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RESEARCH PAPER

Enantiospecific responses of southern pine beetle (*Dendroctonus frontalis*) and its clerid predator, *Thanasimus dubius*, to α-pinene

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Abstract Multi-trophic interactions between pine bark beetles, their host trees, and predators are mediated in part by volatile terpenes in host tree oleoresin that can influence aggregation and/or host finding by both prey and predator species. The southern pine beetle, *Dendroctonus frontalis* Zimmermann, mass-attacks pine trees in response to its aggregation pheromone combined with host resin odors including α-pinene. We investigated discrimination of α-pinene enantiomers in olfactory responses of *D. frontalis*, and in behavioral responses of *D. frontalis* and its major clerid predator, *Thanasimus dubius* (Fabricius). Trapping trials in the spring and fall assessed attraction of both species to the *D. frontalis* aggregation pheromone components, frontalin and endo-brevicomin, either alone or in combination with α-pinene lures of differing enantiomeric compositions. α-Pinene lures enriched with the (+)-enantiomer (97.5 %) increased *D. frontalis* catches significantly more than (−)-enriched (93 %) lures, but did not differ from racemic lures. Sexes of *D. frontalis* did not differ from one another in their responses to the enantiomers, and there was no difference in lure discrimination by *D. frontalis* and *T. dubius*. *Thanasimus dubius* did not distinguish between different α-pinene enantiomers. Dose–response electroantennogram studies of *D. frontalis* indicated that their antennae had a slightly lower response threshold to (+) than (−)-α-pinene. Each enantiomer habituated the antennae more to itself than to its antipode, implying the existence of olfactory receptors with differing affinities for enantiomers. The preference of *D. frontalis* for (+)-α-pinene may affect its host selection behavior, and should be considered in population monitoring lures.

Keywords α-Pinene · *Dendroctonus frontalis* · endo-Brevicomin · Enantiomer · Frontalin · *Thanasimus dubius*

Introduction

Monoterpenes are among the dominant volatile organic compounds (VOC) present in oleoresin in conifers (Guenther et al. 2000; Janson 1993). These chemicals serve as defensive compounds against insects and pathogens colonizing the trees (Langenheim 1994). Monoterpenes may also act as kairomones for endophytic herbivores such as bark beetles (Coleoptera: Curculionidae: Scolytinae) that assist them with host location and selection (Seybold et al. 2006). The most abundant monoterpenes of conifers (such as α-pinene, β-pinene, camphene, and limonene) are chiral molecules and are produced in enantiomeric ratios

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that may vary among conifer taxa and populations (Wibe et al. 1998; Phillips et al. 1999; Sjödin et al. 2000). The stereochemistry of VOCs may translate into differential olfactory responses of bark beetles and their major predators, thus leading to cascading chemical interactions at multi-trophic levels.

Dendroctonus frontalis Zimmermann (southern pine beetle) is one of the most economically important pests of *Pinus* in the southeastern USA (Billings 2011). This bark beetle colonizes 18 pine species with loblolly (*P. taeda L.*), pitch (*P. rigida* Mill.), pond (*P. serotina* Michx.), shortleaf (*P. echinata* Mill.), and Virginia (*P. virginiana* Mill.) pines being particularly susceptible hosts (Georgia Forestry Commission 2009). During outbreaks, *D. frontalis* successively colonize adjacent trees thereby creating characteristic "spots" of dying and dead pines scattered across forested landscapes (Franklin 1970; Schowalter et al. 1981). Host pines die after beetles mining within the bark girdle the phloem and introduce a pathogenic blue-stain fungus [*Ophiostoma minus* (Hedgcock)] which blocks water movement in the xylem (Hain et al. 2011). In the southeastern USA, *D. frontalis* is endemic within pine landscapes, but produces regional outbreaks at roughly 10–12 year intervals (Birt 2011). Activity by *D. frontalis* has caused an average of 7 million dollars of loss in annual timber sales in Georgia alone (Georgia Forestry Commission 2009).

Mass colonization by *D. frontalis* of host pines is mediated by a complex of semiochemicals that facilitates host location, host selection, mass aggregation, attack density regulation, and interactions between the sexes (Smith et al. 1993; Sullivan 2011). The hindgut of newly emerged *D. frontalis* females (the gallery-initiating sex) contains pheromone components frontalin and trans-verbenol which are released after landing on a host and attract both sexes (Renwick and Vité 1969). Females that successfully penetrate the outer bark begin feeding on phloem; this stimulates increased production of frontalin by the female and triggers the damaged host tissues to release defensive oleoresin containing α-pinene (Sullivan 2011; Pureswaran and Sullivan 2012). α-Pinene is the predominant volatile constituent of the oleoresin of preferred hosts for *D. frontalis* (Mirov 1961; Phillips et al. 1999). Trans-Verbenol and α-pinene are apparently interchangeable attractive synergists of frontalin, and α-pinene presumably replaces trans-verbenol after oleoresin release commences (Renwick and Vité 1969; Payne et al. 1978). Males arriving on a host in response to female-released attractant produce (+)-endo-brevicomin and verbenone (Pitman et al. 1969; Sullivan et al. 2007). Verbenone is a dose-dependent, multifunctional pheromone component which at low doses arrests walking males and at high doses inhibits attraction to frontalin by both walking and flying beetles (Rudinsky 1973; Payne et al. 1978; Salom et al. 1992; Sullivan et al. 2011). endo-Brevicomin can either enhance or reduce attraction of flying beetles depending on concentration and other factors (Vité et al. 1985; Sullivan et al. 2007; Sullivan and Mori 2009; Sullivan unpublished data).

