

Intra-Annual Variation in Responses by Flying Southern Pine Beetles (Coleoptera: Curculionidae: Scolytinae) to Pheromone Component *endo*-Brevicommin

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Abstract

The southern pine beetle *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae) is attracted to an aggregation pheromone that includes the multifunctional pheromone component *endo*-brevicommin. The effect of *endo*-brevicommin on attractive lures varies from strong enhancement to reduction of beetle attraction depending upon release rate, lure component spacing, and proximity of beetle infestations. Anecdotal observations have further suggested that the effects of *endo*-brevicommin vary during the year. We investigated this possibility under nonoutbreak conditions in southwestern Mississippi where for two-and-a-half years we monitored traps baited with frontalin and the host odor *alpha*-pinene either (a) alone, or with an *endo*-brevicommin release device either (b) located directly on the trap, or (c) displaced 6 m away. The *endo*-brevicommin devices in our tests increased *D. frontalis* catches during all times of year, and 6 m displacement of the *endo*-brevicommin release device from the trap did not significantly alter responses except during the spring flight peak when displacement increased catches. Our data suggest that flying *D. frontalis* have a stronger tendency to avoid the immediate proximity of a release point of *endo*-brevicommin during their springtime dispersal flight when catches are greatest. Catches of *Thanasimus dubius* (F.) (Coleoptera: Cleridae), a major predator of *D. frontalis*, were not altered by *endo*-brevicommin, and ratios of *D. frontalis* to *T. dubius* changed over the course of the year. We discuss the possible effects of intra-annual variation in *D. frontalis* response to *endo*-brevicommin both on beetle attack behavior and use of *endo*-brevicommin as a lure adjuvant in *D. frontalis* population monitoring.

Key words: monitoring, bark beetle, trapping, pheromone, phenology

Bark beetles (Coleoptera: Curculionidae: Scolytinae) capable of killing mature, healthy trees generally rely upon aggregation pheromones to elicit mass attacks that can overwhelm the constitutive resin defenses of a host and render it susceptible to colonization (Renwick and Vité 1969, Byers 1989a, Raffa et al. 1993). The individual compounds and blends which compose the aggregation pheromones have been identified and synthesized for most tree-killing species of economic importance in North America, which are predominantly in the genera *Dendroctonus*, *Ips*, and *Scolytus* (Borden 1982, Wood 1982, Skillen et al. 1997). The capacity of aggregation pheromones (typically synergized by odors released from the damaged host tissue) to attract beetles in large numbers has inspired development of pest management technologies that utilize them to disrupt the capacity of the beetles to concentrate their attacks as well as to attract beetles to traps or trap trees where they can be removed from the environment (Borden 1989, 1995; Cook et al. 2006; Strom and Clarke 2011). They have received wider use,

however, as lures in traps for detecting arrivals of exotic insects and for delimiting the geographic range, assessing population size and activity level, and forecasting damage potential of target pests (Schwalbe and Mastro 1988, Weslien et al. 1989, Weslien 1992, Rabaglia et al. 2008). Trap catch data can be used for planning and implementation of management actions (Billings 2011). Intra- and interannual variation in responses by bark beetles to attractant lures—if uncorrelated to local densities of potentially attack-initiating beetles—ostensibly restricts the utility of baited traps in assessing risk.

Southern pine beetle, *Dendroctonus frontalis* Zimmermann, is a major bark beetle pest of pines within a range that stretches from Massachusetts to eastern Texas and from Arizona south to Nicaragua (Clarke and Nowak 2010; Kevin J. Dodds, personal communication). They produce outbreaks (indicated by the appearance of numerous multiple-tree infestations or “spots”) at irregular intervals of generally between five and fifteen years (Turchin et al. 1991, Birt 2011a). Within the southeastern United States, forecasts of

D. frontalis outbreaks and their severity for the coming summer are made on the basis of catches in pheromone traps deployed in spring (at flowering of dogwood, *Cornus florida* L.) and maintained for 4 wk (Billings and Upton 2010, Billings 2011). Both the average daily catches of *D. frontalis* per trap as well as ratios of catches of *D. frontalis* to those of a major predator, *Thanasimus dubius* (F.) (Coleoptera: Cleridae), are data used in a model which forecasts outbreaks with a demonstrated 60–80% reliability (Billings and Upton 2010, Billings 2011). Current efforts at improving the existing monitoring-forecasting program are considering a range of modifications of methodology including revision of the composition of the trap lure and addition of further monitoring periods during the year (Ronald F. Billings, personal communication).

