Electrophysiological and Behavioral Responses of *Dendroctonus frontalis* (Coleoptera: Curculionidae) to Volatiles Isolated from Conspecifics

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ABSTRACT Olfactory sensitivity of the southern pine beetle, *Dendroctonus frontalis* Zimmermann, to compounds isolated from the mid/hindguts of newly emerged conspecific adults was assayed with coupled gas chromatography-electroantennographic detection. All previously reported pheromones for *D. frontalis* plus eight additional compounds (fenchyl alcohol, myrtenal, *cis*-verbenol, *trans*-pinocarveol, acetophenone, *trans*-myrtanol, *cis*-myrtanol, and 2-phenylethanol) consistently elicited antennal responses from at least one sex. The eight additional compounds were assayed individually at three release rates (0.4–0.8, 3–9, and 25–100 mg/d) for the ability to alter *D. frontalis* responses to traps baited with *D. frontalis* attractant (4 mg/d frontalin and 17 mg/d α-pinene). At the high release rate, *cis*-verbenol enhanced attraction of *D. frontalis* females, whereas the other seven compounds significantly reduced attraction of one or both sexes. Acetophenone significantly reduced attraction of male *D. frontalis* at the low release rate, and five compounds (fenchyl alcohol, *trans*-pinocarveol, acetophenone, *cis*-myrtanol, and 2-phenylethanol) reduced attraction of one or both sexes at the intermediate rate. Only acetophenone significantly altered the sex ratio of beetles trapped, decreasing the proportion of males. Attraction of predatory checkered beetles (Cleridae) was enhanced by *cis*-verbenol released at the high rate but was not altered by any compound inhibitory to *D. frontalis*. Analyses of volatiles from individual *D. frontalis* indicated that the majority of the eight compounds were produced in greater quantities by newly emerged beetles than ones attacking pine bolts. Five of the compounds were associated predominantly with one sex. Possible ecological roles of these compounds in the biology of *D. frontalis* are discussed.

KEY WORDS Scolytinae, semiochemical, pheromone, GC-EAD, repellent

BARK BEETLES (Coleoptera: Curculionidae: Scolytinae) can reproduce in healthy, vigorous trees only when they attack in adequate numbers to overwhelm tree resin defenses (Berryman 1972, Paine et al. 1997). Aggressive species rely on pheromones to initiate, focus, and synchronize mass attacks that ultimately result in successful reproduction on resistant hosts (Wood 1982a). Hence, host resources that would otherwise not be susceptible are accessible to bark beetles by virtue of their semiochemical communications systems. "Aggregation pheromones" produced by one or both sexes attract conspecifics of both sexes to a tree during the initial stages of colonization (Birch 1978, Byers 1989b). In addition, some bark beetles (particularly *Dendroctonus* spp.) produce compounds that inhibit attraction of conspecifics to aggregation pheromones (Borden 1982, Skillen et al. 1997). These "antiaggregation pheromones" terminate aggregation on a tree once its constitutive defenses are overcome and attack densities have reached or exceeded the threshold for optimal beetle reproduction (Byers 1989a). The essential role that pheromones play in the biology and destructive potential of bark beetles has inspired extensive investigation of semiochemicals as tools for managing these pests, and a few noteworthy successes have been reported (Borden 1995, Skillen et al. 1997). Particular attention has been focused on developing antiaggregation pheromones and other attraction inhibitors as a means for preventing beetle attacks on individual trees or containing expanding outbreaks (Borden 1996).

The southern pine beetle, *Dendroctonus frontalis* Zimmermann, is the most serious economic pest of conifers in the southern United States and portions of Mexico and Central America (Price et al. 1998, Billings et al. 2004). Outbreaks of *D. frontalis* result in continuous patches of infested, dying, and dead trees that can expand to cover hundreds of acres if uncontrolled (Thatcher 1960, Payne 1980, Clarke and Billings 2003). Landing female beetles release the aggregation pheromone frontalin and the synergist *trans*-verbenol, which, in combination with the host monoterpene α-pinene, induce mass attack (Renwick and Vité 1969, Smith et al. 1993). Arriving males produce the multifunction pheromones verbenone and *endo*-brevicomin, which in sufficient concentrations can inhibit

attraction to frontalin and its synergists (Vité and Renwick 1971, Rudinsky 1973, Payne et al. 1978a). The potential for artificial releasers of verbenone and *endo*-brevicomin to halt infestation growth was explored extensively from the late 1970s through the 1990s (Payne et al. 1977, 1992; Richerson and Payne 1979; Payne and Billings 1989), and successes with verbenone in particular led to registration of this compound by the Environmental Protection Agency in 1999 as a biorational insecticide for management of *D. frontalis* (Clarke et al. 1999). In addition, a compound produced by host trees, 4-allylanisole, also was found to have repellant activity with *D. frontalis* and was subsequently registered (Hayes et al. 1994, 1996, Strom et al. 1995). However, treatments involving verbenone and 4-allylanisole can be prohibitively expensive because high rates of release are required to inhibit beetle aggregation (Salom et al. 1992, Hayes et al. 1994), and the compounds are costly to produce. Application of these treatments is therefore restricted to situations where exceptionally high value trees are at risk or where cutting and removal of infested trees is not possible (Hayes et al. 1996, Clarke et al. 1999, Strom et al. 2004). In addition, conflicting data exist on the efficacy of 4-allylanisole (Strom et al. 2004), and verbenone is recommended for use only in *D. frontalis* infestations of limited size (Clarke et al. 1999). The potential for semiochemical-based management of *D. frontalis* would be enhanced by antiaggregation semiochemicals with lower cost and greater activity than those currently registered as biorational insecticides for this pest.

Recently, researchers have productively used coupled gas chromatography-electroantennographic detection (GC-EAD) to reexamine the semiochemical systems of some bark beetle species whose chemical ecology had been studied exhaustively with other methods (Pureswaran et al. 2000, 2004). GC-EAD analyses of beetle-produced volatiles revealed previously unknown olfactory stimulants for conspecifics (Pureswaran et al. 2000, 2004), and some of these proved to be potent inhibitors of bark beetle attraction (Pureswaran and Borden 2004). This article describes similar studies with *D. frontalis*, and it reports results of GC-EAD analyses and field bioassays designed to screen *D. frontalis*-produced volatile compounds for previously undescribed semiochemicals.

