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Variable Responses by Southern Pine Beetle, *Dendroctonus frontalis* Zimmermann, to the Pheromone Component *endo*-Brevicomin: Influence of Enantiomeric Composition, Release Rate, and Proximity to Infestations

Brian T. Sullivan · Mark J. Dalusky · Kenji Mori · Cavell Brownie

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Abstract The male-produced bicyclic acetal *endo*-brevicomin is a component of the pheromone blend that mediates colonization of host pines by the bark beetle *Dendroctonus frontalis* Zimmermann. Efforts to identify its behavioral function have been complicated by contrasting reports that it either enhances or reduces attraction of flying beetles. Our studies failed to support the hypothesis that this published variability is due to differences in release rate and/or the enantiomeric composition [i.e., the beetle-produced (+)-enantiomer vs. the racemate] of the *endo*-brevicomin used in the experiments. In trapping trials within active *D. frontalis* infestations, racemic and (+)-*endo*-brevicomin did not differ from each other in behavioral effects when tested at seven different release rates ranging from 0.005 to 3 mg/d. At the highest release rates, racemic and (+)-*endo*-brevicomin similarly reduced catches in traps baited with an attractant (frontalin and turpentine), but neither enhanced catches at any release rate. Furthermore, the activity of racemic *endo*-brevicomin baits depended on trap proximity to *D. frontalis* infestations. Addition of these baits to attractant-baited traps located inside active infestations reduced catches, but they enhanced catches at traps located either 100 or 200 m outside these infestations. The contrasting responses may reflect differences in host-seeking strategies by either aggregated or dispersing *D. frontalis,* and may be elicited by differing abundance of natural sources of semiochemicals or differing responsiveness of beetles inside vs. outside of infestations. We suspect that much of the published variability in *D. frontalis* responses to *endo*-brevicomin is attributable to differing proximity of experimental field sites to infestations.

Key Words Aggregation · Attractant antagonist · Coleoptera · Dispersal · Enantiomer · Flight behavior · Pheromone synergist · Scolytidae · Semiochemical · Spatial dynamics · Trapping

B. T. Sullivan (✉)
USDA Forest Service, Southern Research Station,
2500 Shreveport Hwy,
Pineville, LA 71360, USA
e-mail: briansullivan@fs.fed.us

M. J. Dalusky
Department of Entomology, University of Georgia,
Athens, GA 30602, USA

K. Mori
The University of Tokyo,
Bunkyo-ku, Tokyo 113–0023, Japan

C. Brownie
3309 Horton St,
Raleigh, NC 27607, USA

Introduction

Aggressive species of bark beetles (Coleoptera: Scolytidae) overcome host defenses by attacking trees in large numbers, and thus are able to colonize trees that would not be susceptible to infestation by smaller numbers of insects (Paine et al., 1997). These aggressive species stimulate and synchronize such "mass attacks," which inevitably result in death of the host tree, by releasing pheromones whose activity is often modified by compounds from the tree itself (Wood, 1982; Byers, 1989). Pheromones of aggressive bark beetles likely fulfill at least two functions in the mass-colonization process: as aggregation pheromones that attract beetles from the general area and concentrate landings and attacks on one or a few trees, and as

antiaggregation pheromones that terminate aggregation on a fully-colonized host and prevent overcrowding (Borden, 1982). Due to the essential role that these semiochemicals play in the beetles' ability to kill trees, substantial effort has been invested in developing semiochemical-based management techniques (Borden, 1995).

The southern pine beetle, *Dendroctonus frontalis* Zimmermann, is an aggressive bark beetle pest of pines in the southeastern United States, Mexico, and much of Central America (Payne, 1980). Population outbreaks are conspicuous as spatially discrete infestations or "spots," which are zones of contiguous, currently-infested or dead, abandoned trees surrounded by healthy forest (Franklin, 1970; Thatcher and Barry, 1982; Clarke and Nowak, 2010). During summer, brief (i.e., ~40 d), broadly-overlapping generations of brood beetles emerge from previously-infested trees within these infestations and are attracted to semiochemicals released from newly-infested trees at the leading edge of the growing infestation (Gara et al., 1965; Gara and Coster, 1968; Billings and Pase, 1979). These beetles either attack non-colonized areas of the main stem on these newly-infested trees or initiate colonization of adjacent, uninfested trees. Infestations accumulate new trees typically along just one side, and thus normally have a single, well-defined "head" and trajectory of infestation growth (i.e., the direction of newly-infested trees relative to those previously infested) at any given time (Coster et al., 1978; Schowalter et al., 1981).

The bicyclic acetal *endo*-brevicomin appears to be a key component of the semiochemical blend that mediates *D. frontalis* aggregation and infestation growth. Male beetles pairing with attack-initiating females in the host bark release apparently enantiomerically pure (+)-*endo*-brevicomin (Sullivan et al., 2007). Baits of synthetic (+)-*endo*-brevicomin can greatly enhance responses by both sexes to traps baited with pine volatiles and the female-produced attractant frontalin (Vité et al., 1985; Sullivan et al., 2007), implying that (+)-*endo*-brevicomin is a component of the aggregation pheromone. Furthermore, *endo*-brevicomin baits can have an even greater catch-enhancing effect when displaced 4–16 m away from such traps (Sullivan and Mori, 2009). Thus, in addition to enhancing attraction to the tree of origin, male-produced *endo*-brevicomin may mediate switching of the focus of mass attack from fully colonized trees (i.e., ones with many established pairs) to adjacent trees receiving initial attacks by females (Sullivan and Mori, 2009).