Thanasimus dubius (Coleoptera: Cleridae) is a generalist predator of bark beetles that utilizes the pheromone component frontalin and volatiles released by attacked pines as kairomones for locating *D. frontalis* infestations (Vité and Williamson 1970; Billings and Cameron 1984; Mizell et al. 1984; Billings 1985; Costa and Reeve 2011). Adult *T. dubius* feed on arriving adult bark beetles whereas the predators' larvae feed on bark beetle brood within the bark (Thatcher and Pickard 1966; Dixon and Payne 1979). Populations of *T. dubius* typically increase as *D. frontalis* colonization intensifies on the landscape and evidence suggests that they are a significant population regulating force for *D. frontalis* (Moser and Dell 1979; Turchin et al. 1991; Reeve 1997; Turchin et al. 1999).

Semiochemicals of *D. frontalis* are used to monitor their population levels each year in the southeastern USA (Turchin and Odendaal 1996; Reeve 1997; Billings 2011). During 4 weeks following the springtime blooming of dogwoods (*Cornus* spp.), *D. frontalis* and *T. dubius* are sampled by a network of multiple-funnel traps deployed throughout the pine forested areas of the southern United States. These traps are uniformly baited with frontalin and a host-component lure that is composed predominantly of α-pinene. Numbers of captured *D. frontalis* and their ratios to *T. dubius* catches are entered into a predictive model that can forecast the summertime abundance of *D. frontalis* infestations with an estimated 75 % accuracy (Billings 2011).

We conducted field trapping and electroantennogram studies to assess whether responses by *D. frontalis* and *T. dubius* to α-pinene are affected by its enantiomeric composition. Our research objectives were to determine whether enantiomeric preferences exist and whether these differ between species, sexes (for *D. frontalis* only), and seasons (fall vs. spring). Pine species differ in the ratios of α-pinene enantiomers they produce (Marques et al. 2012), hence discrimination of α-pinene enantiomers could potentially influence *D. frontalis* host tree preferences and *T. dubius* prey finding efficiency. Furthermore, if enantiomeric preferences are absent, it should be possible to change the enantiomeric composition of the α-pinene component of the population monitoring lure (as may be dictated by cost or availability) without reducing lure sensitivity or requiring recalibration of the predictive model.

Materials and methods

Study sites

Field bioassays were conducted in the spring (19 April–4 May 2011) and fall (12–27 October 2011) within the Oconee National Forest in Greene County, Georgia (Piedmont region of Georgia; 33°39′N, 83°16′W). Traps were deployed in stands of mature loblolly and shortleaf pines that had a mean (±SD) diameter at breast height (DBH) of 53 ± 4.24 cm and were 31 ± 3 years old. The understory was composed of mixed hardwood trees (*Liquidambar styraciflua* L. and *Quercus* species) in clayey-Udults soil (Griffith et al. 2001, National Resources Conservation Service 2013). The local annual mean temperature was 16.3 ± 0.9 °C and precipitation was 191.3 ± 0.4 cm (data supplied by Georgia Automated Environmental Monitoring Network 2013). At the time of the experiment, *D. frontalis* was not in outbreak status and spots had not been detected in this section of the Oconee National Forest, Georgia since 2000 (Georgia Forestry Commission 2009). Natural sources of semiochemicals that might interact with trap lures (e.g., pines damaged by wind, lightning, or insect attack) were not apparent within our sampled stands.

Lure treatments for field trials

Twelve-unit multiple-funnel traps (Lindgren 1983) (Contech International Inc., Delta, British Columbia) were hung from free-standing metal poles with the collection cup positioned 1–1.5 m above ground. Trap collection cups were filled with 250–350 ml of propylene glycol (Prestone® Low Tox® Antifreeze/Coolant, Prestone Products Corporation, Danbury, CT, USA) and water (3:1 mixture) to arrest beetle movement and preserve specimens. Traps were baited uniformly with racemic endo-brevicomin and frontalin plus either: (1) α-pinene enriched in the (+)-enantiomer; (2) α-pinene enriched in the (−)-enantiomer; (3) racemic α-pinene; (4) racemic α-pinene at twice the release rate of treatment 3 (i.e., two racemic release devices), or (5) no α-pinene (control) (Table 1). The α-pinene release devices were constructed from pieces of 3.2 cm diam. low-density polyethylene (LDPE) tubing (2 mil) sealed with an impulse sealer on both ends to create a 5.1 × 3.8 cm enclosure in the shape of an irregular tetrahedron (each seal was at right angles to the other). Each device was filled with 6 ml of α-pinene before the second seal was made (Table 1). The elution rate of α-pinene devices was ~450 mg/day at 21 °C for approximately 1 week. On each funnel trap, one α-pinene release device and two LDPE microcentrifuge tubes containing frontalin (Synergy Semiochemicals Corp., British Columbia) were secured to the lip of the fourth funnel above the collection cup. To enhance beetle attraction to baited traps, an endo-brevicomin bubble device (Synergy Semiochemicals, British Columbia) (Table 1) was attached to the tip of a bamboo gardening rod and positioned 1.5 m above the ground and 6 m southwest of each trap (Sullivan and Mori 2009).