Materials and Methods

Insects. Bolts of loblolly pine, *Pinus taeda* L., were cut from trees naturally infested with *D. frontalis* in the Bienville, Chickasawhay, and Tombigbee National Forests in Mississippi. Emerging adults were collected in a rearing enclosure (Browne 1972) and maintained at 8°C on moistened paper towel for up to 3 d before use. Beetles were sexed by the mycangial bulge on the female pronotum and the deep median groove on the male frons (Wood 1982b).

Electrophysiological Studies. Responsiveness of *D. frontalis* olfactory receptors to volatile compounds present in the alimentary tract of newly emerged adult beetles was assayed with GC-EAD. Apparatus and general procedures are described in Asaro et al. (2004). For each assay, a glass pipette Ag/AgCl reference electrode (containing Beadle-Ephrussi saline and 0.5% polyvinylpyrrolidone) was inserted into the foramen of a beetle's excised head. The tip of a similar recording electrode was cut to match the diameter of the antennal club, and the club was laid flat against the electrode opening so that one entire side made contact with the saline. The club's opposite side was positioned in an airstream receiving effluent from the GC. The hindgut and posterior midgut of 100 newly emerged male and female beetles were each extracted into 1.5 ml of pentane (Byers and Wood 1980), and the extract was subsequently concentrated under nitrogen to 0.5 ml. One microliter was injected splitless into the GC-EAD, hence ≈0.1 beetle equivalents were delivered to each antennal preparation [a 1:1 split was maintained between the antennal preparation and a flame ionization detector (FID)]. The GC-EAD column was an HP-INNOWax (60 m by 0.25 mm by 0.25 μm film; Agilent Technologies, Wilmington, DE), and the oven program was 40°C for 1 min, 16°C/min to 80°C, then 7°C/min to 230°C for a final 10 min. Male and female antennal preparations were exposed to GC-analyzed hindgut extracts of both sexes (14–17 replications of each combination). Spikes in the EAD trace were classified as antennal responses only if they occurred at the same retention time in at least four runs. Identifications of GC peaks coinciding with EAD responses were made with an Agilent 6890–5973 coupled gas chromatograph-mass spectral detector (GC-MS) operating with the same column and operating parameters as the GC-EAD. Identifications were confirmed by retention time matches with known standards and by GC-EAD analyses of these standards with *D. frontalis* antennae.

Trapping Bioassays. Eight hindgut compounds that elicited antennal responses were assayed separately for their ability to alter responses of *D. frontalis* to traps baited with an attractant mixture. Randomized complete blocks of Lindgren 12-unit multiple-funnel traps were erected inside active *D. frontalis* infestations within mixed *P. taeda*/*Pinus elliottii* Engelm. forests of the Chickasawhay National Forest in southeastern Mississippi. Traps were positioned within portions of infestations occupied by trees with predominantly larval *D. frontalis* brood (Payne et al. 1978b). Traps were suspended from metal standards ≈1 m above the ground and spaced >5 m apart, >1 m from the nearest pine, and >10 m from any trap in an adjacent block. Trap collection cups were filled with propylene glycol and water (1:3) to preserve insects. Bait composition, release rate, and release device construction are presented in Table 1. In general, neat test compounds were released from heat-sealed packets of polyethylene sheeting of varying thickness and density. Release rates were controlled by adjusting packet dimensions (between 1 by 0. 4 cm and 8 by 5 cm) and number per trap (1–2). Release rates were measured by suspending baits in a fume hood (median 22°C) and measuring weight loss over 1 wk. Test compounds that

Table 1. Baits used in trapping tests of electrophysiologically active compounds identified from mid/hindguts of newly emerged *D. frontalis* males and females

Chemical name	Source	Purity (%)[a]	Chirality (%)[b]	Release device[c]	Release rate (mean mg/d ± SD)[d]		
					Low	Medium	High
Standard attractant							
Frontalin	Phero Tech Inc.	>99	Racemic	PE tube	3.7 ± 0.2		
α-Pinene	Aldrich, Milwaukee, WI	97	52 (−)	PE tube	17 ± 1		
Test compound							
Fenchyl alcohol[e]	Aldrich	>99	94 (+)	PE pouch	0.6 ± 0.3	4.9 ± 1.7	34 ± 2
Myrtenal	Aldrich	98	97 (−)	PE pouch	0.6 ± 0.1	6.9 ± 0.4	66 ± 3
cis-Verbenol[e]	Aldrich	98	91 (S)	PE pouch	0.5 ± 0.1	4.0 ± 0.1	59 ± 3
trans-Pinocarveol	Fluka, Buchs, Germany	>99	98 (−)	PE pouch	0.8 ± 0.1	5.8 ± 0.4	50 ± 5
Acetophenone	Aldrich	>99	Nonchiral	PE pouch	0.5 ± 0.1	8.5 ± 0.5	96 ± 12
trans-Myrtanol	Fluka	98	98 (−)	PE pouch	0.5 ± 0.1	3.1 ± 0.1	27 ± 1
cis-Myrtanol	Fluka	99	95 (−)	PE pouch	0.4 ± 0.1	3.6 ± 0.6	29 ± 2
2-Phenylethanol	Fluka	>99	Nonchiral	PE pouch	0.8 ± 0.2	7.6 ± 2.0	79 ± 33

[a] Measured by GC.
[b] Determined by analysis on a chiral GC column.
[c] PE tube was a 400-μl capacity polyethylene microcentrifuge tube; PE pouch was a heat-sealed polyethylene bag. Bag thickness and dimensions determined release rate.
[d] Measured gravimetrically in a fume hood at room temperature (mean ≈22°C).
[e] Fenchyl alcohol and *cis*-verbenol were dissolved in isopropyl myristate (Sigma, St. Louis, MO) in 1:1 and 1:6 ratios, respectively.

were solids at room temperature were first dissolved in isopropyl myristate. In pilot trials, release devices filled with isopropyl myristate alone did not significantly alter *D. frontalis* response to attractant-baited traps; hence, this solvent was presumed to be behaviorally neutral. Baits were suspended adjacent to one another outside the fourth funnel above the collection cup. Twelve to 14 blocks (lasting 3–7 d each) were executed for each test compound between 10 June and 27 August 2004. The five bait treatments (one trap each per block) were 1) an unbaited control, 2) a known *D. frontalis* attractant (the female-produced aggregation pheromone frontalin plus the synergistic host monoterpene α-pinene), and (3–5) the attractant plus the test compound released at one of three rates: approximately the same rate as frontalin in the attractant ("medium rate") or an order of magnitude slower ("low rate") or faster ("high rate"). Catches of *D. frontalis* and predators in the family Cleridae were quantified.

