Attraction of the Southern Pine Beetle, *Dendroctonus frontalis*, to Pheromone Components of the Western Pine Beetle, *Dendroctonus brevicomis* (Coleoptera: Curculionidae: Scolytinae), in an Allopatric Zone

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ABSTRACT Subtle differences in pheromone components of sympatric species should be attractive only to the producing species and unattractive or repellent to the nonproducing species, and thereby maintain reproductive isolation and reduce competition between species. Bark beetles *Dendroctonus brevicomis* and *D. frontalis* (Coleoptera: Curculionidae) are known to have common pheromone components, except for *exo*-brevicomin, which is produced by *D. brevicomis*. We predicted that *D. frontalis* would not respond to *exo*-brevicomin outside of the zone of sympathy with *D. brevicomis*. We conducted a field experiment to determine the effect of *exo*-brevicomin on attraction of *D. frontalis* and associated species in Mississippi. We determined whether *D. frontalis* pheromone production differed inside and outside the sympatric zone and compared the pheromone profiles with *D. brevicomis* within the sympatic zone. Trapping studies revealed that *D. frontalis* can perceive and respond positively to *exo*-brevicomin, an aggregation pheromone of a sympatric congener (*D. brevicomis*), at locations hundreds of kilometers outside the sympatric zone. Qualitative pheromone profiles showed that both species emit similar pheromone components: frontalin, *endo*-brevicomin, *exo*-brevicomin, *trans*-verbenol, verbenone, and myrtenol. Although not previously reported, *D. frontalis* males from Arizona produced *exo*-brevicomin. The predator *Thanasimus dubius* did not discriminate traps baited with *exo*-brevicomin and was most attracted to traps with frontalin. *Hylastes* beetles were significantly attracted to traps baited with *exo*-brevicomin in combination with other compounds. Our results raise new practical and evolutionary questions on the role of *exo*-brevicomin in the behavioral ecology of *D. frontalis*. The addition of *exo*-brevicomin to the current lure might increase the efficiency of trapping programs in the southeastern United States.

KEY WORDS pheromones, interspecific communication, reproductive isolation, *exo*-brevicomin, trapping

Pheromone-mediated communication in bark beetles (Coleoptera: Curculionidae: Scolytinae) enables host and mate location, aggregation, and resource partitioning (Wood D. L. 1982, Borden et al. 1986, Byers 2004). Interspecific interactions occur when heterospecific beetles (Svihra et al. 1980), predators (Byers et al. 1984, Reeves 1977), and parasitoids cue into a colonized resource (Ayres et al. 2001, Dahlslet et al. 2004) that is usually rare or patchy in distribution. When two or more species are sympatric and inhabit the same tree, pheromones serve to partition the resource and minimize the deleterious effects of interspecific competition (Byers and Wood 1980, 1981, Light et al. 1983, Rankin and Borden 1991) by maintaining adequate spacing among galleries. The genus *Dendroctonus* includes major killers of pine trees that often occur in outbreaks, during which they can overcome and kill healthy trees (Wood S. L. 1982). Females initiate attack, excavate galleries in the phloem, and release aggregation pheromones that are attractive to both sexes (Borden et al. 1986, Raffa et al. 1993). In most *Dendroctonus* species, females are joined by males that may also produce aggregation pheromones that further facilitate aggregation. Successful colonization and reproduction by beetles in living trees thus requires release of enough aggregation pheromone to ensure the attraction of sufficient conspecifics to overwhelm host defenses (Raffa et al. 1993).

Many bark beetle species have common pheromone components, although the sex that produces them and their function varies. For example, frontalin is the female-produced aggregation pheromone in the southern pine beetle, *D. frontalis* (Kinzer et al. 1968, Renwick and Vité 1969), the Douglas-fir beetle, *D.
psuedotusvogae (Pitman and Vité 1970), and the spruce beetle, *D. rufipennis* (Dyer 1973, 1975). However, in the western pine beetle, *D. brevicomis*, frontal is a male-produced aggregation pheromone (Kinzer et al. 1969), and in the mountain pine beetle, *D. ponderosa*, which is sympatric with all the above species, it is a male-produced (Ryker and Libbey 1982) multi-functional pheromone, facilitating aggregation in low doses and functioning in antiaggregation in high doses (Borden et al. 1987).

