁶Brand and others 1977, ¹⁷Hayes and others 1994, ¹⁸Strom and others 1999, ¹⁹Dickens and others 1992.

consequent minimal contribution of each individual to the total pheromone plume from mass-attacked trees, genotypes that produce very small amounts of pheromone may be able to persist despite the obvious fitness benefits conferred to individuals in the population by the total pheromone plume.

Southern pine beetle-produced semiochemicals fall into three chemical classes: 1. bicyclic ketals, 2. oxygenated monoterpenes, and 3. non-monoterpene aromatics. Whereas many—perhaps most—of these compounds are probably true pheromones for the SPB (i.e., compounds produced specifically for the purpose of influencing behavior of conspecifics), tests have not been performed that unequivocally prove this for any. Most studies on the behavioral activity of SPB-produced compounds have not determined whether the trial dose is one that occurs in nature, and thus, whether an active dose represents a normal or a supernormal stimulus. It is doubtful that every one of the 14 behaviorally active compounds isolated from the SPB plays a role in intraspecific communication; some may be ancestral pheromone remnants or function for the SPB primarily or solely as interspecific signals (i.e., as kairomones). Bark beetles excrete volatile metabolic waste products along with true pheromones, and some of these waste products are common to a wide range of organisms in the normal environment of the SPB (Gries and others 1990, Hunt and others 1989).

Bicyclic ketals

These compact, eight- and nine-carbon molecules occur commonly in the genus *Dendroctonus* and some other coniferophagous and hardwood-infesting species of bark beetles. They are synthesized *de novo* by means of the mevalonate pathway and, unlike many common bark beetle pheromones, are not derived from any known host-derived precursors (Seybold and Tittiger 2003). Although bicyclic ketals have been isolated from the tissues of some hardwood trees (Huber and others 1999), in the normal environment of the SPB these compounds apparently have no origins other than conspecific and occasionally heterospecific bark beetles, and thus they presumably are relatively free of signal interference from alternative background sources.

Frontalin (1,5-dimethyl-6,8-dioxybicyclo [3.2.1] octane). This eight-carbon bicyclic ketal is produced predominantly or exclusively by females, and is the only bicyclic ketal produced

in significant quantities by this sex (Renwick and Vité 1968). Small quantities have sometimes been reported from males (Grosman and others 1997, Rudinsky and others 1974). Newly emerged females contain small quantities of this compound in their hindgut, which largely disappear once they begin feeding in host tissue (Coster and Vité 1972). However, aerations and combined aerations/extractions made from actively feeding or fed beetles indicate that mining into a host actually increases production and release of frontalin as much as 10 fold (Pureswaran and others 2008b). The hindguts of feeding females likely contain lower quantities than newly emerged individuals because the former are defecating (and thus emptying their hindgut of pheromones) with much greater frequency. Whereas feeding appears to enhance production of frontalin in the SPB, application of juvenile hormone (a stimulant of bicyclic ketal production in females of some *Dendroctonus* species) appears to have no effect on this compound (Bridges 1982). Female production of frontalin declines somewhat following pairing (Hofstetter and others unpublished data, Sullivan and others 2007b).

Frontalin is considered the major component of the aggregation pheromone of the SPB because it is the only semiochemical capable of attracting flying beetles in the absence of other semiochemicals, and no combination of other semiochemicals is attractive in its absence (Smith and others 1993). Nonetheless, frontalin is merely weakly attractive to flying SPBs in the absence of beetle- and host-produced synergists (Kinzer and others 1969, Payne and others 1978). Traps baited with frontalin either alone or with host odors typically catch more males than females (often two to four times more). The skewed sex ratios may be due to differential attraction of the sexes, but also may be an artifact of trapping procedures. Female SPBs do not orient as directly to sources of frontalin as do males but preferentially land some distance away (Hughes 1976), and large trapping surfaces catch the sexes in ratios that approach 50:50. Thus the sexes may be attracted by frontalin in similar numbers, but traps of a size convenient for research studies (i.e., <1 m long) may be more efficient at trapping males than females. The tendency for females to land some distance from sources of frontalin likely propels the expansion of mass attack both along the bole of each individual host (Coster and others 1977a, Fargo and others 1978) and onto adjacent trees (Hughes 1976, Vité 1970). In

walking bioassays, frontalin appears to be much less attractive to females than males (McCarty and others 1980), suggesting that, for beetles walking on the bark surface, this compound may function primarily as a sex pheromone that attracts males to entrances established by solitary females.