This interpretation of the biological function of *endo*-brevicomin is based upon results of behavioral studies performed with the beetle-produced (+)-enantiomer (Vité et al., 1985; Sullivan et al., 2007; Sullivan and Mori, 2009). However, the potent attractant synergism observed for (+)-*endo*-brevicomin in published studies contrasts sharply with several reports that racemic *endo*-brevicomin baits can inhibit *D. frontalis* responses to traps baited with frontalin and host odors (Vité and Renwick, 1971; Payne et al., 1978a; Salom et al., 1992). The inhibitory activity of racemic *endo*-brevicomin was reported earlier than the synergistic capacity of (+)-*endo*-brevicomin, and this led to the original classification of *endo*-brevicomin as a component of an antiaggregation pheromone for *D. frontalis* (Borden, 1982, 1985; Skillen et al., 1997). Vité et al. (1985) hypothesized that the reported inhibitory activity of *endo*-brevicomin was due to earlier studies' use of high release rates of the racemate and the presence of the (−)-enantiomer, which they demonstrated could inhibit *D. frontalis* responses to its attractant. However, no single, controlled study has evaluated this hypothesis by directly comparing *D. frontalis* responses to racemic and (+)-*endo*-brevicomin across a range of doses.

Furthermore, there are other possible reasons for the disparity among published results of field studies on *D. frontalis* responses to *endo*-brevicomin. Those trapping studies that demonstrated potent attractant synergism for *endo*-brevicomin generally were performed in uninfested tracts of forest (i.e., Sullivan et al., 2007; Sullivan and Mori, 2009; half of replicates in Vité et al., 1985); whereas those that demonstrated inhibition were performed mostly inside of growing infestations (i.e., Payne et al., 1978a; Salom et al., 1992; not detailed in Vité and Renwick, 1971). Host seeking behavior of *D. frontalis* ostensibly differs inside and outside of active infestations (Gara, 1967; Payne and Coulson, 1985); hence flying *D. frontalis* may respond differently to sources of *endo*-brevicomin located within or close to infestations rather than some distance away.

In this study, we tested the hypothesis that variation in the reported behavioral activity of *endo*-brevicomin can be attributed simply to variation in the enantiomeric composition and/or dose of the baits by comparing the flight response of *D. frontalis* to a range of release rates of racemic and (+)-*endo*-brevicomin in the field. Secondly, we tested the hypothesis that proximity of an active infestation affects the flight response of *D. frontalis* to *endo*-brevicomin by simultaneously operating attractant-baited traps inside and outside of infestations and altering the presence of an *endo*-brevicomin bait.

Methods and Materials

All experiments utilized multiple-funnel traps (12-unit; ChemTica Internacional, S.A., San Jose, Costa Rica) that were suspended from vertical standards consisting of 1.7 cm diam pieces of electrical conduit staked into the ground. Each trap cup was positioned 1–1.5 m above the ground and contained several centimeters of propylene glycol and water (1:3) to retain and preserve captured insects.

Experiment 1: Variable Dose and Enantiomeric Composition of endo-Brevicomin Baits Deployed Inside of Infestations Sixteen different trap bait treatments were compared: an unbaited trap, a standard attractant consisting of frontalin and pine turpentine, or the standard attractant plus one of seven logarithmically increasing release rates of either (+)- or racemic *endo*-brevicomin (Table 1). *endo*-Brevicomin was released either from glass capillaries with one heat-sealed end, or from open, 100 μl-capacity glass autosampler vial inserts (Table 1). Capillaries and inserts were secured open-end-up inside of an uncapped, inverted 4 ml-capacity screw-cap glass vial (Sullivan and Mori, 2009). One device was deployed per trap for the (+)-*endo*-brevicomin treatments; two devices were deployed for the racemic treatments. Hence, the release of (+) was equal for both enantiomeric treatments at each level of release rate. (+)-*endo*-Brevicomin was synthesized (Sullivan et al., 2007) and was >99% pure (enantiomerically and chemically) by NMR spectroscopy and GC. There were no detected contaminants with known behavioral activity. Racemic *endo*-brevicomin [Phero Tech International (now Contech Enterprises Inc.), Delta, BC, Canada] was 95% chemically pure by GC (<1% *exo*-brevicomin contamination). Racemic frontalin was released from a pair of capped 400 μl-capacity LDPE microcentrifuge tubes each containing 200–300 μl frontalin [>95% chemical purity (contaminants with no known behavioral activity), ChemTica]. Turpentine (steam distilled from loblolly pine, *Pinus taeda* L.; Hercules Inc., Brunswick, GA, USA; composition determined by GC-FID as 72% α-pinene, 1% *trans*-verbenol, 1% *trans*-pinocarveol, <1% myrtenal, myrtenol, and verbenone, with the remainder consisting of 5% β-pinene, 6% camphene, and other monoterpenes of no known behavioral activity) was released from a pair of LDPE transfer pipettes filled to capacity (4 ml) and heat-sealed at the tip. Bait release rates were measured in a fume hood at 24±2°C (23±2°C for frontalin), either gravimetrically for turpentine (0.12 g/d) and frontalin (5 mg/d) or by volume loss for *endo*-brevicomin (Table 1). Baits were attached at the fourth funnel from the bottom of the trap.

Sixteen traps were placed inside each of two actively-growing *D. frontalis* infestations (located >1 km apart) in stands of *P. taeda*, in the Homochitto National Forest, Mississippi. Traps were clustered (i.e., with no particular geometric arrangement) within portions of the infestations occupied by trees with predominantly larval-stage *D. frontalis* brood (Payne et al., 1978b), and were <10 m away from a brood tree and <50 m away from trees undergoing mass attack and presumably releasing aggregation semiochemicals. Traps were spaced >1 m from the nearest pine and 5–10 m apart; this close trap spacing was required in order to test all 16 treatments simultaneously within each infestation. The 16 bait treatments were assigned randomly and without duplication to the traps of each infestation. Trap catch was collected after 1–4 d, and then the treatment assignments were re-randomized without replacement to any previous position. Randomization and catch collection were repeated 9 times at one of the infestations (5–19 July 2005) and 7 at the other (5–13 July 2005; we were required to abandon this latter infestation so it could be controlled).