In Arizona, several *Dendroctonus* species occur in sympatry (Wood S. L. 1982, Gaylord et al. 2006), and *D. frontalis* and *D. brevicomis* often coexist on the same tree (Breece et al. 2007; R.W.H., unpublished data). In the southern United States, however, *D. brevicomis* is not found east of western Texas (DeMarch and Roetgering 1982), whereas *D. frontalis* occurs from East Texas eastward to the Gulf and Atlantic coasts and has a geographically isolated population in central Arizona (Payne 2006). During host colonization, female *D. frontalis* produce aggregation pheromones frontal and trans-verbelenol when they land on a tree and initiate galleries, attracting both males and females (Payne et al. 1978). Males join females and produce endo-brevicom (Pitman et al. 1969), which augments aggregation and may facilitate switching of attack focus to adjacent trees (Vité et al. 1985, Sullivan et al. 2007). Females may also produce endo-brevicom in minute amounts (Grosman et al. 1997, Sullivan et al. 2007). Both sexes produce anti-aggregation pheromones verbeneone (predominantly males; Grosman et al. 1997) and myrentol, which may function in terminating aggregation (Payne et al. 1978) and reducing intraspecific competition. In *D. brevicomis*, aggregation is mediated by female-produced exo-brevicom (Silverstein et al. 1969, Browne et al. 1979, Byers 1983a) and male-produced frontal (Kinzer et al. 1969). Endo-Brevicomin has been reported to be produced by female *D. brevicomis* and may play a role in aggregation (Libbey et al. 1974). In both *D. brevicomis* and *D. frontalis*, aggregation is terminated by trans-verbelenol and verbeneone produced by females and males, respectively (Renwick 1967, Byers and Wood 1980, Byers et al. 1983, Byers et al. 1984). However, Byers (1983a) found that verbeneone is released early in colonization and hypothesized that it is not used in terminating the attack.

There are conflicting reports on the role of exo-brevicom in the behavior of *D. frontalis*. Vité et al. (1964) observed that volatiles emanating from *D. brevicomis*-infested pine bolts were attractive to *D. frontalis* in the field. However, Payne et al. (1977) found that mixtures of endo- and exo-brevicom reduced the attraction of *D. frontalis* to frontal baits. Both species have common pheromone components (frontalin, endo-brevicom, trans-verbelenol, and verbeneone). Thus, one would expect that pheromones occurring in one species but not the other (e.g., exo-brevicom produced by *D. brevicomis* but not *D. frontalis*) might mediate species specificity in pheromone communication by being attractive only to the producing species and unattractive or repellent to the nonproducing species, and thereby maintain reproductive isolation between the two species where they occur in sympatry (Byers 1989). For instance, *D. brevicomis*, *I. paraconfusus*, and *I. pini* (Say) are sympatric in California and Oregon and compete for ponderosa pine. *Ips paraconfusus* produces (+)-ipsdienol that inhibits response of both *D. brevicomis* and *I. pini* to their aggregation pheromones (Birch et al. 1990, Lainier et al. 1980, Byers 1982, Byers et al. 1984). Investigation of cross-attraction of *D. frontalis* to exo-brevicom in Arizona, where this species and *D. brevicomis* co-occur, revealed a significant increase in trap catch when exo-brevicom was added to the pheromone bait of *D. frontalis* (Gaylord et al. 2006, Hofstetter et al. 2007). This suggests that *D. frontalis* might use exo-brevicom as an attractive cue to locate infested trees and raises the question of whether such cross-attraction persists in Mississippi where the two species are currently geographically isolated from each other.