Both newly emerged and feeding SPB produce frontalin in an enantiomeric composition of 85-95 percent (-) (Stewart and others 1977, Sullivan and others 2007b). Olfactory sensilla of both sexes of SPB are substantially more sensitive to the (-) than the (+) enantiomer of frontalin (Sullivan and others 2007b). Both the (+) and (-) enantiomers of frontalin are attractive to SPB, but the (-)-enantiomer is significantly more active, particularly with flying beetles (Payne and others 1982). The SPB is not more strongly attracted to the beetle-produced enantiomeric ratio than to a racemic mixture of the enantiomers. Modification of the structure of frontalin by either deleting or altering the position of methyl groups reduced antennal and behavioral responses by the beetles, and only one of seven such analogs (*endo*-5,7-dimethyl-6,8—dioxabicyclo [3.2.1] octane) was significantly attractive to the SPB in trapping trials (Payne and others 1988, Renwick 1970). Opening of frontalin's ring structure likewise eliminated its behavioral activity in the field (Renwick 1970).

Frontalin apparently plays a major role in mediating mass attack, since this behavior can be stimulated on uninfested trees merely by attaching frontalin baits to or near them. Frontalin appears to define the locus of mass attack more precisely than other attraction-mediating semiochemicals for the SPB, since dislocation of frontalin baits from a trap appears to effect a far greater reduction in catch than dislocation of frontalin's synergists (Sullivan and Mori 2009). However, when released at 1 g/h, frontalin stimulated a similar number of landings at poles from which it was released as unbaited poles located 10 m downwind (Vité 1970), suggesting that at high doses frontalin may release landing responses to visual (or other) cues but not stimulate orientation toward its point of origin (Hughes 1976).

***endo*-Brevicomin** (*endo*-7-ethyl-5-methyl-6,8-dioxibicyclo [3.2.1] octane). This nine-carbon molecule is produced almost exclusively by male beetles and is the major bicyclic ketal produced by this sex (Hughes 1973, Rudinsky and others 1974, Vité and Renwick 1971). In

the Southeastern United States it is present in newly emerged SPBs in small amounts, but pairing with a female in a host increases the quantities isolated from males nearly 10 fold (Sullivan and others 2007b). This increase in production is apparently stimulated by pairing rather than entrance into a host, since solitary males forced to attack pine bolts do not produce *endo*-brevicomin in significantly greater quantities than newly emerged males. Exposure of callow adult males to juvenile hormone II or the juvenile hormone analog methoprene likewise resulted in increased production of *endo*-brevicomin (Bridges 1982). In contrast to populations in the Southeastern United States, newly emerged male SPB in Mexico and Central America contain relatively large quantities of *endo*-brevicomin (Vité and others 1974). Although conflicting reports exist (Grosman and others 1997, Redlich and others 1987, Sullivan and others 2007b), it appears that the SPB produces predominantly or solely the (+)-enantiomer (see Sullivan and others 2007b for detailed explanation).

GC-EAD studies suggest that the SPB antennae have a lower response threshold to *endo*-brevicomin than to any other volatile compound isolated from this species or its host (Sullivan 2005, Sullivan and others 2007b). Both sexes can sense (+)-*endo*-brevicomin at concentrations less than 1 ng/l air, or approximately one order of magnitude less than their response threshold to frontalin's more active (-)-enantiomer, and four orders of magnitude lower than to (-)-*endo*-brevicomin. In EAG habituation studies, *endo*-brevicomin ranked second to frontalin in the percentage of olfactory acceptors that could be occupied by it (approximately 75-87 percent) (Dickens and Payne 1977). The sexes do not differ in their relative sensitivity to the enantiomers.

The SPB's exceptional olfactory sensitivity to (+)-*endo*-brevicomin suggests that this compound has major ecological importance for this species. Nonetheless, despite much research, the precise behavioral activity and ecological function of *endo*-brevicomin is unclear and a subject of ongoing investigation. Releasers of racemic *endo*-brevicomin can inhibit SPB responses to attractant-baited traps (Payne and others 1978, Salom and others 1992, Vité and Renwick 1971), and it stimulates walking males to produce their rivalry chirp while inhibiting their arrestment by female odors (Rudinsky and others 1974). Consequently, *endo*-brevicomin was initially

concluded to be an antiaggregation pheromone for the SPB and a short-range indicator of male presence. However, subsequent studies showed that pure (+)-*endo*-brevicomin baits can strongly (i.e., 5-45 fold) enhance SPB responses to traps baited with frontalin and host odors, while under the same conditions the (-)-enantiomer is inhibitory (Vité and others 1985, Sullivan and others 2007b). Low doses of the racemate also appeared to have some capacity to enhance responses to female-produced attractant. These data led to the hypothesis that the inhibitory activity of synthetic racemic mixtures of *endo*-brevicomin enantiomers was due to the inhibitory (-)-enantiomer overriding the synergistic activity of the (+)-enantiomer (Vité and others 1985). Since the SPB produces little or none of the (-)-enantiomer (Sullivan and others 2007b), this hypothesis also implied that *endo*-brevicomin functions as a synergistic component of the SPB aggregation pheromone.