To date the lure for this annual survey has consisted of two components: a device releasing the *D. frontalis* female-produced aggregation pheromone component frontalin (at a rate of ~5 mg per day) and a high-release source of host pine odors in the form of turpentine or purified *alpha*- and *beta*-pinene (at ~5 g per day; Ronald F. Billings, personal communication). Since initiation of the survey during the 1980s, it has been recognized that the relatively inexpensive, racemic form of the male-produced pheromone component *endo*-brevicomin has the potential to substantially increase the attractiveness of the two-component lure (Vité et al. 1985, Sullivan et al. 2007, Sullivan and Mori 2009). The addition thus appears to allow detection of beetles at lower population densities than possible previously. However, the effect on catches of adding *endo*-brevicomin devices to traps baited with the two-component lure can vary from strong enhancement (i.e., more than 10-fold) to substantial reduction (i.e., to less than one-third; Salom et al. 1992; Sullivan et al. 2007, 2011). This variability may be due at least in part to *endo*-brevicomin possessing a “multifunctional” dose response curve (Rudinsky 1973); that is, it is a potent enhancer of beetle attraction at low release rates, but becomes less attractive and finally inhibitory at high rates (Sullivan 2016). Although some variability in the effects of *endo*-brevicomin can be attributed to release rate of the devices and their arrangement in space (Sullivan and Mori 2009, Sullivan et al. 2011, B.T.S. unpublished data), it is also influenced by factors that are extrinsic to the traps and the qualities and spacing of the lures. Notably, *endo*-brevicomin released at rates which enhance attractiveness of traps located outside beetle spots reduce catches in identical traps located inside spots (Sullivan et al. 2011).

The synergistic effects of an *endo*-brevicomin release device of a particular rate can potentially be enhanced by displacing the device a few meters away from the trap baited with frontalin and host odors (Sullivan and Mori 2009). The author’s (B.T.S.) unpublished data indicate that, for pairs of 6-m separated traps in which both possess frontalin and host-odor lures but only one has an *endo*-brevicomin device, the *endo*-brevicomin release rate that maximizes catches in the amended trap nonetheless generates a higher response to the adjacent, unamended trap. Additionally, displacement of the *endo*-brevicomin release device from the trap has a practical advantage in that it can greatly reduce by-catches of irrelevant, *endo*-brevicomin-attracted species that could be mistaken for *D. frontalis* and *T. dubius* during catch counting. This is particularly true for *Hylesinus* spp. bark beetles which are strongly attracted to *endo*-brevicomin and somewhat resemble *D. frontalis* under low magnification (Shepherd et al. 2010). Thus, deployment of the *endo*-brevicomin lure component (at a rate of 0.1–1 mg/d) a few meters away from, rather than on, the trap is the author’s (B.T.S.) recommended procedure for enhancing the two-component lure for *D. frontalis*.

Experiments conducted by the authors at different seasons have suggested that the response of *D. frontalis* to *endo*-brevicomin

(particularly with regard to displacement-produced catch enhancement) varies during the course of the year and possibly in synchrony with the annual behavioral cycle of this multivoltine insect. To investigate this possibility, we monitored traps over the course of approximately two-and-a-half years to document the annual cycle of responses by *D. frontalis* and its predator *T. dubius* [which responds strongly to the aggregation attractant of *D. frontalis* (Vité and Williamson 1970)] to baited traps with or without *endo*-brevicomin either on or displaced from the trap. We hypothesized that catch enhancement due to *endo*-brevicomin lure displacement should be greatest under conditions (and associated times of year) when susceptible host trees are in greatest abundance relative to the abundance of searching beetles and are thus a less limiting resource. At these times beetles can presumably afford to be more discriminating and thus more prone than host-limited individuals to avoid cues indicating high local densities of conspecific competitors. *endo*-Brevicomin, which is produced by arriving and paired male *D. frontalis*, could function at higher concentrations as an indicator of complete resource occupation by previously arriving conspecifics. We also hypothesized that intra-annual variability in catches of the predator *T. dubius* should track that of *D. frontalis* since dispersal of the predator should be triggered by factors that trigger dispersal of their prey [i.e., loss of centers of attractant production coincident with *D. frontalis* infestation collapse and winter generational synchronization; temperatures conducive to flight and survival outside the host (Moser and Thompson 1986, Ayres et al. 2011)].

Materials and Methods

The experiment was performed in the Sandy Creek Wildlife Management Area of the Homochitto National Forest, MS (i.e., within 5 km of W91.200, N31.419), in stands consisting predominantly of a mixture of mature loblolly pines (*Pinus taeda* L.) and hardwoods and having generally little understory due to regular prescribed burning. The forest was not undergoing an outbreak during the period (beetles were at low and static numbers), and no infestations were detected in the National Forest during the trapping period (personal communication James Meeker, USFS-Forest Health Protection). All tests utilized Lindgren-type, 12-unit multiple funnel traps suspended from standards constructed of electrical conduit. Trap heights were adjusted so that catch collection cups were located 0.5–1.5 m above the ground, and trap cups were filled with a blend of propylene glycol and water (~1:3) to preserve captured insects. Traps were positioned >10 m from the nearest potential host tree and >5 m from forest edges.