Mean daily catch for both males and females was calculated for every collection. All trap catch data for both experiments 1 and 2 were cube root transformed to meet the distributional assumptions of the tests; conformity to assumptions was confirmed by examination of residuals plots. Catches by the unbaited traps were omitted from the statistical analyses (Reeve and Strom, 2004). A mixed model ANOVA was carried out by using PROC MIXED (SAS 9.0, SAS Institute Inc., Cary, NC, USA) with effects for treatment, sex, and sex by treatment assumed fixed. Infestation and date within infestation were considered random sources of replication, and so treatment by infestation and treatment by date within infestation were also random. Sex was viewed as a repeated measures factor within each trap and date combination, hence sex by treatment by infestation, and sex by date within infestation, were also included as random effects. Treatment was partitioned to test for a significant effect of release rate, enantiomeric composition of *endo*-brevicomin, and interactions between these factors. Treatment by sex was partitioned analogously. An ESTIMATE statement was used to test for an interaction between sex and presence/absence of *endo*-brevicomin (averaged for both enantiomeric compositions). Degrees of freedom were obtained by using the default containment option. Since no significant interactions between treatment and sex were found, total catches of *D. frontalis* (i.e., with males and females pooled) were cube root transformed and subjected to the same mixed model ANOVA with omission of all effects involving sex. ESTIMATE statements were used to compare mean catches (averaged over enantiomeric compositions) at each release rate to catches by the standard attractant alone, with a Bonferroni correction applied to the resulting *P*-values (α=0.05).

Experiment 2: endo-Brevicomin Activity Inside vs. Outside of Infestations Tests were performed in 16 *D. frontalis* infestations; 8 in the Oconee National Forest, Georgia (25 June–26 July 2007) and 8 in the Homochitto National Forest, Mississippi (9 August–21 September 2007). Selected infestations had at least 5 recently-infested trees (i.e., with green crowns) and at least one tree visibly undergoing mass attack at the infestation head when trapping was initiated. All but one of the infestations continued to accumulate new, infested trees during the trapping period, but in all cases, the infestation heads advanced less than 20 m. Three traps were placed at each infestation: (1) inside the infestation head (surrounded by trees containing egg-stage brood and 4–20 m

Table 1 Construction and elution rate of *endo*-brevicomin release devices for experiment 1

Dose level	Glass insert/capillary characteristics				Elution rate[a] (mg/d ± s.d.)
	Diameter (i.d. mm)	Length (mm)	Height filled (mm)	Number per device	
1)	0.200	32	10	1	0.0049±0.0001
2)	0.346	32	10	1	0.015±0.001
3)	0.631	32	10	1	0.049±0.001
4)	1.17	32	10	1	0.16±0.01
5)	1.17	32	10	3	0.47±0.02
6)	3.66	30	10	1	1.2±0.1
7)	3.66	30	8	3	3.0±0.2

Each device consisted of 1–3 glass autosampler vial inserts or glass capillaries secured open-end-up inside of an inverted, uncapped 4 ml capacity screw-cap vial

[a] Measured with the racemate by volume loss in a fume hood over 26 d at 24±2°C (N=3). One device was deployed per trap for the (+)-*endo*-brevicomin treatments; two devices were deployed per trap for the racemic *endo*-brevicomin treatments. Hence, this rate represents the release of each enantiomer of *endo*-brevicomin

from trees being mass attacked); 2) 100 m; and 3) 200 m beyond the limit of infested trees and within forests with a pine component within the general trajectory of infestation growth. In some instances, trap locations diverged from the trajectory of infestation growth as much as 90° if land within the infestation trajectory did not contain susceptible pines or was not publicly owned. Due to the inherently limited size of forest management units ("stands"), it was often necessary to locate the 2nd or 3rd (i.e., the 100 or 200 m) traps within adjacent forest stands that may not have been identical in tree composition, age, density, and other characteristics to the stand containing the beetle infestation itself. In some cases, the stand containing the infestation and those containing the 2nd or 3 rd traps were separated by treeless zones such as roads and utility right-of-ways. No multiple-tree infestations were closer to the 2nd and 3rd traps than the selected infestation, and no solitary colonized trees were closer than 50 m. Traps typically were placed no closer than 4 m from healthy pines to reduce chances of artificially inducing new infestations in these trees, but distances as small as 2 m were unavoidable in high-density pine stands.

All three traps at an infestation were consistently baited with frontalin and turpentine. A device releasing racemic *endo*-brevicomin was either attached simultaneously to all 3 traps assigned to an infestation or not attached to any, with the initial treatment (*endo*-brevicomin present/absent) chosen by a coin toss. Catch was collected at intervals of 1–5 d, and the treatment assignment was reversed for all 3 traps at an infestation on each date that catch was collected (3–10 collections over a 5–26 d trapping period). The frontalin bait was the same as experiment 1 except a single tube was used (i.e., 2.5 mg/d at 23°C). The turpentine bait (4–7 g/d at 24°C) was a single 250 ml-capacity brown glass bottle with a 1 cm diam piece of cotton dental wick immersed in the liquid (200–250 ml, same source and chemical composition as in experiment 1) and extending 1–2 cm through a hole in the cap. The *endo*-brevicomin bait (0.5–0.8 mg/d at 24°C) was an LDPE "bubble"-type device (ChemTica) containing 49 mg racemic *endo*-brevicomin (>95% purity with 2–3% *exo*-brevicomin). Frontalin and *endo*-brevicomin baits were attached near the center of each trap, whereas the turpentine bottle was placed within the funnel immediately below the trap top to protect the wick from rain.