In apparent conflict with this hypothesis, recent studies have shown that releasers of (+)-*endo*-brevicomin may nonetheless cause flying SPB responses to collocated attractant baits to be less than responses to identical attractant baits located several meters away (Sullivan and Mori 2009), a result that might be interpreted as demonstrating inhibitory activity for (+)-*endo*-brevicomin. However, this effect was shown to be due to enhanced attractiveness of the distant bait rather than reduced attractiveness of the collocated bait. Apparently (+)-*endo*-brevicomin can create a “halo of synergism” that enhances responses of flying SPBs to surrounding point-sources of female-produced attractant (in experiments, frontalin and host odors) more than to attractant sources located at the point of its release. The precise size and shape of the halo, which can be dozens of meters in diameter, appears to be influenced by the release rate of (+)-*endo*-brevicomin, as well as other variables. Presumably, one effect of SPB-produced (+)-*endo*-brevicomin in nature is to render attacks by solitary females relatively more attractive than attacks by pairs. In SPB spots, this activity might promote movement of the focus of mass attack from completely colonized trees (i.e., ones with many established pairs and thus a high density of (+)-*endo*-brevicomin-producing males) to adjacent trees being newly colonized by females. The halo of synergism produced by *endo*-brevicomin may also help define the spatial boundaries of an active SPB spot, since pioneer female attacks

on uninfested trees will be more attractive, and colonization presumably more likely to be successful, if they occur within the synergistic zone associated with the (+)-*endo*-brevicomin released from previously colonized trees.

***exo*-Brevicomin (exo-7-ethyl-5-methyl-6,8-dioxybicyclo [3.2.1] octane).** This nine-carbon bicyclic ketal occurs in trace quantities (0.2 ng/beetle) in emerged male SPBs in Mississippi but was detected in somewhat higher concentrations (3 ng/beetle) in Arizona populations (Pureswaran and others 2008a). This compound has been reported to significantly inhibit (Vité and Renwick 1971), enhance (Pureswaran and others 2008a), or not effect SPB responses to baits composed of frontalin and host volatiles (Payne and others 1978). The reason for this variability of SPB responses is unknown. The SPB possesses a high degree of olfactory sensitivity to *exo*-brevicomin (Pureswaran and others 2008a), and *exo*-brevicomin and frontalin apparently stimulate the same olfactory receptors (Payne 1975). However, given the very small quantities produced by the SPB, it seems unlikely that this compound is itself a pheromone for the SPB. Rather, SPB behavioral responses to *exo*-brevicomin may be an accident (e.g., resulting from an incidental capacity to stimulate olfactory receptors for other semiochemicals) or may be an adaptation to sympatric, pine bark beetle species that produce *exo*-brevicomin, including *Dendroctonus brevicomis* in Arizona and *Dendroctonus terebrans* in the Southeastern United States (Payne and others 1987, Vité and Pitman 1969a). The SPB may utilize *exo*-brevicomin to identify and exploit host resources colonized by such potentially competing species.

Manipulation of host colonization behavior with bicyclic ketals

Deployment of competing, artificial sources of insect attractant has often been a successful strategy for disrupting orientation by insect pests, particularly mate-seeking moths, thereby reducing their damage significantly (Bartell 2008, Shorey 1970). In an attempt to disrupt orientation by the SPB, rice seed soaked with a mixture of frontalin and *alpha*-pinene was spread by aircraft over an active SPB infestation at a rate of 45 g and 450 g semiochemical/ha (Vité and others 1976). Although this treatment caused captures in attractant-baited traps to decrease, it simultaneously caused landings and attacks on pines already under attack in

the treatment area to increase substantially, suggesting that saturation of the atmosphere with aggregation pheromone does not cause SPBs within spots to become disoriented. It is possible that the beetles oriented to synergistic aggregation semiochemicals (e.g., odors of fresh resin) released by the attacked pines but not provided by the semiochemical saturation treatment or the traps. Furthermore, visual cues may become the predominant host location cues for the SPB under conditions of aggregation pheromone saturation (Gara and others 1965).

Baits of frontalinal and *alpha*-pinene deployed within the interior of active SPB infestations (i.e., attached to pines with developing brood stages and nonhost trees) can reduce aggregation on newly infested trees at the infestation's advancing head and thereby reduce or stop infestation growth (Richerson and others 1980). However, this technique appears to be ineffective when beetle densities are very high (Payne and others 1985). Attempts to manipulate SPB flight activity with point sources of attractive semiochemicals have been greatly complicated by the fact that even modest release rates can stimulate mass attacks on nearby host trees, and high release rates can stimulate attacks on trees as far as 40 m distant (Vité 1970). The tendency for synthetic SPB attractants to stimulate such spillover attacks is likely the most significant technical obstacle to using baited traps to reduce SPB populations.