Experimental design for field trials

Four replicate transects of five traps each were established, and one of the five treatments was assigned at random to each trap within each transect. Interference among transects and traps was limited by installing transects ≥150 m apart and traps ≥100 m apart within transects (Turchin and Odendaal 1996). Lure-induced attacks on adjacent trees were limited by installing traps >9 m from all pine trees and >15 m from any pines located in a northeastern (prevailing downwind) direction from each trap. Every 3 days catches were collected and treatments re-randomized without replacement to any previous position within transects so each treatment occurred at each trapping site once during five collection periods. Hence, the experimental design was four complete Latin squares with each square containing five traps (columns) and five consecutive trapping intervals (rows). A random number table was used to re-assign treatments to columns and rows (Winer et al. 1991; SAS 2007).

Table 1 Semiochemical lure components used in trapping trials for *D. frontalis* and *T. dubius* in the Oconee National Forest, Georgia

Semiochemical	Chemical purity[a] (%)	Proportion of major enantiomer[a]	Quantity	Release rate	Release device
(+)-α-Pinene	98.9	99.38 %	6 ml	~450 mg/day at 21 °C	Sealed LDPE sachet
(−)-α-Pinene	99.2	98.25 %	6 ml	~450 mg/day at 21 °C	Sealed LDPE sachet
(±)-α-Pinene	99	(1:1 blend of above)	6 ml	~450 mg/day at 21 °C	Sealed LDPE sachet
(±)-α-Pinene	99	(1:1 blend of above)	2 × 6 ml	~900 mg/day at 21 °C	Two sealed LDPE sachets
(±)-Frontalin	98	Racemic	2 × 300 mg	5 mg/day at 20 °C[a]	Two LDPE microcentrifuge tubes
(±)-endo-Brevicomin	96	Racemic	40 mg	0.3 mg/day at 20 °C[a]	Bubble cap

[a] Product data provided by Sigma-Aldrich, Milwaukee, WI, USA

Captures of adult *D. frontalis* and *T. dubius* were recorded and preserved in 70 % ethanol. Female *D. frontalis* were identified by the presence of a bulging callus partially encircling the anterior portion of the pronotum, and males were identified by the deep medial groove and paired tubercles of the frons (Osgood and Clarke 1963; Payne 1980). *Thanasimus dubius* were not sexed. Voucher specimens were deposited at the Georgia Museum of Natural History, Athens, GA, USA.

Electroantennogram assays

Electroantennogram (EAG) assays were performed to identify differences in olfactory responsiveness by *D. frontalis* to the enantiomers of α-pinene when presented over a range of concentrations. To obtain test subjects, Lindgren multiple-funnel traps were deployed on 5–9 August 2013 in the same location as field trials in Oconee National Forest in Georgia and baited either as treatments 1 or 2 of the field trials. Assayed beetles were derived in equal numbers from traps baited either with (+) or (−)-enriched α-pinene to detect whether individuals trapped with either enantiomer had inherently different olfactory sensitivities to the enantiomers. Trap cups were lined with moistened paper towels, and the mouth of the bottom trap funnel of each trap was covered by a ~20 cm diameter disk of 3.2 mm mesh hardware cloth to prevent entry of beetle predators. Live *D. frontalis* were collected every 1–2 days, and held in refrigeration on pieces of moistened paper wipers until used in EAG tests (i.e., after 6–12 days).

Antennal preparations and signal recording apparatus for *D. frontalis* were as described previously in Sullivan (2005). Antennal preparations were exposed to a stream of humidified, purified air (400 ml/min) delivered from a 1 cm i.d. glass tube into which brief "puffs" (50 ml/min for 2 s) of test odors were introduced from Pasteur pipettes. Each Pasteur pipette contained a 0.5 × 7 cm piece of Whatman #1 filter paper to which was added 35 μl of water (to equalize humidity with the delivery tube airstream) and 10 μl of test solution. This solution consisted of either α-pinene dissolved in mineral oil (Smart Sense™, Hoffman Estates, IL, USA) at 10^{-1}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} by volume; frontalin dissolved in mineral oil (10^{-2}, as a positive control); or pure mineral oil (negative control). A separate dilution series was created for both (+) and (−) α-pinene (for both: Aldrich, 99 % purity and 99:1 enantiomeric ratio). In each 46-min trial, an antennal preparation was exposed sequentially to the positive and negative controls followed by all 12 α-pinene dilutions in random order; this was followed again by the two controls. Each exposure was separated by 3 min, and the antennal voltage amplitudes coinciding with each exposure were recorded. Preparations of eight males and eight females [with half of each trapped with either the (+) or (−) enantiomer of α-pinene] were tested. A single insect was used once for each trial.