At each trap collection, pines adjacent to the 100 and 200 m traps were checked for the presence of pitch tubes and other evidence of *D. frontalis* attack. Small numbers of attacks (1–5) were sometimes observed on one or more nearby pines. However, if a mass attack were observed on one or more adjacent trees (as occurred at one infestation in Mississippi), catch data from this trap collection were discarded, and the trap was relocated >50 m away from the infested trees while maintaining the assigned treatment distance from the infestation.

For each trap, catches were pooled across collections to obtain a single mean value for daily catch with *endo*-brevicomin either absent or present. We performed a mixed-model ANOVA on these cube root transformed means by using PROC MIXED (SAS 9.0) with main and interaction effects for four fixed factors (*endo*-brevicomin treatment, trap position, state, and beetle sex) plus the following four random effects: infestation within state, trap position by infestation within state, *endo*-brevicomin treatment by infestation within state, and trap position by *endo*-brevicomin treatment by infestation within state. Thus, infestation within state was the random source of replication, whereas trap position and *endo*-brevicomin

treatment were considered stripped (i.e., applied uniformly) across each other within infestations. Sex was considered a repeated measures factor within each combination of trap position and *endo*-brevicomin treatment. To examine significant interactions, means for levels of one factor were compared at each fixed level of another factor by using the SLICE option with the LSMEANS statement. A Bonferroni correction was applied to the resulting *P*-values separately for each significant interaction (α=0.05).

Results

Experiment 1: Variable Dose and Enantiomeric Composition of endo-Brevicomin Baits Deployed Inside of Infestations No significant interactions were revealed between sex of the responding beetles and bait treatment overall (F=1.34; df=14,15; P=0.29), or between sex and the various treatment components, specifically, between sex and the presence/absence of *endo*-brevicomin (t=0.42; df=15; P=0.68); between sex and *endo*-brevicomin dose (F=1.87; df=7,15; P=0.15); between sex and the enantiomeric composition of the *endo*-brevicomin bait (F=0.00; df=1,15; P=0.97); or among sex, dose, and enantiomeric composition of *endo*-brevicomin (F=0.94; df=6,15; P=0.50). With both sexes of *D. frontalis* pooled and averaging over dose, the enantiomeric composition of the *endo*-brevicomin bait had no significant effect on trap catches (F=0.00; df=1,14; P=0.98), and catches were not influenced by an interaction between dose of *endo*-brevicomin and its enantiomeric composition (F=1.04; df=6,14; P=0.44). However, averaging over enantiomeric composition, the release rate of *endo*-brevicomin had a highly significant effect on trap catches (F=17.5; df=6,14; P<0.001). Traps with the two highest release rates of *endo*-brevicomin caught significantly fewer beetles than traps baited with the standard attractant alone (F≥14.8; df=1,14; Bonferroni adjusted P≤0.013; Fig. 1). Traps with lower release rates of *endo*-brevicomin did not catch beetles in numbers that differed significantly from the attractant-only traps (P>0.45).

Experiment 2: endo-Brevicomin Activity Inside vs. Outside of Infestations A highly significant two-way interaction was revealed between trap location relative to the infestation head and presence/absence of the *endo*-brevicomin bait (F=40.3; df=2,28; P<0.001). Averaging over states, *endo*-brevicomin significantly reduced *D. frontalis* catches at frontalin/turpentine baited traps located inside the head of the infestation, but significantly increased catches in such traps located 100 and 200 meters beyond the head of the infestation (Table 2). However, a marginally-significant three-way interaction was detected between presence/absence of *endo*-brevicomin, trap location relative to the infestation head, and the state where the infestations were located (F=2.97; df=2,28; P=0.068). With the two states considered separately, *endo*-brevicomin significantly reduced catches at traps within the infestation in both states, but it significantly enhanced catches at traps outside the infestation only at the 100 m distance in Georgia and the 200 m distance in Mississippi (Table 2). However, this interaction with state was a quantitative (i.e., non-crossover) interaction, and the mean trend in both states was catch enhancement by *endo*-brevicomin for both trap locations outside the infestations. There was no significant interaction between sex and the effect of *endo*-brevicomin: there was not a significant three-way interaction among sex, *endo*-brevicomin presence, and trap location relative to the infestation head (F=0.91; df=2,84; P=0.406) nor a significant four-way interaction among sex, *endo*-brevicomin presence, state, and trap location relative to the infestation head (F=1.92; df=2,84; P=0.153). However, there was a highly significant two-way interaction between trap location relative to the infestation head and sex (F=35.2; df=2,84; P<0.001), apparently due to more strongly male-skewed sex ratios trapped within the infestation than outside. Furthermore, there was as a highly significant interaction between state and sex (F=83.0; df=1,84; P<0.001), apparently from more strongly male-skewed sex ratios trapped in Georgia than in Mississippi.

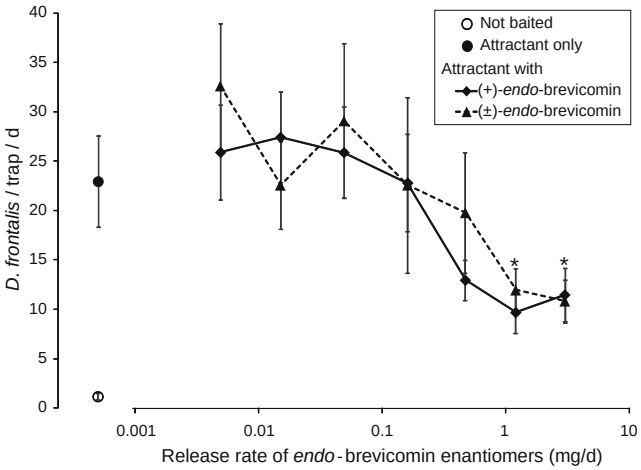

Fig. 1 Catches of *Dendroctonus frontalis* in multiple-funnel traps baited with an attractant (racemic frontalin and turpentine) alone or with varying release rates of either racemic or (+)-*endo*-brevicomin, Homochitto National Forest, Mississippi, July 2005. Catches at release rates of *endo*-brevicomin denoted with an asterisk were significantly different from catches by the attractant-only control trap (Proc Mixed ANOVA on transformed data; CONTRASTS with Bonferroni correction; α=0.05). The release rate indicated by the X-axis is for each individual enantiomer, thus the total release of *endo*-brevicomin [that is, the (+)- and (−)-enantiomers summed] by the racemic bait was twice that for the (+)-*endo*-brevicomin bait