Regular distribution of low-rate releasers of *exo*-/*endo*-brevicomin in an area encompassing the front edge of an advancing SPB infestation significantly reduced landing of the SPB on host trees, but did not alter the densities of beetles flying through the stand (Payne and others 1977). In a separate study, releasers of either a 50:50 or 85:15 mixture of *endo*:*exo* brevicomin were evenly distributed over the bole of trees located within the path of an advancing SPB infestation. Although this treatment reduced SPB landings and prevented SPB mass attack of the treated trees, it failed to prevent their ultimate mortality, possibly due to greatly increased attacks by the associate *Ips avulsus* (Richerson and Payne 1979). While brevicomin failed as a protectant for individual trees, brevicomin-induced competitive displacement of the SPB by *I. avulsus* was proposed by these authors as a possible tool for inducing spot collapse (Payne and Richerson 1985).

Oxygenated monoterpenes

In common with other coniferophagous bark beetles, the SPB produce a diversity of cyclic ten-carbon unsaturated alcohols, aldehydes, and ketones. These generally share the same carbon backbone as one of the major resin monoterpenes of the host pines, and beetles derive them at least in part from the oxidative detoxification of resin compounds through the action of cytochrome P-450 enzymes (Seybold and others 2006). Exposure of the SPB to high atmospheric concentrations of host volatiles results in accumulation of elevated quantities of oxygenated monoterpenes in the hindgut (Hughes 1973, Renwick and others 1973). Individually these compounds may possess little potential message specificity as semiochemicals, since they have numerous alternative sources in the environment of the SPB. The capacity to oxidize hydrocarbon monoterpenes enzymatically is possessed by a diversity of microorganisms including symbionts of the SPB and other bark beetles (Bhattacharyya and others 1960, Brand and others 1975, Dhavilkar and others 1974, Leufven 1991). Many of the oxygenated monoterpenes produced by the SPB are likewise produced by sympatric species of *Ips* and *Dendroctonus* bark beetles (Skillen and others 1997), or can be generated spontaneously during the exposure of resin monoterpenes to air (Hunt and others 1989).

The hindguts of newly emerged and reemerged SPBs contain very large quantities of oxygenated monoterpenes; they may compose more than 1 percent of beetle body weight and are visible as oil droplets within the dissected tissue (Vité and Crozier 1968, author's unpublished data). Adult emerged beetles apparently emit these compounds continuously, and enclosures containing large numbers of SPBs possess a strong odor of two major hindgut constituents, verbenol and verbenone (author's personal observations). The beetles' hindguts are largely depleted of oxygenated monoterpenes within a day after they enter a host, suggesting that these compounds are released rapidly while attacking beetles are either still on the bark surface or are initiating galleries (Coster and Vité 1972; Pureswaran and others 2006, 2008b; Sullivan 2005). The SPB continues to generate and release oxygenated monoterpenes once they enter a host and begin feeding; however, the extent of this likely depends upon the concentrations of host monoterpenes to which the beetles are exposed while mining.

***trans*-Verbenol (trans-4,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-ol).** *trans*-Verbenol is present in very large quantities in the hindguts of newly emerged female SPBs (approximately 2-10 µg), but it occurs in 1-2 orders of magnitude smaller quantities in males (Grosman and others 1997; Pitman and others 1968, 1969; Pureswaran and others 2006; Renwick 1967). This monoterpene alcohol shares the same carbon backbone as *alpha*-pinene, the predominant resin monoterpene of the major host pines for the SPB (Coyne and Keith 1972). The southern pine beetle presumably can generate this compound by direct oxidation of *alpha*-pinene derived from its host, since exposure of females to high atmospheric concentrations of *alpha*-pinene results in elevated concentrations of *trans*-verbenol in the hindgut (Hughes 1975, Renwick and Hughes 1975, Renwick and others 1973). The mean enantiomeric composition produced by the SPB has been reported to be 60:40 (+)/(-) (Plummer and others 1976) and 25:75 (+)/(-) (Grosman and others 1997); however, these differences could have resulted from examination of beetles reared on species or populations of pines with differing enantiomeric ratios of the *alpha*-pinene precursor (Byers 1983).