We predicted that *D. frontalis* would not respond to exo-brevicom outside of its zone of sympathy with *D. brevicomis*; therefore, we conducted a field experiment to determine the effect exo-brevicom has on the behavior of *D. frontalis* in Mississippi. Additionally, to determine whether *D. frontalis* pheromone production differed inside and outside the sympatric zone, we used gas chromatography–mass spectrometry (GC-MS) to examine the pheromone profiles of *D. frontalis* from Mississippi and Arizona and compared these to pheromone profiles of *D. brevicomis* from Arizona. We performed gas chromatographic–electroantennographic detection analyses (GC-EAD) of the exo-brevicom baits on the antennae of *D. frontalis* from Mississippi to confirm that this species perceived the exo-brevicomin these baits. We also examined the effects of exo-brevicom in the attraction of baited traps to other insect species—in particular, the attraction to *Thanasimus dubius* (Coleoptera: Ceridae), a common predator of bark beetles in Mississippi. Possible evolutionary and ecological mechanisms for *D. frontalis* attraction to exo-brevicom are discussed.

**Materials and Methods**

**GC-MS Analyses of Volatiles from Beetles.** Both sexes of *D. brevicomis* (*n* = 15 females and 13 males) and *D. frontalis* (*n* = 18 females and 14 males) were collected in flight traps near Flagstaff, AZ, in August 2005 and May 2007. Beetles were placed individually in 1-ml centrifuge tubes and shipped on ice to the USDA Forest Service, Southern Research Station (Pineville, LA). Beetles that appeared healthy and vigorous were used in aerations to collect emitted volatiles. Volatiles were collected from individual beetles by confining them in 100-µl conical glass vials whose tips were filled with ~0.3 mg of adsorbent Super Q (80–100 mesh) (Sullivan 2005). Individual beetles were inserted abdomen-first into the vials and immobilized using perfluoroalkoxy (PFA) tubing so that the tip of the abdomen was 1–2 mm from the
adsorbent. Vials were loosely closed with a polytetrafluoroethylene (PTFE) lined cap to allow adequate gas exchange for beetle respiration. Volatiles released from the beetles were passively collected on the adsorbent Super Q until they died at room temperature. A stream of purified, humidified air was passed over the vials during gutation of beetles (Sullivan 2005). Once aeration were complete, beetles were removed, and 50 μl redistilled pentane spiked with 3.5 ng/μl heptyl acetate (internal standard) was added to the adsorbent and allowed to sit for 15 min at room temperature. The supernatant was pipetted out, transferred to a GC autosampler vial, and stored at −80°C for further analysis. Because we sampled newly emerged beetles, our results likely represent the pheromone release by insects during attack initiation (Sullivan 2005, Pureswaran et al. 2006, Sullivan et al. 2007).

GC-MS. Samples were analyzed on an Agilent 6890–5973 coupled gas chromatograph–mass spectral detector (GC-MS) using a HP-5MS (Agilent Technologies, Santa Clara, CA) at 50 mm by 0.25 mm (5 μm-film) column. The temperature program was 40°C for 1 min, 16°C/min to 80°C, and then 7°C per min to 230°C and held for 10 min. Carrier gas (helium) flow was a constant 1.0 ml/min, and the injector and detector ports were held at 200 and 240°C, respectively. The amounts of five major pheromones of D. frontalis and D. brevicomis, frontalin, exo-brevicomin, endo-brevicomin, trans-verbolnen, verbeneone, and myrtenol (Payne et al. 1978), were quantified against a standard curve of detector responses to known concentrations of synthetic pheromones by comparing the relative abundance of diagnostic ions in analytes to the internal standard (MSD ChemStation software; G1701DA Version D.00.00.38; Agilent Technologies). Means and SEs of pheromone quantity isolated per beetle were calculated to compare their qualitative profiles between the two species.