Discussion

Experiment 2 demonstrated that an *endo*-brevicomin bait with a fixed release rate can produce apparently opposite behavioral effects depending on whether it is deployed inside or outside the head of an active *D. frontalis* infestation. The *endo*-brevicomin bait of experiment 2 consistently inhibited attraction and/or landing at point sources of attractant within infestations, but generally enhanced these responses outside. Furthermore, inside infestations we failed to observe attraction enhancement by either racemic or (+)-*endo*-brevicomin at any tested dose (experiment 1), including doses within a range that significantly enhanced catches in previous studies that we conducted outside of infestations [approximately 0.2–3 mg/d for (+); 0.5 mg/d for racemic; Sullivan et al., 2007; Sullivan and Mori, 2009].[1]

This variability may in part explain the alternatively synergistic or inhibitory activity reported by the six published trapping studies with *D. frontalis* that investigated the attractant-modifying capacity of *endo*-brevicomin alone. Although there was variation in the enantiomeric composition and release rate of the deployed baits, two of these studies performed away from infestations reported attraction enhancement or no effect by *endo*-brevicomin (Sullivan et al., 2007; Sullivan and Mori, 2009), whereas two performed inside infestations reported either attractant inhibition or no effect (Payne et al., 1978a; Salom et al., 1992). Vité and Renwick (1971) observed attractant inhibition, but did not report proximity of their traps to infestations. However, our results appear inconsistent with those of Vité et al. (1985), who observed significant attraction enhancement by both racemic and (+)-*endo*-brevicomin with half of their replicates conducted "within (a *D. frontalis*) infestation" and half in "uninfested stands." Although they pooled data from these two locations for their statistical analyses, their means reported for each site suggest similar responses to *endo*-brevicomin.

Furthermore, modest inconsistencies in our data from experiment 2 suggest that caution be used in inferring that *endo*-brevicomin invariably has opposite activities inside vs. outside of *D. frontalis* infestations. Significant catch enhancement by *endo*-brevicomin at both distances outside of infestations (100 m and 200 m) was detected only when data from both Mississippi and Georgia were pooled, and between the two states there appeared to be no consistent relationship between distance from the infestation and the degree of the enhancement effect of *endo*-brevicomin. The

[1] It should be noted that experiment 1 and the tests cited in Sullivan et al. (2007) and Sullivan and Mori (2009) differed substantially in the rate of turpentine released from the standard bait (i.e., 0.12 vs. 7 g/d, respectively).

Table 2 Effect of *endo*-brevicomin baits on *Dendroctonus frontalis* flight responses to attractant-baited traps positioned either inside or outside of active infestations

Trap location	*endo*-Brevicomin bait	Homochitto Nat. For., Mississippi Aug.–Sept., 2007 (N=8)[a]			Oconee Nat. For., Georgia June–July, 2007 (N=8)[a]			States combined (N=16)[a]		
		Catch/trap/d	Proportion females	P[b]	Catch/trap/d	Proportion females	P[b]	Catch/trap/d	Proportion females	P[b]
Inside infestation	Present	38.5±14.9	0.35	0.010	67.3±29.8	0.12	<0.001	52.9±16.5	0.23	<0.001
	Absent	114.9±63.1	0.43		221.0±108.8	0.16		168.0±62.3	0.30	
100 m outside	Present	9.7±4.1	0.47	0.197	97.8±47.1	0.29	0.005	53.8±25.5	0.38	0.001
	Absent	3.9±2.5	0.45		26.3±10.8	0.30		15.1±6.1	0.37	
200 m outside	Present	26.3±7.7	0.51	<0.001	34.5±16.3	0.26	0.370	30.4±8.8	0.39	<0.001
	Absent	1.6±0.4	0.53		17.3±10.8	0.50		9.5±5.6	0.51	

Table entries represent untransformed trap catches (mean ± s.e.)

Attractant consisted of racemic frontalin and steam distilled *Pinus taeda* turpentine (see Methods and Materials for details)

[a] Each replicate was a single infestation of *D. frontalis*

[b] P-value for test of hypothesis that catch was the same with *endo*-brevicomin either present or absent at the trap (SLICE option of the LSMEANS statement, SAS PROC MIXED on cube root transformed data, with Bonferroni correction, α=0.05)

marginally-significant interaction detected between treatment effect and state may be related to large differences in the abundance of infestations in the forests utilized in either state: during the Summer of 2007 approximately 1.6 infestations/km^2 were detected on the Oconee National Forest, Georgia, but only 0.12/km^2 in the Homochitto National Forest, Mississippi (Valli Peacher, USFS-FHP, personal communication).

The contrasting activities of *endo*-brevicomin possibly reflect the very different demands on host/mate-seeking *D. frontalis* either inside or outside of infestations. Beetles dispersing outside infestations may be hundreds of meters from the nearest suitable host (Coulson et al., 1985), and natural selection should presumably favor strategies that reduce energy and time consumed during host location. For these insects, the combination of pheromone components of both sexes (i.e., frontalin and *endo*-brevicomin) with host odors may serve as a more reliable indicator of an established infestation—where high densities of attacking conspecifics assures continuous host and mate availability—than frontalin and host odors alone (Sullivan and Mori, 2009). For beetles flying within infestations, by contrast, host trees are immediately available through the continuous cycle of new mass attacks on adjacent, healthy trees at the infestation head (Thatcher, 1960; Gara et al., 1965). In this circumstance, individual reproductive success may be maximized through discrimination of phloem resource availability and quality within individual hosts undergoing mass attack. *endo*-Brevicomin may aid beetles in avoiding trees that have reached their colonization capacity (i.e., ones having many established pairs and presumably releasing elevated quantities of *endo*-brevicomin) in favor of incompletely colonized trees (i.e., ones with at least some solitary females releasing frontalin and fewer or no *endo*-brevicomin producing males). Thus, *endo*-brevicomin may have distinct functions for dispersed and aggregated *D. frontalis*, aiding the former in locating active infestations and the latter in assessing resource availability within individual hosts.