A line of six traps spaced 450–720 m apart was established along a woods road, and all traps were baited uniformly with frontalin and *alpha*-pinene. The line was divided into two groups of three adjacent traps, and one of the three treatments was assigned randomly to one trap in each group: 1) no additional lure, 2) an *endo*-brevicomin device attached directly to the trap, or 3) an *endo*-brevicomin device secured at 1.5 m height on a plastic rod positioned 6 m east of the trap. All lure components were purchased from Synergy Semiochemicals, Vancouver, British Columbia, Canada (who provided purity information given below). The frontalin lure consisted of two, capped LDPE microcentrifuge tubes (containing ~250 µl each; >95% purity, racemic); the *endo*-brevicomin lure was a single, capped LDPE microcentrifuge tube (~250 µl contents; >95% purity, racemic); and the *alpha*-pinene lure was a single, sealed plastic bag of 7 by 23 cm [~250 ml contents; >95% purity, with 25% of the (+)-enantiomer]. Microcentrifuge tube devices were replaced

when it was observed that less than approximately one centimeter of compound remained in the tube ($\sim 60 \mu\text{l}$); these never ran dry before replacement. The *alpha*-pinene lures were replaced when we assessed that they would be dry by the next catch collection; in only three cases (i.e., two traps on 7 May 2009 and one on 20 May 2010) was an *alpha*-pinene lure found entirely dry at the time of catch collection. Replacement did not necessarily occur simultaneously for the different lures or at regular times during the year. Release rates of lures were measured gravimetrically while they were suspended inside a running fume hood (at room temperature, i.e., @ 20–24°C). Lures had been stored in a freezer (-19°C) up to two years prior to deployment on traps and up to five years prior to gravimetric release rate tests. The pooled ranges of release rates of unused lures from storage, emptied/cleaned/refilled lures (tested for microcentrifuge tube lures only; these measurements were intended to obtain a prestorage and predeployment release rate), and those replaced during the experiment were, for frontalin, 1 to 5 mg per day (tube pair); for *endo*-brevicomin, 0.25 to 1.5 mg/d; and for *alpha*-pinene, 1 to 5 g per day. Although replacement of lures with fresh ones should have resulted in an immediate increase in release rate from traps (devices would have declined in release rate as their contents were depleted), we did not observe abrupt changes in catches (Fig. 1) simultaneously with lure replacement [e.g., 19 August 2009 and 27 May 2011 (both frontalin and *endo*-brevicomin lures replaced) and 6 July 2010 and 17 December 2010 (*alpha*-pinene lures replaced)]. The *alpha*-pinene lures were suspended from the uppermost funnel of the trap, whereas frontalin and *endo*-brevicomin lures (when the latter were attached directly to the trap) were suspended on the fourth from the lowest funnel. Catches were collected at biweekly intervals at which time the treatments were rotated among traps within each group; only the *endo*-brevicomin lure was moved during the rotation. The order of rotation was reversed for traps within either of the two groups (i.e., 1, 2, 3, 1, etc. vs. 3, 2, 1, 3, etc.). Traps were maintained in the field 6 February 2009 to 6 September 2011. Catches of *D. frontalis* and its predator *T. dubius* were separated and counted (*D. frontalis* were also sexed).

For statistical analyses, averaged daily catches were transformed by cube root or log transformation depending on which produced sufficient normality and homoscedasticity based on examination of residuals plots. Mixed-model ANOVAs were executed with PROC MIXED (SAS 9.3). The possibility of treatment \times sex and treatment \times sex \times collection date interactions occurring across the entire trapping period for *D. frontalis* was examined by ANOVA for a stripped split plot design with sex as the subplot factor (note: assumption of a first-order autoregressive structure for errors did not improve fit based on the Bayesian Information Criterion). The cube root transformed catches per day were analyzed by using a mixed model ANOVA with treatment, sex, date, and all possible interactions as fixed factors and trap, trap \times treatment, and trap \times treatment \times date as random factors. Since no treatment \times sex \times date interaction was detected, sexes of *D. frontalis* were pooled in subsequent analysis of date \times treatment interactions. The cube root transformed (pooled) data were analyzed with a mixed-model ANOVA with treatment, date, and their interaction as fixed factors, trap as a random factor and within-trap errors over time assumed to be serially correlated according to a first-order autoregressive process.

As there was a strong treatment \times date interaction, and no objective way to partition the total collection period into smaller meaningful intervals, we performed an ANOVA on cube root transformed catches for each of 64 overlapping data sets formed from every three successive collection dates. Contrasts of the moving

average of every three successive trap collections allowed for comparisons of treatment means within the shortest time interval in which treatments were balanced among traps (i.e., every treatment had been at every trap; normally this was 6 wk). Individual data sets in this series each mimic those of a more typical short-term trapping study with respect to experimental design and statistical power, and we believed that such tests would indicate the ability to repeat results of short-term trapping tests executed at different times during the year. For each data set the mixed model ANOVA was similar to that for two Latin squares with a single fixed factor (treatment) and two random factors (trap and date). All-pairwise contrasts of least squares means were performed via a t-test with Tukey-corrected *P*-values. Catches of *T. dubius* per day were cube root transformed and analyzed as for pooled sexes of *D. frontalis*.