Dickens and Payne (1977) reported that *trans*-verbenol occupied a larger percentage of antennal acceptors in males than females. However, dose-response studies indicated a similar threshold of olfactory response to both enantiomers by both sexes of the SPB (author's unpublished data).

trans-Verbenol is an attractive synergist of frontalin for flying SPBs (Renwick and Vité 1970). It appears to be interchangeable with *alpha*-pinene in this function, since *trans*-verbenol is ineffective as a synergist when concentrations of *alpha*-pinene are also present (Payne and others 1978, Renwick and Vité 1969). Pioneer females initially arriving on a host may release *trans*-verbenol to synergize conspecific attraction to frontalin before they have penetrated the phloem and triggered release of *alpha*-pinene from the host. Turpentine rich in *alpha*-pinene, as well as purified *alpha*-pinene, is generally much less expensive than synthetic *trans*-verbenol; thus, the former have been used rather than the latter as synergists for frontalin in bait formulations for the SPB (Billings 1988). *trans*-Verbenol may also play some role during courtship and in close-range communication by beetles on the bark surface (Rudinsky 1973, Rudinsky

and others 1974). It is not known whether the enantiomeric ratio of *trans*-verbenol influences SPB behavioral responses.

Verbenone (4,6,6-trimethylbicyclo [3.1.1]hept-3-en-2-one). Verbenone is present in very large quantities in hindguts of male beetles that are either newly emerged or newly arriving on a host (approximately 0.5-10 µg/insect) but in much smaller quantities in females (5-100 ng/insect; Grosman and others 1997, Pitman and others 1969, Pureswaran and others 2006). Entrance into host tissue reduces these quantities considerably in males but has minimal effect on females (Pureswaran and others 2008b, Sullivan and others 2007b). Verbenone, like verbenol, has the carbon backbone of *alpha*-pinene, and it can be derived from verbenol by a further oxidation of the hydroxyl group (Renwick 1970). As with *trans*-verbenol, the SPB apparently can generate verbenone also through the oxidation of *alpha*-pinene derived from the host (Hughes 1975). Verbenone may also arise through conversion of beetle-produced *trans*-verbenol by the action of SPB-associated microbes (Brand and others 1976) or spontaneously by contact of either *alpha*-pinene or verbenol with the air (Hunt and others 1989). Males newly emerged from loblolly pine contain the two enantiomers in a mean ratio of 36:64 (+):(-), whereas females may contain nearly the reciprocal ratio (Grosman and others 1997).

Verbenone can influence behavior of the SPB in a variety of ways. In trapping trials, verbenone at high doses (>5 mg/h) reliably reduces response of flying male beetles to combinations of frontalin and host odors, but inhibition in flying females has proven less consistent (Payne and others 1978). As a consequence of the typically greater inhibition of males, verbenone tends to shift the sex ratio of responding beetles toward females (Renwick and Vité 1969). In the laboratory, verbenone also inhibited upwind anemotaxis by walking male and female SPBs to triplicate (a 1:1:12 mixture of frontalin, *trans*-verbenol, and *alpha*-pinene) when concentrations exceeded the concentration of attractant, but at 1/100 the attractant dose, it significantly increased frequency of female responses (McCarty and others 1980). At very low concentrations, verbenone is reported to increase the frequency with which walking males are arrested and produce an attractant chirp at a release point of female attractant, whereas concentrations 100 times the arresting dose caused males to pass the attractive source

and emit a rivalry chirp (Rudinsky 1973). Verbenone has been called a multifunctional pheromone based upon its dose-dependent, alternatively attractive/inhibitory activity with walking SPBs (McCarty and others 1980, Rudinsky 1973); however, there is minimal evidence suggesting that verbenone enhances attraction of flying SPBs at any dose (however, see Salom and others 1992, Test 2).

Salom and others (1992) examined the effect of verbenone chirality on its capacity to inhibit attraction of flying SPBs in the field. In a test in which a low dose of host odors (2 mg/hr *alpha*-pinene) was used as synergist with frontalin, (+)-enriched verbenone was more effective at inhibiting male beetles than (-)-enriched verbenone. However, in tests where a high dose of host volatiles (3600 mg/hr turpentine) was used as a synergist for frontalin and in which SPB responses to attractant-only control traps were generally 10 fold greater, (+)-enriched verbenone was equally or less inhibitory than racemic verbenone or a 34 percent(+):66 percent(-) mixture.

Verbenone's capacity to inhibit SPB attraction has inspired efforts to use this semiochemical to stop SPB mass attacks within infestations. Devices releasing a total of 80 mg verbenone/day/tree were attached at 2 m intervals along the bole of uninfested pines located in the trajectory of a growing SPB spot, but this failed to reduce SPB landing and attack density or prevent mortality of the treated trees. Multiple studies have shown that verbenone releasers placed on freshly infested and uninfested trees located along the advancing head of an SPB spot can slow or stop spot growth (addressed in detail in chapter 26). However, treatment of the spot head with verbenone does not alter the abundance or distribution of flying beetles within the spot (Johnson and Coster 1980, Salom and others 1995); hence, the treatment apparently neither prevents beetles emerging within the spot from locating and aggregating at the spot head nor causes beetle dispersal from the spot. Since verbenone also apparently does not significantly reduce SPB landing rates on or near treated trees (Richerson and Payne 1979, Salom and others 1995), the mechanism by which verbenone treatment causes infestation collapse remains a matter of speculation.