Volatiles Analyses of Individual Beetles. Production of the eight bioassayed hindgut compounds was quantified in both male and female *D. frontalis* that were either 1) newly emerged, 2) feeding singly in a pine bolt <1 d, or 3) paired in a pine bolt <1 d. Pine bolts (50–70 cm in length, 15–25 cm in diameter) were cut from healthy *P. taeda* felled in the previous 5 d. To initiate attacks, single beetles were confined inside half of a #00 gelatin capsule secured over a hole drilled through the outer bark with a 1-mm diameter bit. Only beetles that entered drill holes and expelled frass within 1 d after introduction were used for treatments 2 and 3. For treatment 3, females were confined onto the bark 1 d before introduction of males to permit construction of a nuptial gallery. Introductions were spaced >10 cm apart on the bark surface, and solitary male and female attacks were induced on separate bolts. Beetles in treatments 2 and 3 were excised from the bark and maintained on moist filter paper at 1–4°C up to 4 h before placement into static headspace sampling enclosures (described below).

Individual beetles were inserted abdomen first into vertically oriented 100-μl conical vials with a 2 mm-depth (≈0.3 mg) of clean Super Q adsorbent (80–100 mesh; Alltech, Deerfield, IL) in their tip (Fig. 1). The beetles were confined to the bottom 6–8 mm of the vials by a semicircle of 2 mm o.d. PFA tubing secured into the aperture of each vial. The vial mouths were

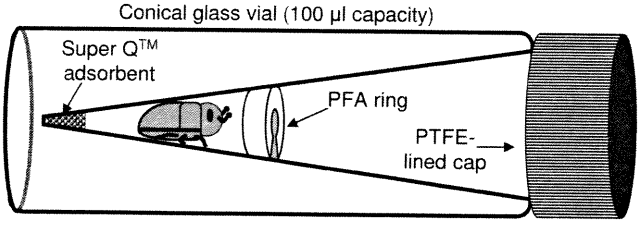

Fig. 1. Enclosure for collecting volatiles from individual *D. frontalis* adults. Volatiles released by the beetle were adsorbed on ≈0.3 mg of Super Q in the tip of a conical 100-μl glass vial. A PFA ring secured the beetle in the tip of the vial, and the vial was capped loosely to concentrate odors within the vial while allowing limited gas exchange. Volatiles were collected for 18 ± 2 h, and then the beetle's excised gut and the adsorbent were extracted together in pentane spiked with an internal standard.

closed with a PTFE-lined cap that was threaded but not tightened to allow minimal air exchange to occur. The volatiles released from the beetles were passively collected on the adsorbent at room temperature during the next 16–20 h, while the capped vials were maintained in a stream of purified, humidified air. The beetle and PFA retainer were then removed from each vial, and 50 μl of redistilled pentane spiked with 180 ng of heptyl acetate was added. The beetle's hindgut was excised, placed into the pentane, and macerated against the inside vial wall with the tip of the forceps. The combined hindgut and adsorbent were then allowed to extract passively for at least 15 min at room temperature in the sealed vial. The extract was removed, and the vial contents were rinsed with a further 50 μl of nonspiked pentane that was subsequently combined with the original extract.

Two microliters of extract were analyzed splitless by GC-MS with both nonchiral phase (HP-INNOWax; 60 m by 0.25 mm by 0.25-μm film) and chiral phase (BetaDex-120 and/or GammaDex-225; 30 m by 0.25 mm by 0.25-μm film; Supelco, Bellefonte, PA) GC columns. For nonchiral GC-MS runs, instrument conditions were same as GC-EAD runs above; for chiral GC-MS runs, the oven program was 40°C for 1 min, 5°C/min to 70°C, 2°C/min to 155°C, and then 25°C/min to 220°C for a final 7 min. Analytes were quantified using response curves calculated from analyses of a dilution sequence of known quantities of synthetic standards. These analyses were replicated on 31–34 individual beetles of each sex and treatment. As a check, analyses were performed on extracts of adsorbent from vial preparations that lacked beetles, and none of the eight target compounds were found.

Statistical Analysis. Raw trap catch numbers were transformed by $\log(X + 1)$ and analyzed with a two-way analysis of variance (ANOVA) by using block and treatment as the two factors (SigmaStat 3.0, SPSS Inc. 1997). All pairwise comparisons of treatments were performed with Tukey's test ($\alpha = 0.05$). Unbaited traps were omitted from statistical analyses (Reeve and Strom 2004). Sex ratios for catch totals in treatments with test compound were compared against catch totals with attractant alone using a χ^2 test and an α level of 0.016 (i.e., experiment-wise error of $\alpha = 0.05$ spread among three comparisons). Quantities of compounds collected from individual beetles were analyzed by a Kruskal–Wallis one-way ANOVA on ranks