GC-EAD Analyses of D. frontalis. We collected volatiles released from the exo-brevicomin baits (Synergy Semiochemicals, Burnaby, British Columbia, Canada) used in field trapping studies in Mississippi. Two baits were placed into a sealed glass enclosure (50 ml) whose inlet received air from an activated-charcoal filter and outlet was connected to a FID cartridge containing conditioned Porapak Q (0.1 g; 50–50 mesh). Air (15 ml/min) was drawn through the enclosure and cartridge for 6 h at 23°C, and the cartridge was extracted with 1.5 ml redistilled pentane. This extract was analyzed by GC-EAD using antennal preparations of D. frontalis reared from infested lobolly pine bolts collected within the Homochitto National Forest in western Mississippi. Techniques for antennal preparation and details of the GC-EAD apparatus are given in Sullivan (2005) and Asaro et al. (2004), respectively. The extract (1 μl) was injected split (1/20) onto an identical column as that used for the GC-MS analyses but with a temperature program of 80°C for 0.1 min, ramped 4°C/min to 140°C, and then 7 min at 250°C to purge the column. This program produced a 1.5-min separation between the elution of (and thus preparation exposure to) exo- and endo-brevicomin. The heights of signal voltage deflections in each GC-EAD run were corrected for time-dependent loss of responsiveness in the antennae by measuring the change in electroantennogram (EAG) responses to a mixture of D. frontalis semiochemicals at the beginning and end of each run (Sullivan et al. 2007). Corrected response voltages of five male and five female antennae responding to exo- or endo-brevicomin in the bait aeration were compared with a paired t-test. The relative proportions of exo- and endo-brevicomin in the bait aeration were determined by flame ionization detector (FID) integration areas.

Field Trapping Studies. Pheromone lures were acquired from Synergy Semiochemicals and released from devices at rates of 4 g/d, 5.2 mg/d, and 1.7 mg/d at 23°C for turpentine (steam-distilled lobolly turpentine; Hercules, Brunswick, GA), frontalin, and exo-brevicomin, respectively. The experiment used 12-unit multiple funnel traps (Lindgren 1983), set up in a straight line along the sides of logging roads, >1 mi from the nearest outbreak, in 10 randomized complete blocks. Traps were ≥50 m apart and were >5 m from any pine to minimize possible effects on experiments of host volatiles or spill-over attacks. Treatments were (1) turpentine, (2) turpentine + frontalin, (3) turpentine + exo-brevicomin, and (4) turpentine + frontalin + exo-brevicomin. Blank controls were not used as a treatment because the attractiveness of our positive control bait (frontalin and turpentine) and the lack of attraction to the negative control (turpentine alone) has been shown numerous times in the literature (Payne 1975, Payne et al. 1978, Billings 1985, Sullivan 2005). The addition of the blank control would have made the tests more prone to error (Strom and Reeve 2004). Captured beetles were frozen until they were identified, sexed (Osgood and Clark 1963), and counted. All data were transformed by log10(x + 1) to meet the assumptions of normality and homoscedasticity and analyzed by analysis of variance (ANOVA) and the Ryan–Einot–Gabriel–Welsch multiple range test (Day and Quinn 1989, SAS Institute 1991–2000). In all cases, α = 0.05.

Results

GC-MS Analyses of Volatiles from Beetles. The qualitative volatile profiles of D. frontalis and D. brevicomis revealed that both species emit frontalin, endo-brevicomin, exo-brevicomin, trans-verbolnen, verbeneone, and myrtenol (Table 1). Although not previously reported, 11 of 14 D. frontalis males from Arizona contained minor amounts (<5 ng) of exo-brevicomin. Trace amounts (0.6–1.9 ng) of exo-brevicomin were also detected in 4 of 31 male D. frontalis collected in Mississippi. However, exo-brevicomin in D. frontalis occurs as a very small percentage (0.2 and 0.002%) in Arizona and Mississippi, respectively) of total pheromone production.

Consistent with previous reports, female D. frontalis and male D. brevicomis are the main producers of frontalin, whereas the roles of the sexes are reversed.
predator *T. dubius* did not discriminate between traps baited with turpentine + frontalin or turpentine + frontalin + exo-brevicomin, but both those treatments caught more beetles than either turpentine alone or turpentine + exo-brevicomin (Fig. 3A; $F_{3,27} = 76.49$, $P < 0.0001$). The number of *Hylastes* captured in traps baited with turpentine + exo-brevicomin was significantly different from turpentine alone ($F_{3,27} = 3.29$, $P = 0.0355$), but there were no significant differences among any of the other treatments (Fig. 3B).