Myrtenol (4,6,6-trimethylbicyclo [3.1.1] hept-3-en-10-ol). Myrtenol is present in large quantities (0.4-2 µg/insect) in emergent and in lesser quantities (3-50 ng/insect) in attacking

male and female SPB (Pureswaran and others 2006, Renwick and others 1973, Sullivan and others 2007b). Like verbenone and *trans*-verbenol, the SPB generates myrtenol in greater quantities after exposure to the host monoterpene *alpha*-pinene (Hughes 1973, Renwick and others 1973). Both the attractant chirp and arrestment were elicited more frequently from walking male SPBs when low concentrations of myrtenol were added to an attractive blend (frontalin, *alpha*-pinene, and *trans*-verbenol), whereas at high concentrations myrtenol inhibited these responses (Rudinsky and others 1974). This led the authors to conclude that myrtenol, like verbenone, was a multifunctional pheromone for the SPB. At a release rate of 1.5-3 mg/d, myrtenol significantly reduced response of SPBs to traps baited with *alpha*-pinene and frontalin within active SPB infestations (Sullivan and others 2007a).

Other oxygenated monoterpenes. Several additional oxygenated monoterpenes have been identified from newly emerged or pine resin-exposed SPBs, including fenchyl alcohol, 6-hydroxycamphene, myrtenal, *cis*-myrtanol, *trans*-myrtanol, *cis*-3-pinen-2-ol, *trans*-pinocarveol, pinocarpone, and *cis*-verbenol (Hughes 1973; Renwick and Hughes 1975; Renwick and others 1973, 1976; Rudinsky and others 1974; Sullivan 2005; Vité and others 1974). They generally have been detected in both sexes, but with significant quantitative dimorphisms with respect to *cis*-verbenol and *trans*-pinocarveol (predominantly females) and myrtenal and pinocarpone (predominantly males) (Grosman and others 1997, Renwick and others 1973, Sullivan 2005). Antennae of both male and female SPBs are sensitive to fenchyl alcohol, myrtenal, *cis*-myrtanol, *trans*-myrtanol, *trans*-pinocarveol, and *cis*-verbenol, and all six of these compounds were found to modify SPB responses to traps baited with a combination of frontalin and *alpha*-pinene. Fenchyl alcohol, *cis*-myrtanol, and *trans*-pinocarveol significantly reduced responses of one or both sexes when released at 3-50 mg/day, whereas myrtenal and *trans*-myrtanol were inhibitory at 27 and 66 mg/day, respectively, but not at lower doses. *cis*-Verbenol baits releasing 59 mg/day but not lower doses significantly increased SPB responses to frontalin and *alpha*-pinene, but significant levels of *trans*-verbenol contamination in these baits may explain this result (Sullivan 2005). None of these oxygenated monoterpenes altered the sex ratio of SPBs responding to the standard attractant.

Non-monoterpene aromatics. 2-phenylethanol is produced in small quantities (<30 ng per insect) by both newly emerged and attacking SPBs of both sexes, although it is detected in greatest quantities in solitary, feeding females (Sullivan 2005). It likely arises as a waste product from beetle metabolism of the amino acid phenylalanine (Gries and others 1990). It is also produced by yeasts and filamentous fungal associates of SPB galleries (Brand and others 1976, Sullivan 1997). 2-Phenylethanol reduced the frequency of upwind anemotaxis by walking SPBs to a source of attractant (Brand and others 1977), and devices releasing 3-80 mg/day significantly reduced catch of SPBs in traps baited with frontalin and *alpha*-pinene (Sullivan 2005, Sullivan and others 2007a).

Acetophenone is produced in small quantities (<40 ng/insect) by both sexes and in relatively greater amounts by newly emerged males (Sullivan 2005). Devices releasing 0.5-27 mg/day significantly reduced responses of males or both sexes to traps baited with frontalin and *alpha*-pinene, and relatively higher release rates shifted the responding sex ratio significantly toward females.

Regional variation in pheromone production and response

Both production of and response to pheromones appears to vary regionally for the SPB. Grosman and others (1997) compared semiochemical content of SPBs from populations in three States and found significant differences in the quantities and enantiomeric ratios of several constituents. In addition, SPBs in a walking olfactometer appeared to prefer volatiles produced by host-attacking beetles from their own region over those produced by beetles from distant States (Berisford and others 1990).