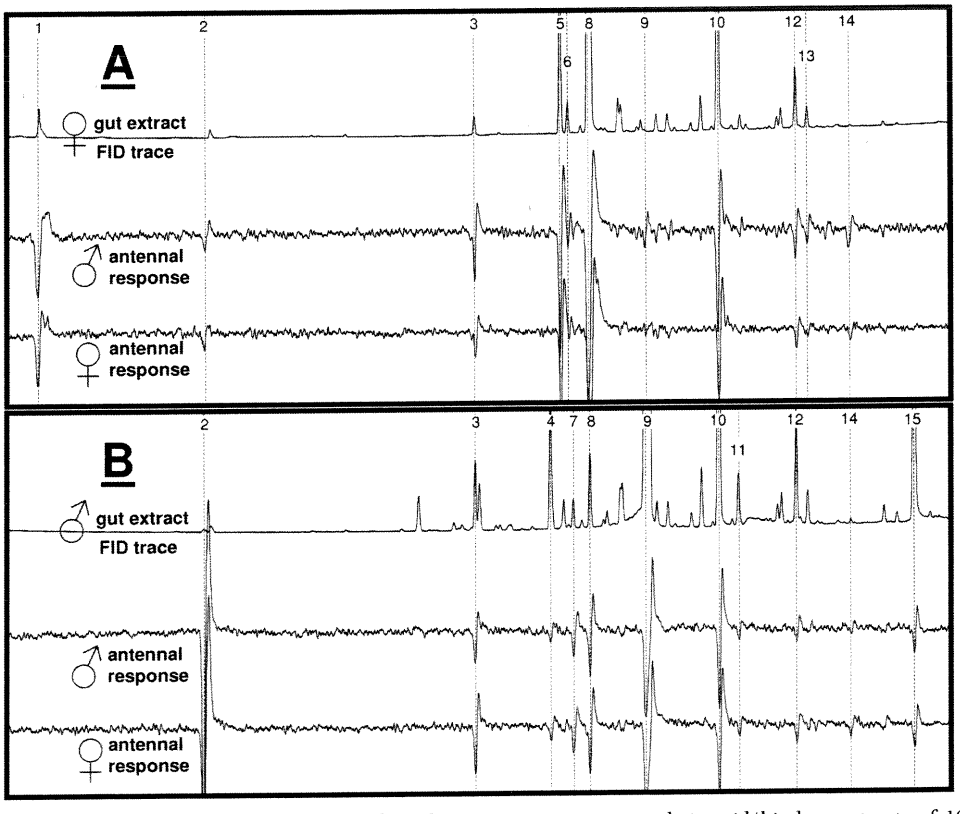

Fig. 2. Electrophysiological responses of *D. frontalis* antennae to compounds in mid/hindgut extracts of 100 newly emerged female (A) or male (B) conspecifics as measured by GC-EAD. Antennal traces represent the combined responses from 14 to 17 individual insects (single EAD traces were digitized and summed in a spreadsheet to produce a composite trace). Compounds eliciting consistent antennal voltage spikes were frontalin (1), *endo*-brevicomin (2), fenchyl alcohol (3), myrtenal (4), *cis*-verbenol (5), *trans*-pinocarveol (6), acetophenone (7), *trans*-verbenol (8), verbenone (9), myrtenol (10), unknown (11), *trans*-myrtanol (12), *cis*-myrtanol (13), 2-phenylethanol (14), and unknown (15).

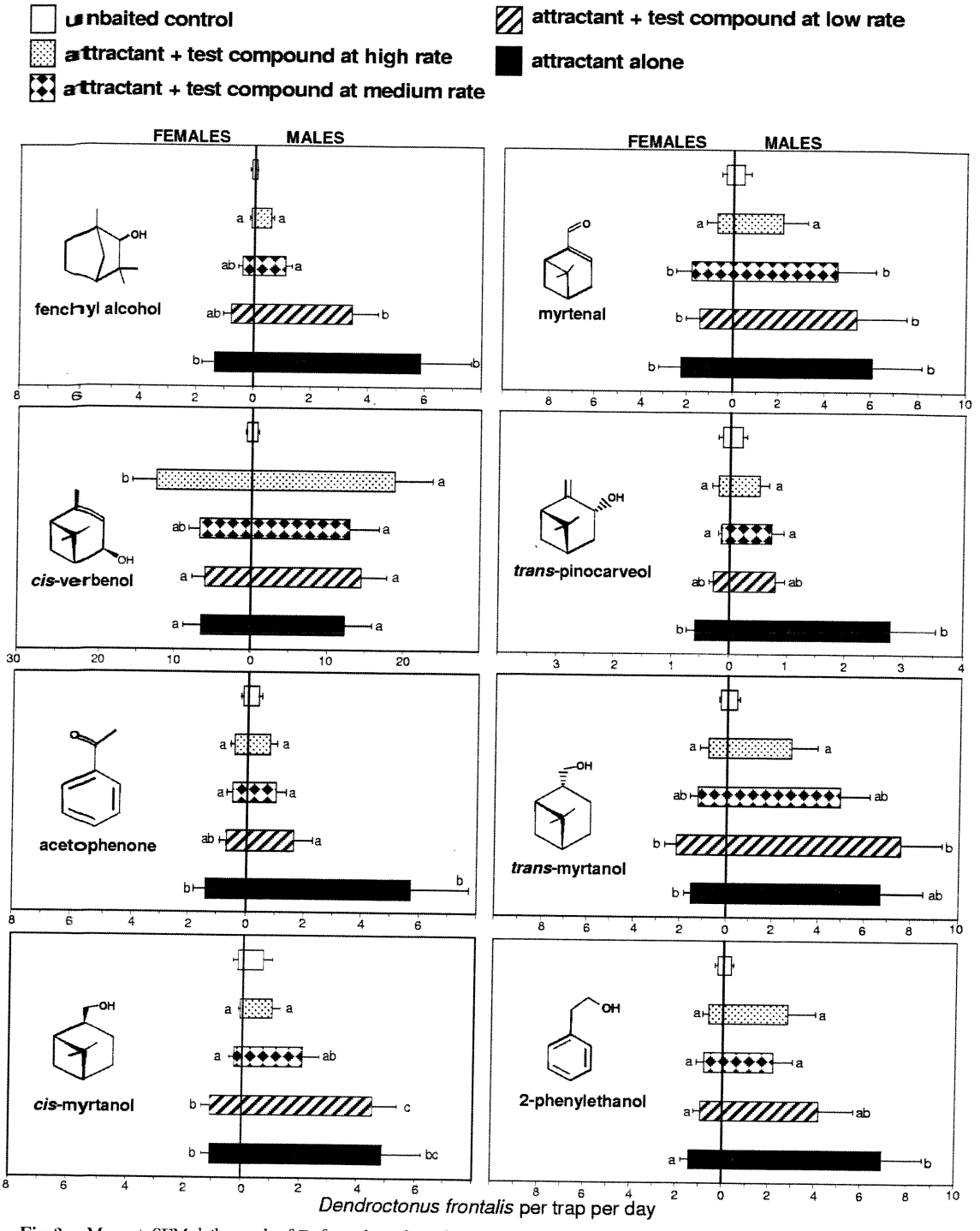

Fig. 3. Mean + SEM daily catch of *D. frontalis* in funnel traps baited with an attractant (frontalin and α-pinene) either alone or in combination with a candidate semiochemical for *D. frontalis*. Within sex, means associated with the same letter were not significantly different ($\alpha = 0.05$; Tukey's test). Catches in unbaited control traps were excluded from statistical analyses. Tests were replicated 12 times with fenchyl alcohol, *trans*-myrtanol, and *cis*-myrtanol, and 14 times with all other compounds.