A different analysis was applied to the log transformed ratios of catches of *D. frontalis* (sexes pooled) to *T. dubius* because of the substantial number of missing ratios corresponding to the large number of trap collections in which no *T. dubius* were trapped. A factor “period” was defined to index the 22 successive, nonoverlapping periods each of ~ 6 wk in length and consisting of three collection dates. A mixed-model ANOVA was performed with treatment, period, and their interaction as fixed factors and with trap and date nested in period as the random factors. Least squares means for treatment \times period combinations and a test for variation among periods separately for each treatment were obtained using the LSMEANS statement and SLICE = TREATMENT option. In all tests $\alpha = 0.05$.

Results

When data were analyzed for the entire two-and-a-half year trapping period as a stripped split plot design with beetle sex as subplot factor, the ANOVA indicated a significant interaction between treatment and beetle sex ($F = 4.37$; $df = 2, 198$; $P = 0.014$), but there was not a three-way interaction among treatment, sex, and collection date ($F = 1.16$; $df = 130, 198$; $P = 0.18$). For each of the three treatments, the catch for males was significantly greater than for females ($F > 8.79$; $df = 1, 198$; $P < 0.010$). Because there was not a three-way interaction among treatment, sex, and collection date, sexes were pooled for analysis of treatment effects across the entire sampling period. With the successive catches at each trap analyzed assuming serially correlated errors, factors treatment ($F = 620.3$; $df = 2, 82.8$; $P < 0.001$), collection date ($F = 22.8$; $df = 65, 140$; $P < 0.001$), and the interaction between treatment and collection date ($F = 6.37$; $df = 130, 109$; $P < 0.001$) were significant. Across the entire study period, traps without an *endo*-brevicomin lure (which caught a total of 1,011 beetles; 36.7% females) trapped significantly fewer total *D. frontalis* than either traps with *endo*-brevicomin attached directly to them (total catch = 5,577; 41.5% females; $t = 24.2$, $df = 82.8$, $P < 0.001$) or traps with an *endo*-brevicomin lure located 6 m away (total catch = 17,943; 36.4% females; $t = 34.3$, $df = 82.8$, $P < 0.001$). Traps with the *endo*-brevicomin lure directly attached to them also caught significantly fewer *D. frontalis* than traps with the *endo*-brevicomin lure at 6 m distance ($t = 10.1$, $df = 82.8$, $P < 0.001$). When treatments were compared for each set of three successive trap collections (i.e., one complete rotation of the treatments through all trap positions), at least one of the three possible pairwise contrasts was significant for all but four of the 64 ANOVAs, and these corresponded to four periods that occurred in August through October when catches were extremely low (Fig. 1). Catches by all treatments were highest from late winter through