In addition, an EAG bioassay was performed to detect the presence of olfactory receptors with differing affinities for either enantiomer of α-pinene. Antennal preparations were partially adapted by exposure for 20 s to one of the enantiomers delivered from a pipette into the airstream passing over the preparation. A test stimulus consisting of a 2-s puff of the same or opposite enantiomer was delivered into the airstream from a second pipette 15 s after initiation of the adapting stimulus. Both pipettes contained a folded, 1 × 7 cm piece of filter paper treated with 35 μl purified water and 25 μl of a 10^{-2} dilution of α-pinene enantiomer in mineral oil, and the air flow through each was 50 ml/min during odor delivery. All four possible combinations of adapting and test stimuli were assayed once on each of 10 preparations (equal numbers of either sex), with a randomized order of presentation for each enantiomer of the adapting and test stimuli. The preparation was allowed to recover for 3.5 min between exposures to olfactory stimuli.

Statistical analyses of field trials

Poisson regression models were used to analyze main effects and interactions for lure treatments, beetle species, sex (*D. frontalis*), and season. Variable parameters included transect, site, and season per collection time period (sample days). Point estimates were approximated using beetle responses (counts) of the mean numbers of beetles captured per trap per sampling period (3 days) ($n = 200$) (SAS 2007). The natural log link function (inherent to Poisson regression) tempered overdispersion and numerous zero counts (no beetles captured within traps) within the data (O'Hara and Kotze 2010). Calculation approximations of effective degrees of freedom of each linear combination of independent sample variances were calculated using Welch–Satterthwaite. Tukey–Kramer tests were used as post hoc tests to analyze treatment main effects within species and within sex of *D. frontalis* and season ($\alpha = 0.05$) (SAS 2007).

Statistical analyses of electrophysiological experiments

To compensate for typical decline in antennal responsiveness over time and its effects on amplitudes of responses to test stimuli during each trial, responses to α-pinene dilutions were normalized using the responses to the positive and negative controls. This was done by calculating the linear equations (with X as time and Y as response amplitude) which joined the positive and negative (respectively) control responses at the beginning and end of each trial. The Y values of these equations which coincided with delivery time of each test stimulus (X value) were used as the time-corrected amplitudes for positive and negative control responses. Estimated negative control response amplitudes were subtracted from the α-pinene dilution response, and then this difference was divided by the estimated positive control response. Normalized responses were cube root transformed to remove heteroscedasticity and analyzed by a mixed-model analysis of variance test (ANOVA) (PROC MIXED; SAS 2007) with dilution concentration (dose), dilution enantiomer, sex, live-trap lure enantiomer, and all possible interactions as fixed factors; and trial nested within live-trap enantiomer by sex as a random factor. A SLICE statement was used to generate pairwise contrasts ($\alpha = 0.05$) of responses to enantiomers within each dose, and the resulting P values were adjusted with a Bonferroni correction. A t test was performed on the means of the cube root transformed responses of each dose and enantiomer to determine whether they exceeded the threshold of response (i.e., were they significantly different from zero with Bonferroni correction of P values for 12 contrasts). The arithmetic differences in amplitude of EAG peaks produced by the (−) and (+) test stimuli during exposure to either (+) or (−) adapting stimuli were contrasted with a paired t test using antennal preparation as subject. Similarly, the raw response amplitudes to (−) and (+) test stimuli were contrasted when either (−) or (+) was the adapting stimulus.

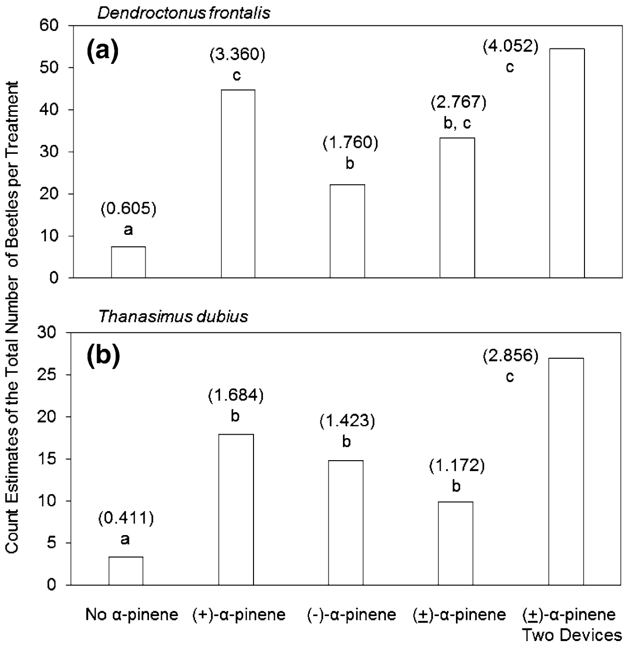

Fig. 1 Values of the least square means table (used in the Poisson analysis) have been converted to the count scale. Natural log (ln) of count estimates of the total number of **A** *Dendroctonus frontalis* and **B** *Thanasimus dubius* captured per treatment during spring (19 April–4 May 2011) and fall (12–27 October 2011) in the Oconee National Forest, Georgia. All traps were baited with frontalin and endo-brevicomin, and either a single device of (+)-α-pinene (containing 4.5 % of the minor enantiomer), (−)-α-pinene (containing 6.5 % of the minor enantiomer), or (±)-α-pinene [a 1:1 mix of the (+) and (−) enantiomers]; two devices of (±)-α-pinene, or no additional lure. Means associated with the *same letter* were not significantly different ($\alpha = 0.05$; Tukey–Kramer Test). The natural log (ln) of count estimates of the total number of beetles captured per treatment is given in *parentheses*