Table 2. Sex ratios of *D. frontalis* trapped with an attractant released either alone or in combination with different release rates of a candidate semiochemical

Test compound		Attractant alone	Dose of test compound			
			Low	Medium	High	Unbaited
Fenchyl alcohol	♂/♀	4.5	4.3	2.8	6.0	0.8
	(n)	(286)	(170)	(60)	(28)	(7)
Myrtenal	♂/♀	2.8	3.8	2.5	2.9	1.5
	(n)	(466)	(412)	(371)	(179)	(50)
cis-Verbenol	♂/♀	1.9	2.3	2.0	1.5	1.1
	(n)	(924)	(998)	(962)	(1534)	(64)
trans-Pinocarveol	♂/♀	4.8	2.9	5.3	2.8	1.7
	(n)	(167)	(51)	(44)	(34)	(16)
Acetophenone	♂/♀	4.3	2.6	2.1[a]	1.9[a]	2.4
	(n)	(528)	(159)	(108)	(84)	(37)
trans-Myrtanol	♂/♀	4.5	3.5	4.3	3.5	1.5
	(n)	(350)	(419)	(268)	(144)	(28)
cis-Myrtanol	♂/♀	4.8	4.1	7.0	10.3	3.7
	(n)	(254)	(235)	(104)	(45)	(33)
2-Phenylethanol	♂/♀	4.8	4.2	2.7	4.5	1.3
	(n)	(213)	(131)	(71)	(77)	(14)

[a] Sex ratio was significantly different from that responding to the attractant-only treatment (χ^2 test; $\alpha = 0.016$).

followed by Dunn's test for all pairwise comparisons (SigmaStat 3.0, SPSS Inc. 1997). Treatments for which a given compound could not be detected were excluded from the analysis.

Results

Electrophysiological Studies. At least 15 compounds detected by FID in the hindguts of newly emerged male or female *D. frontalis* produced consistent antennal responses in one or both sexes (Fig. 2). Thirteen of these FID peaks could be identified by mass spectral and retention time matches with identified standards. These included the known *D. frontalis* semiochemicals frontalin, *endo*-brevicomin, *trans*-verbenol, verbenone, and myrtenol (Payne 1980) as well as eight compounds not previously reported as semiochemicals produced by this species: fenchyl alcohol, myrtenal, *cis*-verbenol, *trans*-pinocarveol, acetophenone, *trans*-myrtanol, *cis*-myrtanol, and 2-phenylethanol. GC-EAD tests with synthetic versions of these compounds confirmed their electrophysiological activity with both sexes.

Trapping Bioassays. All eight compounds modified responses of one or both sexes of *D. frontalis* to traps baited with attractive lures (Fig. 3). *Cis*-verbenol was the sole test compound that enhanced the standard attractant, and it did so only at the high release rate and only with female beetles. The other seven compounds were inhibitory, but the minimum dose necessary to elicit inhibition varied. Only one of the seven (acetophenone) significantly reduced catch of either sex at the low release rate; at the medium rate, four more compounds (fenchyl alcohol, *trans*-pinocarveol, *cis*-myrtanol, and 2-phenylethanol) exhibited inhibition; and at the high release rate, all seven (i.e., those aforementioned plus myrtenal and *trans*-myrtanol) were inhibitory to at least one sex. The sex ratio trapped by the standard attractant was not altered by combination with any test compound except aceto-

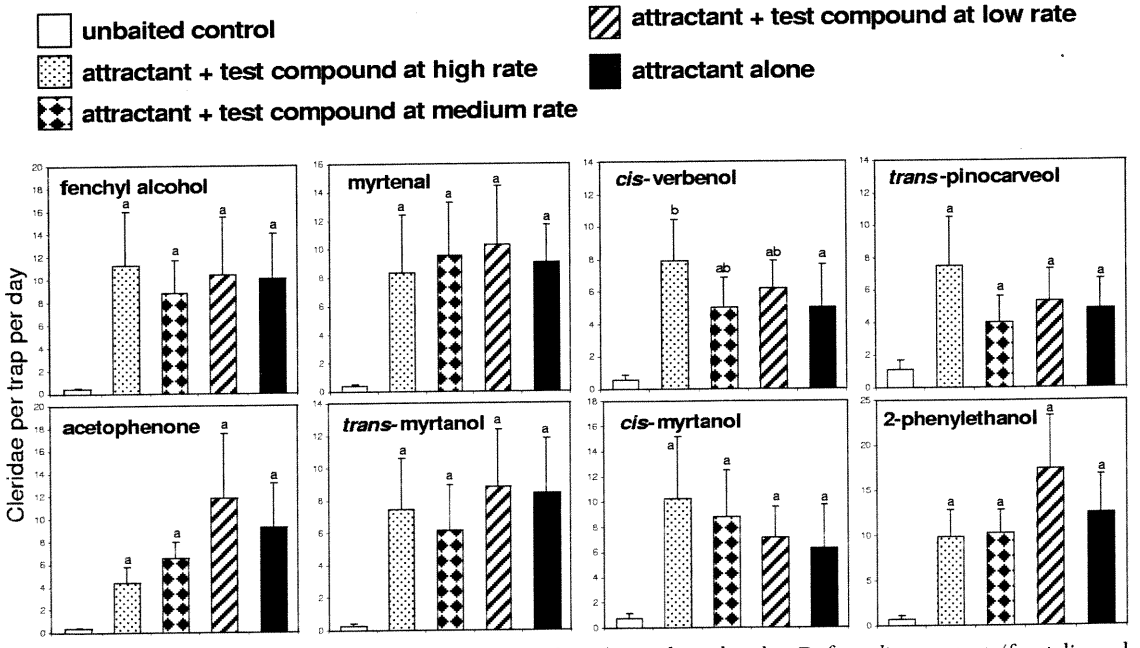

Fig. 4. Mean + SEM daily catch of clerid predators in funnel traps baited with a *D. frontalis* attractant (frontalin and α-pinene) either alone or in combination with a candidate semiochemical for *D. frontalis*. Means associated with the same letter were not significantly different ($\alpha = 0.05$; Tukey's test). Catches in unbaited control traps were excluded from statistical analyses. Tests were replicated 12 times with fenchyl alcohol, *trans*-myrtanol, and *cis*-myrtanol, and 14 times with all other compounds.