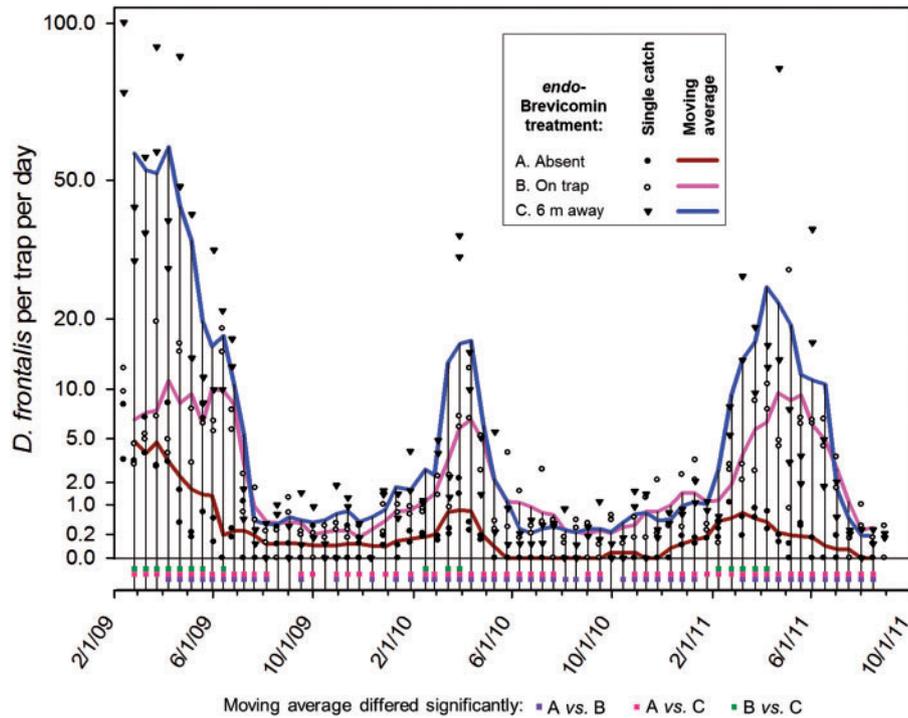


Fig. 1. Catches of the southern pine beetle, *D. frontalis*, in six widely spaced (>400 m) multiple funnel traps during an approximately two-and-a-half year interval in the Homochitto National Forest, Mississippi. Traps were uniformly baited with frontalin and α -pinene and at any given time two traps each had either no additional lure (A) or an *endo*-brevicomin lure that was located either on the trap itself (B) or 6 m away (C). Trap catches were collected and the lure treatments were rotated at each trap position at approximately biweekly intervals. Points along colored lines indicate the moving average value of three successive collection dates (i.e., a complete rotation of all three treatments through every trap position) with the X-axis value representing the midpoint of this ~6-wk trapping interval. Colored squares beneath the X-axis indicate dates on which the value of the moving average differed significantly between lure treatments as determined by an ANOVA of data from the three trap collections (all pairwise treatment contrasts by t-test of least squares means with Tukey correction, $\alpha = 0.05$).

spring with relatively low catch numbers during the rest of the year. Except during those 6-wk intervals when overall catches were very low (fewer than one beetle/trap/day) and the sensitivity of the tests were consequently reduced, presence of an *endo*-brevicomin device located 6 m from the trap significantly increased the catches. Catch enhancement by the *endo*-brevicomin device was similar when either on the trap or 6 m away except during several weeks in the spring when catches were significantly increased by displacement of the *endo*-brevicomin device.

With catches of *T. dubius* analyzed with the assumption of serially correlated errors, response of this predator was significantly influenced by date ($F = 18.4$; $df = 65,140$; $P < 0.001$) and by treatment ($F = 3.21$; $df = 2,98$; $P = 0.045$); however, there was not a treatment by date interaction ($F = 1.16$; $df = 130,114$; $P = 0.204$) and there were no significant pairwise differences among treatments ($P \geq 0.076$ with Bonferroni correction of all-pairwise LS means contrasts). When *T. dubius* catches were compared among treatments as the moving average of three trap rotations, the treatment effect was statistically nonsignificant in all of the 64 ANOVAs ($F < 2.62$; $df = 2,8$; $P > 0.133$). Highest *T. dubius* catches occurred from the late winter through early spring and roughly coincided with the peak flight of *D. frontalis* (Fig. 2). A lesser peak in catches by *T. dubius* was observed in fall. Lowest catches occurred in July and August. Low power to detect treatment differences with catch means averaged over three collection dates in the individual ANOVAs (when compared to greater power for comparison of study-long averages) explains the apparent difference in results from the two types of analysis. With the ratio of *D. frontalis* to *T. dubius* for the entire study analyzed with time summarized by the fixed factor period,

with 22 levels for the 22 successive, (nonoverlapping) 6-wk long periods, there was a significant effect for treatment ($F = 186$; $df = 2,240$; $P < 0.001$), period ($F = 4.14$; $df = 21,35.2$; $P < 0.001$), and the interaction of treatment by period ($F = 2.81$; $df = 42,208$; $P < 0.001$). On average, traps without an *endo*-brevicomin device had a significantly lower *D. frontalis*/*T. dubius* ratio than traps associated with a device (for without vs with device on trap, $t = 15.8$, $df = 243$, $P < 0.001$; for without vs with displaced device, $t = 17.6$, $df = 237$, $P < 0.001$), but the ratio was not significantly affected by whether the device was on the trap or 6 m away ($t = 1.07$, $df = 241$, $P = 0.28$). The *D. frontalis*/*T. dubius* ratio varied significantly among periods when *endo*-brevicomin was absent ($F = 2.97$; $df = 21,87.9$; $P < 0.001$) or when a device was located directly on ($F = 3.10$; $df = 21,87.9$; $P < 0.001$) or 6 m away ($F = 4.92$; $df = 21,84.8$; $P < 0.001$) from the trap. In general, the *D. frontalis*/*T. dubius* ratio was highest in spring and summer and lowest during fall and winter, with this intra-annual variation spanning at least an order of magnitude for all three treatments (Fig. 3).

Discussion

We detected only one flight peak per year by *D. frontalis*, and this occurred from late winter through spring. In 2009 and 2011, elevated catches continued until mid-summer. Catches were consistently low from mid-summer through fall. Past research indicates that the annual activity cycle of *D. frontalis* in the southern United States includes a major dispersal flight in spring and a variable, typically lesser dispersal flight in fall (Moser and Dell 1979b, Ronald F.