A range of variables relating to the environment, the physiological state of responding insects, and the chemical signal itself can ultimately determine how bark beetles respond to semiochemicals (Borden et al., 1986; Raffa, 2001; Miller et al., 2005; Pureswaran et al., 2008). Pheromone components that alternately exhibit either attractive or inhibitory activity within a single population have been identified in several bark beetle species (Rudinsky, 1973; Borden et al., 1987; Schlyter et al., 1987; Seybold et al., 1992). In these reports, the precise activity of such "multifunctional" pheromone components is governed by a quality intrinsic to the pheromone source, namely the release rate of the compound, with low release rates being attractive but high ones inhibitory (Borden, 1996). In our experiment 2, however, the experimentally deployed *endo*-brevicomin baits were identical both inside and outside of the *D. frontalis* infestations, hence the reversal of activity we observed in either situation ostensibly had causes extrinsic to the baits themselves. These might include:

(a) *Differing background semiochemicals associated with the traps.* A prevailing background of *D. frontalis* semiochemicals presumably exists within the microenvironment of infestations but not in the surrounding forest. Trees within the advancing head of an infestation may contain many thousands of pheromone-producing beetles (Pureswaran et al., 2008), and evidence indicates that the associated semiochemical plume can profoundly influence behavior of *D. frontalis* orienting within and near these infestations (Gara et al., 1965; Gara, 1967; Cronin et al., 1999). These background semiochemicals conceivably could alter beetle responses to point sources of semiochemicals established within the infestation, such as our baits. This possibility is suggested by the observation that a device releasing approximately a single infested tree equivalent of *endo*-brevicomin can alter *D. frontalis* catches in traps within a radius of at least 30 m (Sullivan and Mori, 2009), a distance that is greater than what separated the infestation-imbedded traps of our experiments and the nearest mass-attacked trees. It should be noted that in experiment 1 the presence of *endo*-brevicomin and the other bait components on adjacent treatments also may have influenced trap catches, since intertrap spacing was merely 5–10 m. However, we presume that the 100 m spacing among traps in experiment 2 was sufficient to preclude any significant cross-interference by experimental baits.

(b) *Relative abundance of competing sources of beetle attraction.* Nearby, alternative sources of attractive cues and landing sites (namely, mass-attacked trees) are readily available to beetles inside infestations but not outside. Thus, whether or not an orienting beetle lands in a trap within an infestation is likely influenced by the bait's attractiveness relative to these competing, attractive sources. One effect of *endo*-brevicomin is apparently to render collocated sources of attractant relatively less attractive than adjacent sources of attractant: when one of a group of adjacent frontalin/turpentine-baited traps is baited additionally with *endo*-brevicomin, this trap becomes relatively less attractive to *D. frontalis* than the adjacent traps lacking *endo*-brevicomin, even though catch is enhanced in all traps (Sullivan and Mori, 2009). Thus, in our experiments, the absence/presence of an *endo*-brevicomin bait on traps inside the infestations could have rendered the traps more/less attractive than nearby infested trees, altering the

number of beetles trapped. By contrast, there were no adjacent mass-attacked trees to serve as alternative, attractive targets to the baited traps located outside infestations in experiment 2.

(c) *Differing responsiveness of dispersed* vs. *aggregated individuals*. Evidence suggests that flying beetles within *D. frontalis* infestations are predominantly newly-emerged brood or re-emerged parent adults from trees within the infestation (Gara, 1967; Cronin et al., 1999). Thus, beetles responding to traps located within infestations likely had flown a shorter average distance and spent less time outside of a host than beetles responding to traps located outside the infestations. Flight exercise alters *D. frontalis* responses to aggregation semiochemicals (Andryszak et al., 1982), and both flight exercise and changes in fat reserves have been shown to alter semiochemical responses in other bark beetle species (Borden et al., 1986). Furthermore, one study found that *D. frontalis* trapped outside of infestations express genes in different frequencies than those trapped inside (Florence et al., 1982), suggesting that dispersing and aggregated beetles differ in their inherent physiological and, perhaps, behavioral capacities. However, this finding is cast into some doubt by the study's failure to examine the sexes separately and by our evidence from experiment 2 that sex ratios trapped inside and outside infestations differ substantially.

Vité et al. (1985) reported that pure (+)-*endo*-brevicomin baits enhanced, whereas pure (−)-*endo*-brevicomin baits reduced, *D. frontalis* responses to traps baited with frontalin and host odors. However, in infested stands (experiment 1), we failed to observe a significant difference in beetle response to either racemic or (+)-*endo*-brevicomin at any of seven release rates spanning nearly four orders of magnitude. Likewise, in a previous study performed in uninfested stands, racemic and (+)-*endo*-brevicomin released at approximately 0.23 mg/d did not differ in their capacity to enhance attraction of *D. frontalis* (Sullivan and Mori, 2009). Thus, despite evidence that (−)-*endo*-brevicomin can inhibit attraction by *D. frontalis* (Vité et al., 1985), we did not find that it modified the activity of the (+)-enantiomer in racemic baits. For this reason, we chose to use the more readily-available and less expensive racemic *endo*-brevicomin rather than (+)-*endo*-brevicomin in experiment 2.