Compounds Produced by Host and Nonhost Trees

Although evidence suggests that the SPB may utilize host-produced olfactory cues to locate uninfested, susceptible trees (Payne and Coulson 1985), to date no host-produced compound or blend has been identified that is attractive to flying SPBs in the absence of the pheromone frontalin. Nonetheless, host-produced volatiles are potent synergists for SPB aggregation pheromone components, and evidently play an important role in mediating mass attack. Raw pine resin and the monoterpene fraction distilled from resin (i.e., turpentine) synergize response by both sexes to frontalin-baited traps (Billings

1985, Kinzer and others 1969, Payne and others 1978). When monoterpene components of the resin of *Pinus taeda* were tested separately for their capacity to enhance attractiveness of a blend of frontalin and *trans*-verbenol, *alpha*-pinene had a stronger effect than *beta*-pinene, 3-carene, camphene, limonene, myrcene, terpinolene, or 4-allylanisole (Renwick and Vité 1969). Although unattractive to flying SPBs, *alpha*-pinene by itself is somewhat attractive to walking beetles (McCarty and others 1980). *alpha*-Pinene is the predominant monoterpene constituent of resin of the major host species for the SPB (Coyne and Keith 1972, Mirov 1961), and is likewise the dominant monoterpene constituent of turpentines derived from these trees. Rapid release of loblolly pine turpentine (1.8-3.6 g/day) increases catch of SPBs in traps baited with frontalin (frontalin and *alpha*-pinene in a 1:2 formulation at 50mg/day) by 15-28 times (Billings 1985).

4-Allylanisole, a minor component of the constitutive resin of SPB host pines (Mirov 1961, Drew and Pylant 1966), is the only host-produced compound reported to reduce response of the SPB to sources of attractant. Devices releasing approximately 160-1,200 mg/day significantly reduced beetle responses to traps baited with frontalin, and walking adult beetles were repelled from locations where 4-allylanisole had been painted on the substrate (Hayes and others 1994, Strom and others 1999). When released from pine boles either with a string of release devices or with a microencapsulated form sprayed onto the bark, 4-allylanisole significantly reduced SPB attack density on pines rendered susceptible by treatment with N-methyldithiocarbamate (Strom and others 2004).

Various small organic molecules, particularly six-carbon alcohols and aldehydes, are associated with angiosperms and other nonhosts for coniferophagous bark beetles, and they appear to be used by foraging bark beetles during selection of suitable host habitat and individual hosts (Zhang and Schlyter 2004). Two such compounds, hexanal and 1-hexanol, either separately or in combination, have been shown to significantly reduce SPB responses to traps baited with frontalin and turpentine (Dickens and others 1992). In addition, a blend of four nonhost volatiles (1-hexanol, hexanal, *cis*-3-hexen-1-ol, and nonanal) significantly reduced responses by the SPB to traps baited with frontalin and *alpha*-pinene in one of two trials (Sullivan and others 2007a).

Compounds Produced by Competing Bark Beetle Species

Pines infested by the SPB are usually also infested by other bark beetles in the southern pine bark beetle guild. The SPB and the other members of the guild compete with one another for the phloem resource, but reduce this competition to some extent by each colonizing separate portions of the tree bole (Flamm and others 1987b, Flamm and others 1993). Bark beetles are commonly inhibited but sometimes attracted by pheromones of competing species (Byers 1989b), and evidence suggests such interactions occur among members of the southern pine bark beetle guild (Birch and others 1980, Svihra and others 1980). Cross-attraction is believed to enhance host-finding by secondarily arriving species, whereas cross-inhibition likely reduces direct competition by promoting spatial partitioning of host resources among species (Byers 1989a). Logs infested with males of either one or two species of *Ips* belonging to the guild failed to attract SPBs, whereas male attacks by *I. grandicollis* reduced SPB responses to bolts infested with female SPBs (Birch and others 1980, Svihra and others 1980). Synthetic blends of *Ips* spp. pheromones likewise failed to attract SPBs (Billings 1985), whereas a pheromone blend characteristic of BTB attracted them in small numbers (Smith and others 1990).

Compounds Produced by SPB-Associated Microbes

A rich flora of microorganisms has adapted to life within the galleries and bodies of the SPB, and several of these associates produce SPB semiochemicals. Cultures of three SPB-associated yeasts, *Hansenula holstii* Wickerham, *Pichia pinus* (Holst) Phaff, and *P. bovis* van Uden et do Carmo-Sousa, produce at least three compounds (2-phenylethanol, isoamyl acetate, and 2-phenylethyl acetate) that alter responses by SPB to an attractant mixture in walking bioassays (Brand and others 1977). In addition, cultures of one of the fungal symbionts carried in the mycangium of female SPBs can convert the SPB-produced semiochemicals *cis*- and *trans*-verbenol into another SPB semiochemical, verbenone (Brand and others 1976). However, studies have not yet determined whether these microbes produce semiochemicals under natural conditions or whether this production significantly impacts SPB behavior.