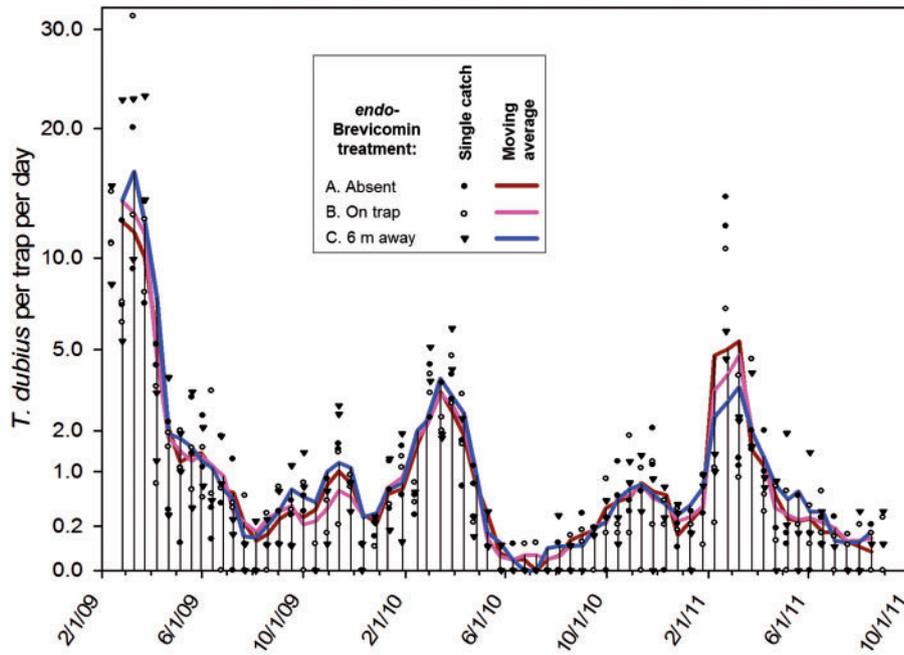


Fig. 2. Catches of the checkered beetle *T. dubius* in multiple funnel traps during a two-and-a-half year interval in the Homochitto National Forest, Mississippi. Traps were uniformly baited with frontalinal and alpha-pinene and at any given time two traps each had either no additional lure (A) or an endo-brevicomin lure that was located either on the trap itself (B) or 6m away (C). Trap catches were collected and the lure treatments were rotated at each trap position at approximately biweekly intervals. Points along colored lines indicate the moving average value of three successive collection dates (i.e., a complete rotation of all three treatments through every trap position) with the X-axis value representing the midpoint of this 6-wk trapping interval. The ANOVAs of the moving averages failed to detect a significant treatment difference with any date.

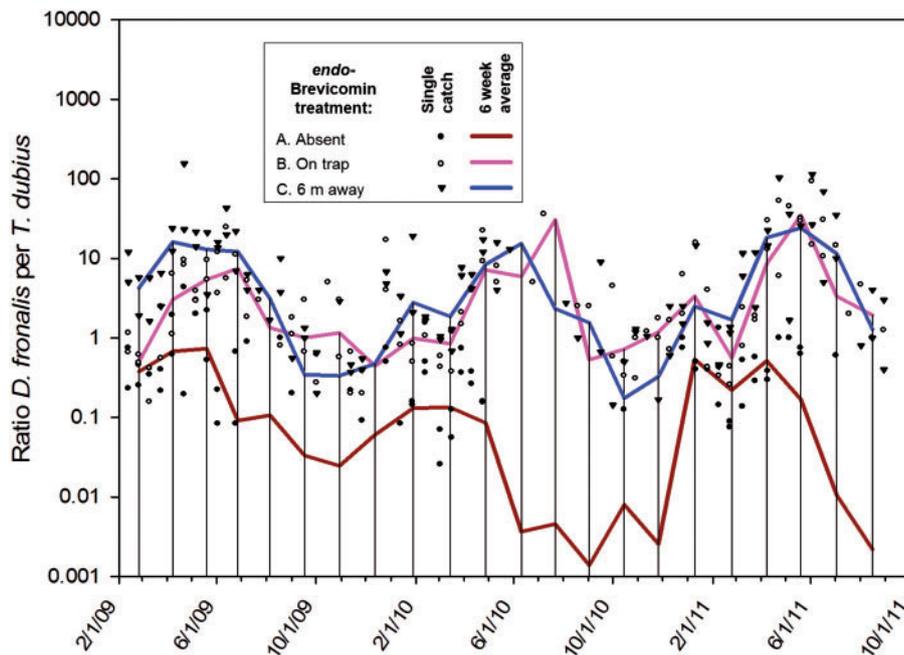


Fig. 3. Numbers of the southern pine beetle *D. frontalis* trapped relative to its predator *T. dubius* in multiple funnel traps during a two-and-a-half year interval in the Homochitto National Forest, Mississippi. Points along the colored lines indicate the average *D. frontalis*/*T. dubius* ratios of three successive trap collection dates with the X-axis value representing the midpoint of the period encompassing three biweekly trap collections. (Averages are back-transformed values of least-squares means estimates calculated in an ANOVA of the log-transformed data. The single catch data are the original, untransformed ratios.)