**Discussion**

Our study showed that *D. frontalis* can perceive and respond positively to exo-brevicomin, an aggregation pheromone of a sympatric congener (*D. brevicomis*) at a location hundreds of kilometers outside the sympatric zone (Figs. 1 and 2), even though some males of the species emit it only in minute amounts (Table 1). Previous studies in Arizona have shown that *D. frontalis* responds positively to exo-brevicomin (in combination with frontalin) within its zone of sympatry with *D. brevicomis* (Gaylord et al. 2006, Hosterter et al. 2007). In this study, females were not significantly more attracted to frontalin + turpentine than to turpentine alone, but there was a three-fold increase in attraction with the addition of exo-brevicomin (Fig. 2A). Males, however, were significantly more attracted to turpentine + frontalin than to turpentine alone, and this attraction doubled with the addition of exo-brevicomin to the combination (Fig. 2B). Turpentine + exo-brevicomin without frontalin were not attractive to either sex, indicating that there is a synergistic attractive function of exo-brevicomin when it occurs in combination with frontalin.

Both behavioral and electrophysiological data indicate that *D. frontalis* are highly sensitive to their aggregation pheromone endo-brevicomin (Sullivan et al. 2007). Simultaneously, commercially available sources of exo-brevicomin are commonly contaminated with 1–2% endo-brevicomin (D. Warkchuk, personal communication), as were our trap baits. Hence, this contamination could confound interpretation of studies of *D. frontalis* olfactory sensitivities and behavioral responses that used synthetic exo-brevicomin. However, our GC-EAD results showed that olfactory sensillae on the antennae of *D. frontalis* are sensitive to exo-brevicomin when chromatographically freed of contamination (Fig. 1), and that, at the quantitative proportions released from our trap baits, exo-brevicomin was a stronger olfactory stimulus than endo-brevicomin. This suggests that beetle responses were, in fact, influenced disproportionately by the exo-brevicomin in our baits. Behavioral assays with exo-brevicomin of extremely high purity may be necessary to completely eliminate the possibly confounding influence of endo-brevicomin contamination.

These results contrast with the early experiments of Vité and Renwick (1971), in which they found that...
exo-brevicomin inhibited the attraction of *D. frontalis* to frontalin + α-pinene. Payne et al. (1977) also found that mixtures of *endo-* and *exo*-brevicomin reduced the attraction of *D. frontalis* to frontalin baits. However, electroantennograms performed by Payne (1975) showed no differences in the antennal responses of *D. frontalis* to *exo*-brevicomin compared with frontalin, and subsequent field tests by Payne et al. (1978) observed no inhibition by *exo*-brevicomin when added to traps baited with frontalin + turpentine, leaving the semiochemical function of *exo*-brevicomin unresolved. Our results raise new practical and evolutionary questions on the role of *exo*-brevicomin in the behavioral ecology of *D. frontalis*. It also seems that addition of *exo*-brevicomin to the lure currently prescribed for *D. frontalis* might increase the efficiency of field trapping programs in the southeastern United States.

Several studies have examined interspecific interactions and competition between bark beetle species that co-attack the same tree. In most cases, when two or more bark beetle species attack the same tree, beetle responses to aggregation pheromones of their own species are inhibited by those of another species (Ayres et al. 2001), and this enables them to partition the tree and minimize interspecific competition (Byers and Wood 1980, Sivira et al. 1980, Light et al. 1983, Rankin and Borden 1991, Amezaga and Rodriguez 1998, Poland and Borden 1998). In the absence of pheromones from their own species, beetles may be attracted to other species' aggregation pheromones, which thereby function as host-finding kairomones (Bowers and Borden 1992, Ayres et al. 2001).