phenone (Table 2), which significantly decreased the relative proportions of males trapped by the standard attractant (M:F, 4.3:1) at both the medium (M:F, 2.1:1; $P = 0.0028$) and high (M:F, 1.9:1; $P = 0.0019$) release rates. Catch of clerid beetles by the standard attractant was not altered significantly by any of the test compounds found to be inhibitory for *D. frontalis*, but attraction of these predators was enhanced by *cis*-verbenol at the high release rate (Fig. 4).

Volatiles Analyses of Individual Beetles. With the exception of 2-phenylethanol and acetophenone in females, the test compounds were isolated in greatest abundance from newly emerged beetles, with typically much smaller levels occurring in solitary-feeding and recently paired individuals (Fig. 5). Greatest quantities of 2-phenylethanol were detected from solitary feeding females, and levels of acetophenone were greater in solitary-feeding and paired females than newly emerged females. *Cis*-verbenol was detected only in females, whereas at least trace amounts of the other seven compounds were detected in both sexes. However, myrtenal and acetophenone were produced disproportionately by males, and *trans*-pinocarveol and 2-phenylethanol by females. GC-MS analyses with chiral capillary columns indicated that *D. frontalis*-produced *trans*-pinocarveol and myrtenal were predominantly the (+)-enantiomer, and fenchyl alcohol and *cis*-myrtanol were a nearly racemic blend of enantiomers (Table 3). *Cis*-verbenol was predominantly the R-enantiomer, and only the (−)-enantiomer of *trans*-myrtanol could be detected.

Discussion

Our GC-EAD analyses revealed that *D. frontalis* possesses olfactory sensitivity to numerous conspecific-produced compounds in addition to compounds reported previously as having either electrophysiological or behavioral activity with this species. In general, the strongest antennal responses in both sexes were elicited by known semiochemicals for *D. frontalis:* frontalin, *endo*-brevicomin, *trans*-verbenol, verbenone, and myrtenol (Payne 1980, Smith et al. 1993). The additional compounds that elicited antennal activity were quantitatively relatively minor mid/hindgut constituents, with the prominent exceptions of *cis*-verbenol and unidentified peak 15 (Fig. 2), which were typically the third most abundant compounds in newly emerged females and males, respectively (unpublished data).

Cis-verbenol was the only attractant for *D. frontalis* identified among the compounds bioassayed in the field, but it exhibited activity only at a relatively high dose (≈60 mg/d at 22°C). *Cis*-verbenol has been isolated repeatedly from female *D. frontalis* (Pitman et al. 1969, Hughes 1973, Renwick et al. 1973, Renwick and Hughes 1975, Grosman et al. 1997), but behavioral activity with *D. frontalis* has not been reported previously. It is an attractive component in the pheromone blend of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Miller and LaFontaine 1991), as well as an important aggregation pheromone component for several *Ips* spp., including ones sympatric with *D. frontalis* (Francke and Vité 1983, Smith et al. 1993, Cognato et al. 1997). GC-MS analyses revealed that the *cis*-verbenol baits in the current study were contaminated with 1–2% *trans*-verbenol, an attractant synergist for *D. frontalis* that has shown activity in traps when released at 12 mg/d (Payne et al. 1978a). The positive results reported here for *cis*-verbenol should therefore be viewed with caution.

Our trapping results represent the first reported evidence that fenchyl alcohol, myrtenal, *trans*-pinocarveol, acetophenone, and *trans*/*cis*-myrtanol can inhibit *D. frontalis* responses to attractants. 2-Phenylethanol was shown to decrease *D. frontalis* responses to an attractant mixture in a pedestrian olfactometer (Brand et al. 1977); however, no field trials were reported. Both myrtenal and *trans*-pinocarveol have been identified previously in extracts of *D. frontalis* hindguts (Hughes 1973, Renwick et al. 1973), but their possible behavioral activity with *D. frontalis* was not addressed. Only three other *D. frontalis*-produced compounds (verbenone, *endo*-brevicomin, and myrtenol) have been shown previously to inhibit this species' responses to attractants (Smith et al. 1993, Skillen et al. 1997).

Several of the *D. frontalis* inhibitors identified in this study have been isolated from or have demonstrated behavioral activity with other scolytine bark beetles. Acetophenone has been identified in volatiles from female *Dendroctonus pseudotsugae* Hopkins, *Dendroctonus rufipennis* (Kirby), and *Dryocoetes confusus* Swaine; male *Taphrorychus bicolor* (Herbst); and both male and female *D. ponderosae* and *Ips pini* (Say) (Conn et al. 1983, Kohnle et al. 1987, Pureswaran et al. 2000, 2004). Field data indicate that it stimulates aggregation in *T. bicolor* and inhibits attraction of female *D. pseudotsugae* (Conn et al. 1983, Kohnle et al. 1987, Pureswaran and Borden 2004). 2-Phenylethanol has been isolated from numerous bark beetles (particularly *Ips* spp.), including at least two other species of *Dendroctonus: D. ponderosae* and *Dendroctonus brevicomis* LeConte (Renwick et al. 1976, Pureswaran et al. 2000). It inhibited response of *D. ponderosae* to attractant-baited traps (Pureswaran et al. 2000), and enhanced attraction of *Ips paraconfusus* Lanier to male-infested logs (Renwick et al. 1976). Myrtenal was identified as a host compound for *D. confusus* that significantly enhanced attraction of this species to traps baited with its pheromone (Comacho et al. 1998). *Trans*-pinocarveol has been isolated from *Conophthorous coniperda* (Schwartz), *D. brevicomis, D. confusus, D. ponderosae,* and *Dendroctonus terebrans* (Olivier) (Hughes 1973, Libbey et al. 1974, Borden et al. 1987, Pierce et al. 1987, Birgersson et al. 1995). *Trans*-pinocarveol was attractive to *D. confusus* in a walking bioassay (Comacho et al. 1998), but field and laboratory bioassays failed to detect any behavioral activity for this compound with *C. coniperda, D. confusus,* or *D. ponderosae* (Libbey et al. 1985, Comacho et al. 1998, De Groot et al. 1998). *Trans*-myrtanol has been reported from extracts of both *Ips typographus* L.