We believe this is the first report of a bark beetle semiochemical bait reversing its apparent behavioral effect (i.e., between attraction enhancement and inhibition) due to its proximity to sites of beetle aggregation. This particular expression of multifunctionality in bark beetle semiochemicals could obviously complicate efforts to use them in beetle management, since failure to deploy such compounds in the appropriate context (i.e., either within infestations or within uninfested stands) could result in the opposite of the intended effect. Efforts to develop *endo*-brevicomin as a management semiochemical for *D. frontalis* have been frustrated by unintended effects, but ones apparently unrelated to *endo*-brevicomin's multifunctionality. Blends of *exo*- and *endo*-brevicomin can greatly reduce *D. frontalis* attacks on trees within infestations (Payne et al., 1977), suggesting these compounds might have value as tree protectants. However, trees within infestations treated with these two isomers of brevicomin experience reciprocal increases in attacks by secondary *Ips* spp. DeGeer bark beetles and therefore no reduction in mortality (Payne and Richerson, 1979; Richerson and Payne, 1979; Watterson et al., 1982). Although, tree protection with *endo*-brevicomin has not been attempted outside of infestations, our results lead us to expect that such treatment would also fail because it would likely enhance, rather than reduce, risk of *D. frontalis* attack.

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References

ANDRYSZAK, N. A., PAYNE, T. L., and BILLINGS, P. M. 1982. Effect of flight activity on laboratory response of the southern pine beetle to an attractant. *J. Georgia Entomol. Soc.* 17:456–460.

BILLINGS, R. F. and PASE, H. A., III. 1979. A field guide for ground checking southern pine beetle spots. USDA Combined Forest Pest Research and Development Program Agriculture Handbook 558.

BORDEN, J. H. 1982. Aggregation pheromones, pp. 74–139, in J. B. Mitton and K. B. Sturgeon (eds.). Bark Beetles in North American Conifers: A System for the Study of Evolutionary Biology. University of Texas Press, Autstin.

BORDEN, J. H. 1985. Aggregation pheromones, pp.257–285, in G. A. Kerkut and L. I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Vol. 9, Pergamon Press, Oxford.

BORDEN, J. H. 1995. From identifying semiochemicals to developing a suppression tactic: a historical review, pp. 3–10, in S. M. Salom and K. R. Hobson (eds.). Application of Semiochemicals for Management of Bark Bbeetle Infestations–Proceedings of an Informal Conference. USDA Forest Service General Technical Report INT-GTR-318, Indianapolis, IN.

BORDEN, J. H. 1996. Disruption of semiochemical-mediated aggregation in bark beetles, pp. 421–438, in R. T. Cardé and A. K. Minks (eds.). Pheromone Research: New Directions. Chapman and Hall, New York.

BORDEN, J. H., HUNT, D. W. A., MILLER, D. R., and SLESSLOR, K. N. 1986. Orientation in forest Coleoptera: an uncertain outcome of responses by individual beetles to variable stimuli, pp. 97–109, in T. L. Payne, M. C. Birch, and C. E. J. Kennedy (eds.). Mechanisms in Insect Olfaction. Clarendon Press, Oxford.

BORDEN, J. H., RYKER, L. C., CHONG, L. J., PIERCE, H. D., JR., JOHNSTON, B. D., and OEHLSCHLAGER, A. C. 1987. Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. *Can. J. For. Res.* 17:118–128.

BYERS, J. A. 1989. Chemical ecology of bark beetles. *Experientia* 45:271–283.

CLARKE, S. R. and NOWAK, J. T. 2010. Southern pine beetle. USDA Forest Service Forest Insect and Disease Leaflet 49.

COSTER, J. E., HICKS, R. R., JR., and WATTERSTON, K. G. 1978. Directional spread of southern pine beetle (Coleoptera: Scolytidae) infestations in East Texas. *J. Georgia Entomol. Soc.* 13:315–321.

COULSON, R. N., FLAMM, R. O., WAGNER, T. L., RYKIEL, E. J., SHARPE, P. J. H., PAYNE, T. L., and LIN, S. K. 1985. Population dynamics of initiation and growth of southern pine beetle infestations, pp. 136–151, in S. J. Branham and R. C. Thatcher (eds.). Proceedings of the Integrated Pest Management Symposium, USDA Forest Service General Technical Report SO-56.

CRONIN, J. T., TURCHIN, P., HAYES, J. L., and STEINER, C. A. 1999. Area-wide efficacy of a localized forest pest management practice. *Environ. Entomol.* 28:496–504.

FLORENCE, L. Z., JOHNSON, P. C., and COSTER, J. E. 1982. Behavioral and genetic diversity during dispersal: analysis of a polymorphic esterase locus in southern pine beetle, *Dendroctonus frontalis*. *Environ. Entomol.* 11:1014–1018.

FRANKLIN, R. T. 1970. Southern pine beetle population behavior. *J. Georgia Entomol. Soc.* 5:175–182.

GARA, R. I. 1967. Studies on the attack behavior of the southern pine beetle. I. The spreading and collapse of outbreaks. *Contrib. Boyce Thompson Inst.* 23:349–354.

GARA, R. I. and COSTER, J. E. 1968. Studies on the attack behavior of the southern pine beetle. III. Sequence of tree infestation within stands. *Contrib. Boyce Thompson Inst.* 24:77–85.

GARA, R. I., VITÉ, J. P., and CRAMER, H. H. 1965. Manipulation of *Dendroctonus frontalis* by use of a population aggregation pheromone. *Contrib. Boyce Thompson Inst.* 23:55–66.

MILLER, D. R., LINDGREN, B. S., and BORDEN, J. H. 2005. Dose-dependent pheromone responses of mountain pine beetle in stands of lodgepole pine. *Environ. Entomol.* 34:1019–1027.