Results

Field trials

Captures of *T. dubius* differed significantly by treatment ($F = 13.19$, $df = 4$, 171.2, $P < 0.001$), but not season ($F = 0.35$, $df = 1$, 8.09, $P = 0.572$), and there was not an interaction between treatment and season ($F = 1.53$, $df = 4$, 170.2, $P = 0.196$). All α-pinene lure treatments significantly increased catches of *T. dubius* (Fig. 1B). *Thanasimus dubius* was significantly more attracted to two racemic α-pinene lures than any other lure treatment [i.e., vs. (+)-α-pinene, $t = -3.07$, $df = 1$, 172.6, $P = 0.021$; vs. (−)-α-pinene, $t = -3.79$, $df = 1$, 173.7, $P = 0.002$; vs. one racemic α-pinene lure, $t = -4.37$, $df = 1$, 172, $P < 0.001$]; but did not distinguish lures differing merely in the enantiomeric composition of α-pinene.

The majority of *D. frontalis* captures occurred in the spring (93 % of total catches) whereas *T. dubius* captures were predominantly in the fall (72 %). There was not a significant interaction between treatment and species, implying that *D. frontalis* and *T. dubius* may not respond differently to the enantiomers of α-pinene ($F = 1.22$, $df = 4$, 345.1, $P = 0.303$) (Fig. 1A, B). There was a significant main effect for lure treatment within *D. frontalis* ($F = 19.43$, $df = 4$, 167.1, $P < 0.001$). With sexes pooled, all α-pinene lure treatments increased the catches of *D. frontalis* (Fig. 1A). The (+)-α-pinene lure and the double racemic lure did not significantly differ from each other, but both increased *D. frontalis* catches compared to the (−)-α-pinene lure. The single racemic lure did not differ significantly from any of the other α-pinene lure treatments in *D. frontalis* catches. There was no season by treatment interaction for *D. frontalis* ($F = 1.04$, $df = 4$, 162.2, $P = 0.388$) indicating that their responses to the enantiomers did not vary significantly between the spring and fall trapping periods; however, fall catches were very low resulting in low power for this test.

More male ($n = 817$) than female ($n = 625$) *D. frontalis* were captured and the main effect of sex was marginally significant ($F = 3.74$, $df = 1$, 22.44, $P = 0.066$). However, there was not a treatment by sex interaction ($F = 0.98$, $df = 4$, 357.9, $P = 0.417$); thus the two sexes of *D. frontalis* did not differ in their discrimination of the enantiomers. With sexes considered separately, responses of both males and females were enhanced by the presence of any α-pinene lure (Fig. 2). Males were more responsive to any α-pinene lures which included the (+)-enantiomer than to the (−)-α-pinene lure [i.e., (−)-α-pinene vs. (+)-α-pinene, $t = 3.60$, $df = 1$, 165.9, $P = 0.004$; vs. one racemic α-pinene, $t = -2.97$, $df = 1$, 166.1, $P = 0.028$; vs. two racemic α-pinene, $F = -5.67$, $df = 1$, 165.8, $P < 0.001$)] (Fig. 2). Traps which included two racemic α-pinene lures were more attractive to female *D. frontalis* than traps with a (−)-α-pinene lure ($t = -3.03$, $df = 4$, 172.8, $P = 0.023$), but otherwise females did not differ in responses to the α-pinene lures.

Electroantennogram (EAG) assays

The mixed-model ANOVA of EAG responses to a dilution sequence of the two enantiomers of α-pinene indicated that the fixed effect dose ($F = 751.3$, $df = 5$, 132, $P < 0.001$), and the interaction between dose and dilution enantiomer ($F = 6.74$, $df = 5$, 132, $P < 0.001$) were highly significant (Fig. 3). Dilution enantiomer was not significant as a main effect ($P = 0.53$). Factors including sex, live-trap enantiomer, and all possible interactions (i.e., their interactions with each other and with any of the other factors in the ANOVA) were not significant ($P > 0.14$). When data were pooled by sex and trap enantiomer (as justified by the lack of significant interactions for these factors), all doses for both stimulus enantiomers exceeded the threshold of detection of the EAG (i.e., produced a response significantly greater than zero) except the 10^{-6} dose for both enantiomers and the 10^{-5} dose for the (−) enantiomer (Fig. 3). Two significant pairwise contrasts were detected between the enantiomers at single doses: (1) response to (−)-α-pinene was significantly greater than to (+)-α-pinene at the highest tested dose of 10^{-1} ($F = 11.38$, $df = 1$, 132, $P = 0.006$); and (2) response to (+)-α-pinene was significantly greater than to (−)-α-pinene at a dose of 10^{-4} ($F = 8.91$, $df = 1$, 132, $P < 0.02$) which was the lowest dose at which both enantiomers exceeded the threshold of detection (Figs. 3, 4).