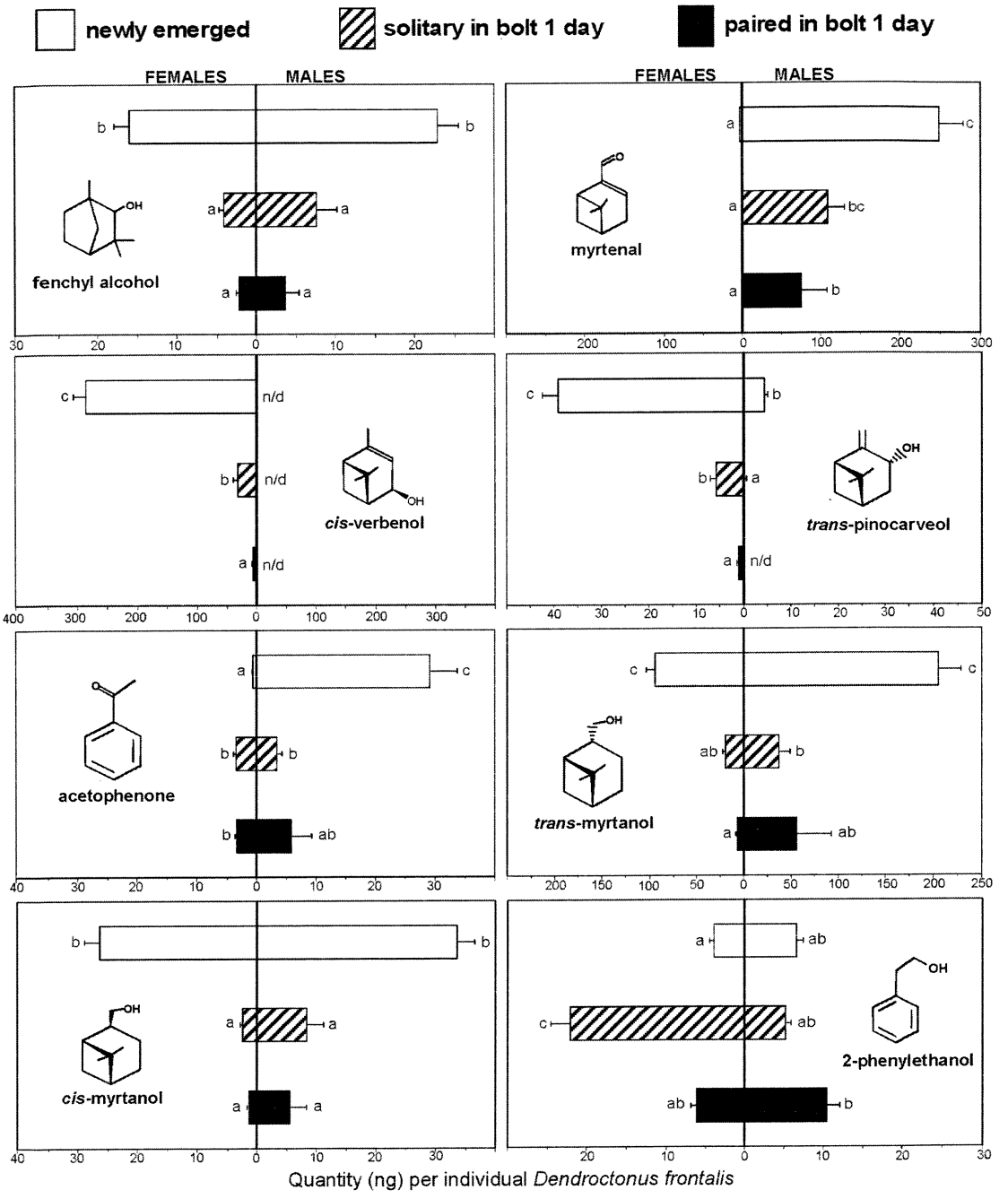

Fig. 5. Mean + SEM quantities of behaviorally active compounds isolated from individual male and female *D. frontalis* ($n = 31$–34). Means associated with the same letter were not significantly different ($\alpha = 0.05$; Dunn's method), and treatments for which a compound could not be detected were excluded from analyses (n/d).

and *Pityogenes chalcographus* L. (Birgersson et al. 1984, 1990), but it did not alter *I. typographus* response to attractant-baited traps (Schlyter et al. 1987). We found no previous reports of fenchyl alcohol or *cis*-myrtanol being isolated from or having behavioral activity with adult bark beetles; however, both compounds were identified in volatiles from larval frass of *Dendroctonus valens* LeConte and *Dendroctonus micans* (Kugelann) (Gregoire et al. 1991).

The seven attractant antagonists identified in this study possibly are antiaggregation pheromones for *D. frontalis*, because they are produced by this species

Table 3. Enantiomeric ratios (as percentage of predominant enantiomer ± SD) of compounds isolated from individual *D. frontalis*.