Billings and Stephen R. Clarke, personal communication). *Dendroctonus frontalis* is multivoltine with up to nine generations per year in the southernmost portions of its range (Birt 2011b), and has overlapping generations through most of the warmer months of

the year (Franklin 1970, Ayres et al. 2011). A higher temperature threshold for eclosion to the pupal stage relative to development of earlier life stages in *D. frontalis* has the effect of synchronizing brood as late instar larvae during the winter (Wagner et al. 1984a,

Trần et al. 2007), thereby also tending to synchronize brood emergence during late winter and spring when temperatures become sufficient for completion of development, adult emergence, and flight (Billings and Kibbe 1978, Friedenberget al. 2007). Furthermore, in summers of outbreak years, *D. frontalis* are concentrated within localized infestations established typically during spring, but they disperse if continuous release of aggregation pheromone from mass-attacked trees within the infestation is interrupted (Gara 1967, Cronin et al. 1999). It has been proposed that prolonged cold temperatures sufficient to prevent flight and attack, combined with synchronization of brood stages in winter, disrupt the continuity of pheromone release by attacking adults within spots and that loss of the aggregating principle triggers dispersal of brood adults from these locations in the spring (Gara 1967, Ayres et al. 2011). Since no infestations were detected in the forests where we performed our tests (i.e., presumably beetles were overwintering predominantly in individual, dispersed trees), our observed spring flight peaks ostensibly were produced not by wintertime collapse of multiple-tree infestations but rather by wintertime synchronization of brood stages, return of temperatures suitable for flight (Moser and Thompson 1986), or some other, unknown drivers of beetle phenology. The very low flight activity of *D. frontalis* that we observed in mid- and late summer is consistent with other reports and was likely influenced by high temperatures which can restrict survival during dispersal and reduce reproduction and brood fitness (Thatcher and Pickard 1964, Moser and Thompson 1986, Hofstetter et al. 2007, Friedenberget al. 2008). However, return of cooler temperatures in the fall failed to restore catches.

A fall flight by *D. frontalis* has been detected in trapping studies conducted at locations both adjacent to and at large distances from active infestations (Moser and Dell 1979b, Ronald F. Billings and Stephen R. Clarke, personal communication). A fall flight peak of *D. frontalis* was not evident in our data. Greater physiological readiness for dispersal in fall as well as spring is implied by elevated responsiveness to pheromone in the laboratory and higher body fat content during both seasons (Hedden and Billings 1977, Roberts et al. 1982, but see Coppedge et al. 1994). Maximum dispersal distance in the field also appears to be greater during fall than summer (Turchin and Thoeny 1993). The present study was conducted during nonoutbreak years, and the absence of multiple-tree infestations as a potential source of dispersing beetles could have reduced fall (and presumably also spring) trap catches relative to what might be observed in outbreak years. It is noteworthy that in the present study the predator *T. dubius*, unlike its prey, did exhibit an increase in catches during the fall, and such a fall increase in *T. dubius* trap catches has been noted previously (Moser and Dell 1979a, Aukema et al. 2005).

endo-Brevicomin appeared to consistently enhance catches of *D. frontalis* during the entire year, although contrasts of moving averages sometimes failed to detect statistically significant increases during periods when catches were very low (i.e., late summer and fall). On average it increased catches to a greater extent when the *endo*-brevicomin device was displaced 6 m from the trap (although it is possible that the same effect would have been at least partially produced by reducing the release rate from the devices attached directly to traps). The total increase in *D. frontalis* catches due to the displaced *endo*-brevicomin device (relative to the absence of *endo*-brevicomin) was ~18-fold during the peak flight months of March through July and ~11-fold during the remainder of the year when catches were low. At no time were catches significantly greater with the lure located on the trap rather than displaced from the trap.

“Multifunctional” bark beetle semiochemicals are those that are attractive or synergistic at low concentrations but become less

attractive and ultimately inhibitory as the concentration is increased (Rudinsky 1973), and pheromone components with this dose-response have been proposed to regulate bark beetle attack densities along the host bole (Hughes 1976, Schlyter et al. 1987, Byers 1989b). *endo*-Brevicomin has been shown to produce a multifunctional dose-response in *D. frontalis* (Sullivan 2016). Presumably, at the low concentrations that are encountered by dispersing bark beetles when they first contact the plume of a multifunctional aggregation pheromone component, attraction is enhanced by a signal indicating an upwind location potentially with ongoing mass attacks, hosts susceptible to colonization through mass attack (due to high local densities of attacking beetles), or mates. However, with closer approach to mass-attacked trees, beetle landing may be deflected by the high concentrations near the semiochemical release point where the host resource may be fully occupied or where no unpaired, prospective mates remain (Hughes 1976, Schlyter et al. 1987, Byers 1989b). Avoidance of locations releasing high levels of pheromone components may also help bark beetles avoid predators, as many predator species are attracted to these compounds (see below). In the current study, the displaced *endo*-brevicomin lure likely provided a long-range synergistic cue to *D. frontalis* but—by being positioned several meters from the attractive, frontalin/host-odor releasing target—not the compound’s short-range, inhibitory effects that would potentially be produced by a co-located *endo*-brevicomin lure.