The arithmetic difference between EAG amplitudes produced by puffs of either the (+) or (−) enantiomers of α-pinene (at a dilution of approximately 2.5×10^{-2}) depended upon the enantiomer used to adapt the antennal preparation prior to and during the puffs ($t = -4.01$, $df = 9$, $P = 0.003$). When the preparation was adapted to the (+)-enantiomer, EAG amplitude was greater to (−) than to (+) puffs (mean difference = 0.118 mv; SE = 0.034; $t = 3.44$, $df = 9$, $P = 0.007$), but the reverse occurred when the preparation was adapted to the

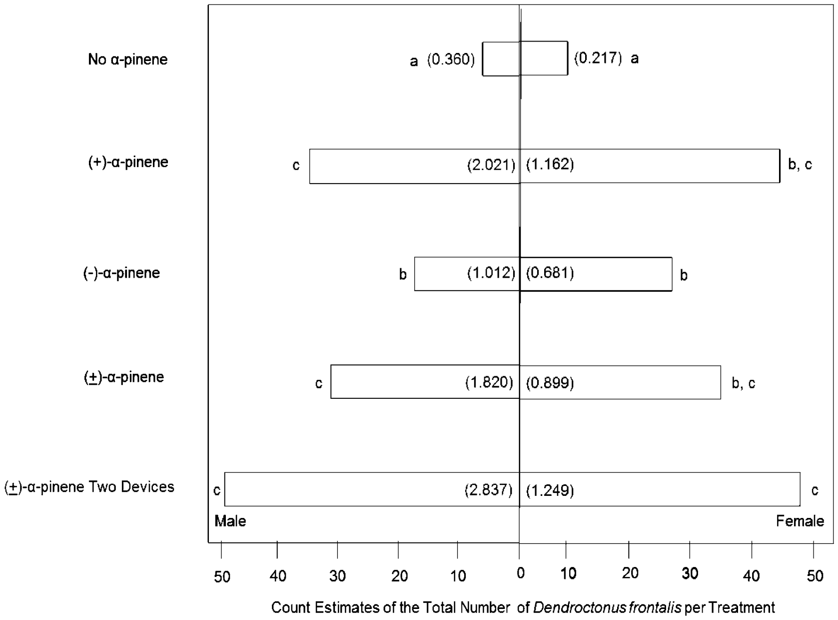

Fig. 2 Values of the least square means table (used in the Poisson analysis) have been converted to the count scale. Natural log (ln) of count estimates of the total number of male and female *Dendroctonus frontalis* captured per treatment during field trials in the spring (19 April–4 May 2011) and fall (12–27 October 2011) in the Oconee National Forest, Georgia. All traps were baited with frontalin and endo-brevicomin, and either a single device of (+)-α-pinene (containing 4.5 % of the minor enantiomer), (−)-α-pinene (containing 6.5 % of the minor enantiomer), or (±)-α-pinene [a 1:1 mix of the (+) and (−) enantiomers]; two devices of (±)-α-pinene, or no additional lure. Means associated with the same letter were not significantly different (α = 0.05; Tukey–Kramer Test). The natural log (ln) of count estimates of the total number of beetles captured per treatment is given in *parentheses*

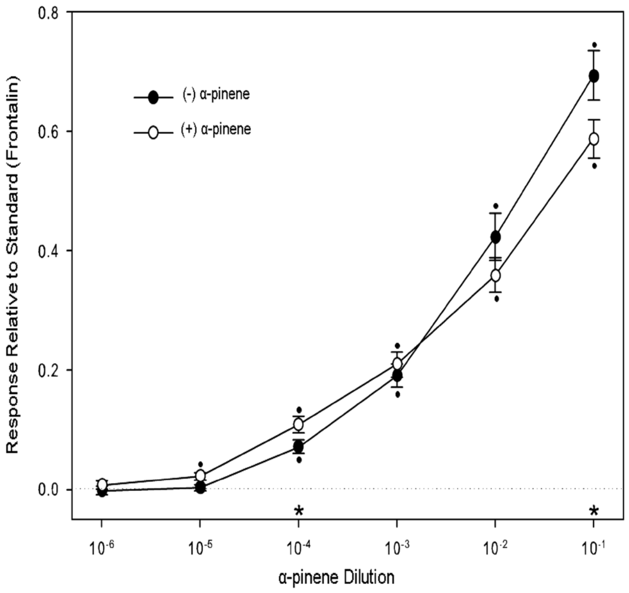

Fig. 3 Electroantennogram amplitudes of *D. frontalis* antenna in response to six concentrations of either (+) or (−) α-pinene ($n = 16$). The *X*-axis labels indicate the proportion of α-pinene dissolved in mineral oil that was used as the odor stimulus. The α-pinene in both the (+)- and (−)-dilutions contained 1 % of the minor enantiomer. Amplitudes were normalized relative to those generated with a standard consisting of the pheromone frontalin (amplitude = 1.0) diluted 1/100 in mineral oil. Data for both sexes ($n = 8$ for each) were combined in this single figure because no sex effects or interactions were detected by ANOVA. *Dots* associated with treatment means indicate that their associated EAG voltage amplitudes were significantly greater than those of the solvent blank (i.e., a response of zero; *t* test with Bonferroni correction of *P* values for 12 contrasts). *Asterisks* indicate that antennal response differed significantly between the two enantiomers at a particular dose (test of effect slices with Bonferroni correction of *P* values for six contrasts). For tests $\alpha = 0.05$

(−)enantiomer (mean difference = −0.060 mv; SE = 0.019; $t = -3.22$, $df = 9$, $P = 0.01$). These differences were relatively small, averaging 26 % of the voltage amplitude of the EAG response to test stimuli.