Chemical name	Newly emerged (%)		Solitary in bolt (%)		Paired in bolt (%)	
	Male	Female	Male	Female	Male	Female
Fenchyl alcohol	54 ± 11 (−) $n = 31$	58 ± 15 (−) $n = 29$	58 ± 11 (+) $n = 12$	—	—	—
Myrtenal	79 ± 7 (+) $n = 31$	—[a]	78 ± 7 (+) $n = 30$	—	77 ± 8 (+) $n = 28$	—
cis-Verbenol[a]	—	96 ± 2 (R) $n = 30$	—	>87 ± 11 (R)[b] $n = 26$	—	>83 ± 15 (R)[b] $n = 6$
trans-Pinocarveol	—	83 ± 11 (+) $n = 30$	—	>63 ± 9 (+)[b] $n = 11$	—	—
trans-Myrtanol	>99 ± 1 (−)[b] $n = 30$	>98 ± 1 (−)[b] $n = 31$	>86 ± 12 (−)[b] $n = 21$	>89 ± 9 (−)[b] $n = 25$	>86 ± 11 (−)[b] $n = 13$	>78 ± 15 (−)[b] $n = 8$
cis-Myrtanol	60 ± 9 (−) $n = 10$	52 ± 6 (−) $n = 10$	—	—	—	—

[a] Compound was not detected in quantities adequate to permit calculation of enantiomeric ratio.
[b] Lesser enantiomer was below the threshold of detection of GC-MS.

and inhibit its response to its aggregation pheromone. Some of the inhibitors also possess qualities that are consistent with a role in mediating sexual interactions in *D. frontalis*. Four (myrtenal, acetophenone, *trans*-pinocarveol, and 2-phenylethanol) were sexually dimorphic in their production by the beetles (Fig. 5). Additionally, one of the inhibitors (acetophenone) disproportionately reduced attraction of the producing sex (males). This trait is shared with verbenone, a predominantly male-produced inhibitory semiochemical for *D. frontalis* that similarly has greater activity with males than females (Salom et al. 1992). Antiaggregation pheromones that disproportionately inhibit the producing sex can act as a negative feedback mechanism for balancing the sex ratio of beetles arriving on a tree, and this has been proposed as an important function of verbenone in the biology of *D. frontalis* (Renwick and Vité 1970, Payne 1980).

However, it may be premature to classify all seven inhibitory compounds as antiaggregation pheromones. The timing of their production in female beetles was inconsistent with the putative function of antiaggregation pheromones, namely, the termination of aggregation once mating pairs are established and tree colonization is complete (Borden 1982). The inhibitor *trans*-pinocarveol was isolated in greatest quantities from newly emerged *D. frontalis* females (Fig. 5), suggesting that its production and behavioral effects would be maximal during attack initiation when females are inducing mass aggregation. In addition, one should have expected to see an increase in female-produced antiaggregation pheromones after mating. However, pairing either decreased or did not alter the levels of inhibitory compounds produced by solitary mining females (Fig. 5).

Additionally, production of at least some of these compounds by *D. frontalis* could be unrelated to intraspecific communication. Bark beetle pheromones are accumulated in the alimentary canal and released through the anus (Byers 1989b). Hence genuine pheromones are presumably accumulated and released in combination with volatile metabolic wastes normally voided during defecation. Five of the attractant antagonists identified in this study were oxygenated monoterpenes, and thus they may represent waste arising from the beetles' detoxification of tree resin monoterpenes (Renwick et al. 1973, White et al. 1980, Francke and Vité 1983). 2-Phenylethanol is produced during metabolism of dietary phenylalanine by *I. pini*, and this conversion may occur generally in bark beetles to prevent accumulation of potentially toxic levels of this amino acid (Gries et al. 1990). However, evidence suggests that in many instances bark beetles have appropriated particular volatile metabolic waste products (especially oxygenated monoterpenes, e.g., verbenone) for use as pheromones (Hughes 1973).

It is not yet known whether any of the attractant antagonists identified in our study are produced by *D. frontalis* in adequate concentrations to alter behavior of conspecifics under natural conditions. Simultaneously, it is possible that responses to these inhibitors evolved in the context of interspecific rather than

intraspecific relationships, hence *D. frontalis* ' production of and responses to these same compounds could conceivably be coincidental. As discussed previously, several of the *D. frontalis* inhibitors examined in this study are produced by other species of bark beetles, including ones that may compete directly with *D. frontalis* for resources. Bark beetle pheromones have frequently demonstrated repellency to sympatric, competing species of bark beetles (Borden 1982, Byers 1989a). Pheromones and other volatile compounds produced by insects infesting pine phloem (including the various detoxification products of host secondary compounds) could potentially signal to foraging hetero- and conspecifics the presence and density of competitors within a prospective host. In addition, some of the newly identified *D. frontalis* inhibitors (specifically, myrtenal, *trans*-pinocarveol, and fenchyl alcohol) are associated with conifer tissue in the later stages of bark beetle colonization and initial decay (Leufvén and Birgersson 1987, Pettersson and Boland 2003, Sullivan et al. 2003). Hence, it is possible that *D. frontalis* ' responses to at least some of these compounds may function primarily for lessening competition with other bark beetle species that produce these compounds or, alternatively, for facilitating avoidance of fully exploited and deteriorating host trees (Byers et al. 1989, Lindgren and Miller 2002).

Commercial availability determined the enantiomeric ratios of chiral compounds tested in our field assays; hence, the enantiomeric ratios in baits differed substantially from the ratios found in the beetles (Tables 1 and 2). For example, the *trans*-pinocarveol and myrtenal baits both contained 96% of the (−)-enantiomer, whereas the beetles contained <40%. The beetle-produced enantiomeric ratios may differ in behavioral activity from the ratios bioassayed in our field trials.

This study identified seven attractant antagonists that may have utility in either protecting trees from *D. frontalis* attacks or disrupting infestations. It is encouraging that some of these compounds produced significant reductions in *D. frontalis* attraction at concentrations substantially lower than reported for the two registered *D. frontalis* inhibitors verbenone and 4-allylanisole (Payne et al. 1978a, Salom et al. 1992, Hayes et al. 1994). Also, none of the inhibitors seemed to repel (and thus potentially lessen the beneficial activities of) clerid beetles, predators that inflict significant mortality on *D. frontalis* (Reeve 1997). However, the numbers of *D. frontalis* caught by the attractant-only traps was small relative to the numbers that typically colonize a tree, and it is possible that the inhibitors would have been less effective against a more potent attractant or higher beetle populations. Additionally, the maximum reduction in *D. frontalis* trap response achieved by the inhibitors in this study was 80–90%; hence, 10–20% of beetles were apparently undeterred. It has been observed that small percentages of undeterred beetles can often inflict mortality on inhibitor-treated trees, causing the achieved reduction in tree mortality to be substantially less than the observed reduction in beetle attraction (Strom et al. 2004). One might possibly achieve higher levels of inhibition by further increasing inhibitor dose or deploying inhibitors in combination rather than singly (Payne et al. 1978a, Borden 1996).

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