The ecological and evolutionary basis for attraction of *D. frontalis* to pheromones of *D. brevicomis* in regions where populations of the two species are allopatric is uncertain. It is possible that the current eastern population of *D. frontalis* descended from western or southern (Mexican) populations that are currently or were historically sympatric with *D. brevicomis*. How premating reproductive isolation mechanisms might separate the two species and enable them to discriminate mates when they attack the same tree in sympatric zones is another question that needs to be addressed. Potential reproductive isolating mechanisms include reproductive incompatibility by means of different seminal rod structures, species-specific differences in acoustic communication, or differences in concentrations of specific pheromone components. Phylogenies of *Dendroctonus* spp. based on the cytochrome oxidase I mitochondrial gene indicate that *D. frontalis* and *D. brevicomis* have different lineages (Kelley and Farrell 1996). Considering that most species of *Dendroctonus* produce *exo*-brevicomin, the attraction to this compound by *D. frontalis* may be a conserved, ancestral trait (Symonds and Elgar 2003). Alternatively, the attraction to *exo*-brevicomin may result from interactions with other sympatric species.
that kill or weaken pines in the southeastern United States. If such species exist, they should have responded to our exo-breviscemin–baited traps, but besides *D. frontalis*, only one pine-infesting beetle (*Hylastes* sp.) responded positively to exo-breviscemin.

The attraction of the bark and stump phloem feeders *Hylastes* spp. to lures with exo-breviscemin has been previously documented in one species (Phillips 1990); however, no known pheromone exits for *Hylastes* spp. (Eidmann et al. 1991). Phillips (1990) found *H. salebrosus* to be attracted to the combination of turpentine, ethanol, and exo-breviscemin. Generally, *Hylastes* spp. are attracted to plant volatiles, such as ethanol and α-pinene (Erbilgin et al. 2001, Petrice et al. 2004, Miller et al. 2005) but not attracted to beetle-produced compounds, such as verbenone and frontalin (Phillips 1990, Lindgren and Miller 2002). Further studies with exo-breviscemin may lead to an effective pheromone lure for *Hylastes* spp. If *Hylastes* in the southeast United States release exo-breviscemin, there is the possibility that *D. frontalis* may use exo-breviscemin to locate *Hylastes*-weakened trees, and thus, provide a mechanism for *D. frontalis* attraction to exo-breviscemin.

Predator responses are often specialized for those bark beetle species that occur within a predator’s geographic range (Bedard et al. 1980, Byers 1982). It is not surprising that the clerid *T. dubius* was most attracted to lures with frontalin and did not discriminate between traps baited with or without exo-breviscemin (Fig. 3A). This pattern varies from observations of the bark beetle predator *Tenechna chlorodina* (Mannerheim) (Coleoptera: Oestridae), which is the most abundant predator where *D. breviscemin* and *D. frontalis* are sympatric in the western United States. *T. chlorodina* is most attracted to lures containing exo-breviscemin (Bedard et al. 1980, Hofstetter et al. 2007) or kairomones of heterospecifics such as *Ips pini* (Gaylord et al. 2006).

Many species of bark beetle produce pheromones that attract large numbers of conspecifics for host colonization and mating (Wood D. L 1982). Therefore, it might be advantageous for colonizing bark beetles to attract as many other beetles, regardless of species, to overwhelm host tree defenses. Interspecific cross attraction to pheromones and multiple species aggregations have been recorded in other systems (Cane et al. 1990, Reid 1999, Ayres et al. 2001) but have received little attention. In these cases, the advantages of aggregation may counterbalance selection for species isolation (Symonds and Elgar 2001). The importance of pheromone specificity could be reduced if species use other cues in mate recognition, such as acoustic signals or behavior. Our qualitative analyses of pheromone blends of sympatric species (*D. frontalis* and *D. breviscemin* in Arizona; Table 1) suggests that interspecific competition has not resulted in a divergence in aggregation pheromone blends among these two species. Additional studies are needed to determine the extent of competition between these species in sympatric zones and whether preexisting isolation mechanisms exist.

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