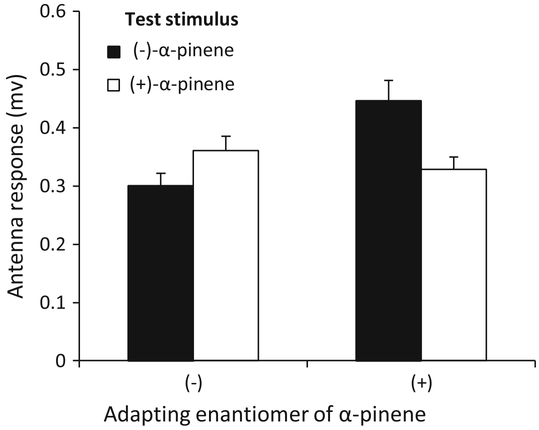

Fig. 4 Mean electroantennogram amplitudes of *D. frontalis* antennae in response to a pulsed test stimulus (2 s) of either enantiomer of α-pinene delivered during partial adaptation of the preparation by the same or opposite enantiomer. Adaptation was accomplished by exposing the antennal preparation for 20 s to an airstream imbued with the adapting odor; the pulsed test stimulus was applied 15 s following initiation of exposure to the adapting odor. Both the adapting odor and test stimulus were released from separate Pasteur pipettes each containing a filter paper strip soaked with a 10^{-2} dilution of the α-pinene enantiomers in mineral oil. The α-pinene in both the (+)- and (−)-dilutions contained 1 % of the minor enantiomer. Data from five individuals of each sex (each exposed to all four possible combinations of adapting and test stimulus in random order) are combined

Discussion

Our results demonstrate that α-pinene can enhance the response of *D. frontalis* and *T. dubius* to traps baited with frontalin and endo-brevicomin. Catches of *D. frontalis* and its predator, *T. dubius*, were on average 2–5 times greater when baited traps included the host monoterpene α-pinene rather than frontalin and endo-brevicomin alone. Previous studies have similarly shown that α-pinene and turpentine derived from *P. taeda* (which has a high α-pinene content; Mirov 1961) can enhance *D. frontalis* and *T. dubius* responses to trap lures which include frontalin (Renwick and Vité 1969; Billings 1985; Sullivan et al. 2007; Hofstetter et al. 2008). Our data show that attraction enhancement can occur across a broad range of enantiomeric ratios of α-pinene [i.e., 7.0–97.5 % (+)-α-pinene], but that sensitivity of lures for detecting *D. frontalis* might be improved by increasing the (+)-composition of the α-pinene component. However, our trapping data were ambivalent regarding whether the (−)-enantiomer has any behavioral activity with *D. frontalis* since the catch enhancement by (−) lures could be attributed merely to their approximately 7 % contamination with the (+) isomer whereas adding (−) did not increase catch enhancement of the (+) lure [i.e., a double release rate of the racemic mixture was not significantly more attractive than (+) alone]. The apparent preference of *D. frontalis* for (+)-α-pinene implies that alterations to the enantiomeric composition of α-pinene in lures used for monitoring *D. frontalis* population levels (Billings 2011) may affect survey results and the forecasting of outbreaks.

Chiral specificity in behavioral responses by *Dendroctonus* bark beetles to α-pinene has been studied extensively only in *Dendroctonus valens* (LeConte) for which α-pinene apparently serves as a primary host location kairomone and is attractive in the absence of other semiochemicals (Erbilgin et al. 2007; Gandhi et al. 2009). *Dendroctonus valens* has alternately displayed significant preference for (+), (−), or neither enantiomer of α-pinene when trapping studies were conducted in different locations within North America and China, and when α-pinene was presented either alone or in combination with other semiochemicals (Hobson et al. 1993; Erbilgin and Raffa 2000; Erbilgin et al. 2007, 2001). Our results with *D. frontalis* involved a single population, and future studies may address the possibility of regional variation in this species' responses to α-pinene enantiomers.

Pine species vary in the enantiomeric ratio of α-pinene present in their oleoresin (Hobson et al. 1993; Marques et al. 2012), although a systematic study of α-pinene chirality in *D. frontalis* host pines has not been published. Thus, the preference of *D. frontalis* for (+)-α-pinene could potentially play a role in host discrimination. This is suggested by the observation that α-pinene in the oleoresin of *P. taeda*, a preferred host of *D. frontalis* (Hain et al. 2011), is predominantly plus (Gambliel et al. 1985; Marques et al. 2012) whereas the resin of an apparently less susceptible species, *P. elliottii* Engelm. (Hodges et al. 1979) is reported to be nearly racemic (Marques et al. 2012). There is little evidence that α-pinene or host odors in general are attractive alone to *D. frontalis* and thus play a role in initial ("primary") host location by this species (Payne and Coulson 1985; Sullivan 2011). Thus, it is unlikely that *D. frontalis*' preference for (+)-α-pinene could directly mediate initial selection or location of a host by pioneering females. However, once beetle colonization is initiated on a host, our study suggests that hosts with relatively higher (+)-α-pinene content will be more attractive and presumably more aggressively colonized than other trees. Furthermore, the chirality of the host's α-pinene can influence the proportions among the monoterpene pheromone components produced by bark beetles (e.g., verbenol; Klimetzek and Francke 1980). Resulting changes in beetle pheromone production could in turn impact the capacity of the beetles to aggregate on and colonize a host, and this could indirectly affect the suitability of trees with specific ratios of α-pinene enantiomers. For example, *Ips paraconfusus* Lanier have been found to produce (+)-*cis*-verbenol when they are exposed to (−)-α-pinene, and (+)-*trans*-verbenol when exposed to (+)-α-pinene, they produced (+)-*trans*-verbenol Renwick et al. (1976). Enantiomeric specificity in bark beetle responses to semiochemicals is common and well documented (Seybold 1993), and, in the case of *D. frontalis*, differing behavioral or olfactory responses have been demonstrated with regard to enantiomers of its pheromone components frontalin (Payne et al. 1982), endo-brevicomin (Vité et al. 1985; Sullivan et al. 2007; Sullivan and Mori 2009; Sullivan et al. 2011) and verbenone (Salom et al. 1992).