The tendency by *D. frontalis* to avoid traps with *endo*-brevicomin devices attached directly to them was greatest during the beetles’ spring dispersal flight, which is when infestations become initiated. This might imply that there is greater avoidance of intraspecific competition and greater discrimination of trees in differing stages of mass attack by beetles during the main dispersal period of late winter and spring than during summer and fall at which time dispersal is greatly reduced (Coulson et al. 1985, Rykiel Jr et al. 1988). The particular timing may also reflect the ostensible capacity of *endo*-brevicomin to induce “switching” [i.e., transition of mass attack from one tree to adjacent trees, thereby causing the establishment and then growth of multiple-tree infestations (Renwick and Vité 1970, Powell et al. 1998)] in *D. frontalis*. Multiple-tree infestations typically are initiated in springtime (Thatcher and Pickard 1964) and greater avoidance of *endo*-brevicomin at this time may reflect a greater propensity by mass-attacking beetles to switch to adjacent hosts and thereby form spots. Also, due to high temperatures and resultant shorter survival outside the host tree during summer (Pope et al. 1980, Wagner et al. 1984b), the fitness costs to dispersing beetles due to time and energy consumed in discriminating prospective hosts may be substantially greater. Seasonal changes in responses to pheromones have previously been reported in species of bark beetle including *D. frontalis* (Wood and Bushing 1963, Roberts et al. 1982, Teale and Lanier 1991).

Responses of *T. dubius* to traps baited with frontalin and host odors were not altered by the presence of *endo*-brevicomin lures at any time during the experiment although these lures were highly synergistic to attraction of *D. frontalis*. The absence of effects of *endo*-brevicomin on *T. dubius* trap catches here agrees with previously published studies which indicate that the compound does not alter *T. dubius* behavior (Richerson and Payne 1979, Salom et al. 1992). Like many bark beetle predators, *T. dubius* are attracted to components of their prey’s aggregation pheromones (Borden 1982); this presumably is because their prey are concentrated at release points of these compounds (Aukema and Raffa 2005). *Thanasimus dubius* is strongly attracted to frontalin (Vité and Williamson 1970, Billings and Cameron 1984, Reeve et al. 2009), the major female-

produced component of the *D. frontalis* aggregation attractant, and furthermore this attraction is synergized by *alpha*-pinene, a component of host resin that likewise synergizes attraction of *D. frontalis* (Stauben et al. 2015). Despite the capacity for *endo*-brevicomin to increase attraction of its prey (and thus its presumable potential utility as a prey-location cue), *T. dubius* is apparently unresponsive to this compound. This may be related to *endo*-brevicomin's multifunctionality for *D. frontalis* and its inability to direct landing of *D. frontalis* to the point of its release (Sullivan and Mori 2009). Thus *endo*-brevicomin should be a relatively imprecise indicator of the location of adult hosts for *T. dubius*. By contrast, frontalin and to a lesser extent host odors do cause landings of *D. frontalis* (particularly males) at or near their point of release (Hughes 1976, Sullivan and Mori 2009).

Our data indicated that annual flight activity for *D. frontalis* and its predator *T. dubius* was not in synchrony (i.e., the catch ratio varied substantially over the year; Fig. 3). Our data also underscore the importance of using flight catch data from the same time each year (e.g., based on degree days, as currently is done approximately in the annual, springtime *D. frontalis* survey by means of timing trap deployment to blooming of dogwood) when considering multi-year trends. Furthermore, the lure and deployment method chosen can profoundly affect inter-annual changes in catch numbers. For example, the ratio of catches between the spring flights of 2009 (catches of 6 February through 30 April) and 2010 (catches of 2 February through 3 May) were 2.2:1 and 7.9:1 for traps with *endo*-brevicomin either on the trap or absent, respectively. Another striking demonstration of how lure selection can influence relative population estimates from traps is provided in Sullivan et al. (2011), in which frontalin/turpentine-baited traps were placed inside and 100 and 200 m outside of active *D. frontalis* infestations during summer. When traps inside the infestations and at 200 m outside simultaneously had no release device of *endo*-brevicomin, the average ratio of catches inside:outside was 18:1; when an identical *endo*-brevicomin device was attached simultaneously to these traps, the ratio was 1.7:1. Thus, estimates of relative beetle abundance among locations, as with times, can vary substantially with the choice of lure combination. Consequently, the shape of functions relating catches against local abundance may differ depending on lure chosen and location of trap deployment. Our studies thus indicate potential complications which must be addressed during formulation of algorithms to calculate an absolute abundance of beetles within a specific radius of a trap, as has been attempted at least once with *D. frontalis* (Turchin and Odendaal 1996). Otherwise, with regard to potential modifications of the current protocol for annual population monitoring and forecast of outbreaks, our data indicate that addition of *endo*-brevicomin has the capacity to increase the sensitivity of the *D. frontalis* lure year-round and enhance detection and quantitation of relatively lower population levels of the beetle. However, population densities sufficient to initiate an outbreak are likely detectable with or without *endo*-brevicomin, at least during the spring (Fig. 1), hence the potential for enhancement of forecasting awaits experimental contrasts of the prediction accuracy of lures with or without *endo*-brevicomin.

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