PAINE, T. D., RAFFA, K. F., and HARRINGTON, T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annu. Rev. Entomol.* 42:179–206.

PAYNE, T. L. 1980. Life history and habits, pp. 31–54, in R. C. Thatcher, J. L. Searcy, J. E. Coster, and G. D. Hertel (eds.). The southern Southern Pine Beetle. USDA Forest Service Science and Education Administration Technical Bulletin 1631.

PAYNE, T. L. and COULSON, R. N. 1985. Role of visual and olfactory stimuli in host selection and aggregation behavior by *Dendroctonus frontalis*, pp. 73–82, in L. Safranyik (ed.). The Role of the Host in the Population Dynamics of Forest Insects. Forestry Canada, Pacific Forestry Centre, Victoria.

PAYNE, T. L. and RICHERSON, J. V. 1979. Management implications of inhibitors for *Dendroctonus frontalis* (Col. Scolytidae). *Bull. Soc. Entomol. Suisse.* 52:323–331.

PAYNE, T. L., COSTER, J. E., and JOHNSON, P. C. 1977. Effects of slow-release formulation of synthetic *endo*- and *exo*-brevicomin on southern pine beetle flight and landing behavior. *J. Chem. Ecol.* 3:133–141.

PAYNE, T. L., COSTER, J. E., RICHERSON, J. V., EDSON, L. J., and HART, E. R. 1978a. Field response of the southern pine beetle to behavioral chemicals. *Environ. Entomol.* 7:578–582.

PAYNE, T. L., COSTER, J. E., RICHERSON, J. V., HART, E. R., HEDDEN, R. L., and EDSON, L. J. 1978b. Reducing variation in field tests of behavioral chemicals for the southern pine beetle. *J. Georgia. Entomol. Soc.* 13:85–90.

PURESWARAN, D. S., SULLIVAN, B. T., and AYRES, M. P. 2008. High individual variation in pheromone production by tree-killing bark beetles (Coleoptera : Curculionidae : Scolytinae). *Naturwissenschaften* 95:33–44.

RAFFA, K. F. 2001. Mixed messages across multiple trophic levels: The ecology of bark beetle chemical communication systems. *Chemoecology* 11:49–65.

REEVE, J. D. and STROM, B. L. 2004. Statistical problems encountered in trapping studies of scolytids and associated insects. *J. Chem. Ecol.* 30:1575–1590.

RICHERSON, J. V. and PAYNE, T. L. 1979. Effects of bark beetle inhibitors on landing and attack behavior of the southern pine beetle and beetle associates. *Environ. Entomol.* 8:360–364.

RUDINSKY, J. A. 1973. Multiple functions of the douglas Douglas-fir beetle pheromone 3-methyl-2-cyclohexen-1-one. *Environ. Entomol.* 2:579–585.

SALOM, S. M., BILLINGS, R. F., UPTON, W. W., DALUSKY, M. J., GROSMAN, D. M., PAYNE, T. L., BERISFORD, C. W., and SHAVER, T. N. 1992. Effect of verbenone enantiomers and racemic *endo*-brevicomin on response of *Dendroctonus frontalis* (Coleoptera: Scolytidae) to attractant-baited traps. *Can. J. For. Res.* 22:925–931.

SCHLYTER, F., BYERS, J. A., and LÖFQVIST, J. 1987. Attraction to pheromone sources of different quantity, quality, and spacing: Density-regulation mechanisms in bark beetle *Ips typographus*. *J. Chem. Ecol.* 13:1503–1523.

SCHOWALTER, T. D., POPE, D. N., COULSON, R. N., and FARGO, W. S. 1981. Patterns of southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation enlargement. *For. Sci.* 27:837–849.

SEYBOLD, S. J., TEALE, S. A., WOOD, D. L., ZHANG, A. J., WEBSTER, F. X., LINDAHL, K. Q., and KUBO, I. 1992. The role of lanierone in the chemical ecology of *Ips pini* (Coleoptera, Scolytidae) in California. *J. Chem. Ecol.* 18:2305–2329.

SKILLEN, E. L., BERISFORD, C. W., CAMAAN, M. A., and REARDON, R. C. 1997. Semiochemicals of forest and shade tree insects in North America and management applications. USDA Forest Service Forest Health Technology Enterprise Team Publication FHTET-96-15. 182 pp.

SULLIVAN, B. T. and MORI, K. 2009. Spatial displacement of release point can enhance activity of an attractant pheromone synergist of a bark beetle. *J. Chem. Ecol.* 35:1222–1233.

SULLIVAN, B. T., SHEPHERD, W. P., PURESWARAN, D. S., TASHIRO, T., and MORI, K. 2007. Evidence that (+)-*endo*-brevicomin is a male-produced component of the southern pine beetle aggregation pheromone. *J. Chem. Ecol.* 33:1510–1527.

THATCHER, R. C. 1960. Bark beetles affecting southern pines: a review of current knowledge. USDA Forest Service Occasional Paper SO-180. 25 pp.

THATCHER, R. C. and BARRY, P. J. 1982. Southern pine beetle. USDA Forest Service Forest Insect and Disease Leaflet 49.

VITÉ, J. P. and RENWICK, J. A. A. 1971. Inhibition of *Dendroctonus frontalis* response to frontalin by isomers of brevicomin. *Naturwissenschaften* 58:418–419.

VITÉ, J. P., BILLINGS, R. F., WARE, C. W., and MORI, K. 1985. Southern pine beetle: enhancement or inhibition of aggregation response mediated by enantiomers of *endo*-brevicomin. *Naturwissenschaften* 72:99–100.

WATTERSON, G. P., PAYNE, T. L., and RICHERSON, J. V. 1982. The effects of verbenone and brevicomin on the within-tree populations of *Dendroctonus frontalis*. *J. Georgia Entomol. Soc.* 17:118–126.

WOOD, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annu. Rev. Entomol.* 27:411–446.