3.4. SPB BEHAVIORS MEDIATED BY VISUAL CUES

Vision appears to play a crucial role in host identification for flying SPBs. The SPB has a presumably visually mediated preference for landing on objects with a vertical rather than a horizontally oriented profile. Pine posts baited with freshly infested log sections attracted few or no SPB attacks when suspended horizontally, but received many attacks when erected vertically (Gara and others 1965). However, black-colored barrier traps with a broadly rectangular profile were equally efficient at trapping SPBs as black multiple-funnel traps possessing a strong vertical profile and a similar trapping surface area (McCravy and others 2000). Traps consisting of plastic panels were more attractive to the SPB when colored black than when transparent, but clear panels were more attractive than white, suggesting that black surfaces are attractive and white ones repellant to the SPB (Strom and others 1999). When pines in the path of a growing SPB infestation were painted with either black or white paint to a height of 4.5 m, white-painted trees received many fewer landings and attacks within the painted portions of the bole than black-painted trees (Strom and others 1999). Fontalure-baited multiple-funnel traps painted either white or yellow caught significantly fewer SPBs than traps painted black, blue, brown, green, or red, whereas these latter five colors did not differ in attractiveness (Strom and Goyer 2001). Surface hue (dominant wavelength) appeared to be much less important to beetle responsiveness than overall reflectance across the visual spectrum, with high reflectance values being the least attractive (Strom and others 1999). Visual responses of host-seeking beetles in the field appear to be substantially different from those of beetles emerging within enclosures, as the latter are strongly attracted to sources of light (Tsao 1965).

3.5. SPB BEHAVIORS MEDIATED BY ACOUSTIC CUES

As with all other species of *Dendroctonus* so far examined, the SPB appears to utilize acoustic signals for short-distance intraspecific communication (Ryker 1988). No tympana or similar organs specialized for the sensing of airborne sound have been identified in the SPB or other bark beetles, and it has been speculated that they may only be able to sense

vibrations received through the substrate or from direct contact with other insects (Lewis and Cane 1992). Both sexes of the SPB stridulate (produce sound or vibrations by rubbing body parts against one another) using cuticular structures on the abdomen and/or the elytra. Males stridulate by moving the tip of the abdomen up and down, causing a pair of sclerotized processes on the posterior margin of the seventh abdominal tergite (the plectrum) to scrape a row of parallel, transverse ridges on the ventral surface of the elytra (the file) (Michael and Rudinsky 1972). The file of SPB males includes an average of 66 teeth spaced 2.8-4.6 μm apart. Female SPBs lack this elytral file; rather, they have a file with 16-18 teeth spaced 3.1-4.1 μm apart located on the posterior margin of the last abdominal sternite. The tip of the final abdominal tergite (the pygidium) acts as the plectrum in females (Rudinsky and Michael 1973). The chirps produced by SPB stridulation are only faintly detectable by the unaided human ear.

Male SPB entering the gallery of a solitary female produce a distinctive attractant chirp that presumably serves as a courtship signal for inducing mating receptivity in the female (Figure 3.2C). Male SPBs likewise produce this sound when encountering female frass or blends of female semiochemicals at artificial entrances (Rudinsky 1973). Male-male encounters elicit a rivalry chirp that is significantly shorter in duration and has a faster rate of toothstrikes than the attractant chirp (Figure 3.2B). The rivalry chirp is likewise produced by males either encountering a blend of male- and female-produced semiochemicals or when confined with other males within an enclosure (Rudinsky and Michael 1974, Rudinsky and

others 1974). Males also stridulate vigorously when handled, producing distinctive stress chirps (Ryker 1988). Gallery-initiating females stridulate as an apparent courtship signal to a male that enters her gallery (Figure 3.2A); in addition, female stridulation may mediate spacing of female attacks and galleries within the bark (Rudinsky and Michael 1973, Grosman and others 1992).

3.6. CONCLUSIONS

The past 50 years have generated much information on the basic biology and behavior of the SPB, particularly regarding the processes of host selection and colonization during outbreaks. Most studies have examined beetles within active spots, whereas much less research has been performed on dispersed or dispersing populations. The reason for this bias is obvious: the bulk of SPB-inflicted tree mortality occurs within spots, and spot suppression remains the core technique used for managing outbreaks. In addition, research within spots typically involves many fewer practical obstacles than studies of dispersed populations. Spots are relatively easy to locate in the forest and typically persist at a single location for many months. However, development of management techniques for preventing SPB outbreaks will likely require major improvements in our understanding of behaviors that allow the SPB to persist in the landscape at suboutbreak population levels.