Our electrophysiological experiments showed that the magnitude of voltage deflections produced by *D. frontalis* antennae differed for the two enantiomers of α-pinene and that this difference depended on the concentration of the α-pinene stimulus. The (−)-enantiomer stimulated a stronger response than (+) at high concentrations, but the reverse occurred at low concentrations. Furthermore, the threshold of detection of the (+) enantiomer (i.e., 10^{-5}) was apparently lower than for the (−)-enantiomer (i.e., 10^{-4}). Relatively stronger antennal responses at low doses and lower response thresholds ostensibly signify greater capacity of an insect to detect a given compound. Since the two enantiomers of α-pinene have identical vapor pressures and other physical characteristics, the implication is that *D. frontalis* can respond to (+)-α-pinene at greater distances from a source than to (−)-α-pinene when these are released at identical rates. A capacity to sense the (+) enantiomer at greater distances than (−) might in part explain the greater responses of flying *D. frontalis* to traps baited with lures enriched with the (+)-α-pinene enantiomer. The stronger antennal responses to the (−)-enantiomer at high concentrations suggest that the relative stimulatory capacity and ecological significance of α-pinene enantiomers may change when the beetle is close to a source of α-pinene, such as when approaching a gallery entrance.

Furthermore, we observed that partial olfactory adaptation to either enantiomer reduced EAG responses to the adapted enantiomer more than the non-adapted enantiomer. This implies the existence of olfactory receptors with differing affinities for the two enantiomers of α-pinene, which is a prerequisite for the capacity of *D. frontalis* to distinguish the enantiomers. Our antennogram results with *D. frontalis* parallel those of an EAG study of enantiomeric specificity of *D. valens* (White and Hobson 1993). As with *D. frontalis* in our study, *D. valens* did not exhibit sexual dimorphism in EAG responses to α-pinene enantiomers, produced larger EAG voltages in response to (−)-than (+)-α-pinene at high concentrations of exposure, and exhibited enantiospecific adaptation (White and Hobson 1993).

It should be noted, however, that approximately 1 % contamination by the antipode in the tested α-pinene dilutions likely altered the amplitudes of EAG responses somewhat from what would have been produced by the pure enantiomers. However, we presume that the low level of antipode contamination should not have altered results of relative comparisons of responses (i.e., which enantiomer produced a greater response) from those produced by the pure enantiomers.

Bark beetle predators commonly seek their prey by responding to beetle aggregation pheromones often in combination with tree volatiles released by beetle damage (Payne 1989). For example, *T. dubius* has been demonstrated an enantiospecific response to frontalin, the major pheromone of *D. frontalis* (Payne et al. 1984). Furthermore, chirality of host tree monoterpenes can influence responses by bark beetle predators to the pheromones of their prey (Erbilgin and Raffa 2001). However, in our trapping study, we failed to detect a significant behavioral preference by *T. dubius* for either enantiomer of α-pinene. Therefore, we found no evidence that chirality of α-pinene from *D. frontalis*' host pines influences the prey-seeking behavior of *T. dubius* when they use *D. frontalis* pheromones as host location kairomones.

We caught 93 % of the trapped *D. frontalis* in the spring, which was expected since higher trap catches in the spring relative to other times of the year are typical pattern for this species (B.T. Sullivan, unpublished data). In the springtime, *D. frontalis* enter mass dispersal during which they abandon dead trees where they have overwintered and seek new hosts (Sullivan 2011). In contrast, 72 % of *T. dubius* catches were in the fall. Mignot et al. (1969) reported that *T. dubius* has faster development and greater survival in the cooler months of the year (spring and fall). Populations of *T. dubius* also vary as a function of *D. frontalis* populations, and there is typically a predator–prey lag (Reeve 1997; Stephen 2011). The apparent seasonal asynchrony in our catches especially in spring time may reflect this lag period.

Overall, we provide evidence that adults of *D. frontalis* responded (both in field and lab) greater to (+)-α-pinene than the (−) antipode suggesting that the enantiomeric composition of monoterpenes may be an important consideration in lure selection. Such a response was absent in *T. dubius* as it did not distinguish between enantiomers of α-pinene. Hence, the finer-level resolution of enantiomeric selection of pine tree volatiles in a chemosphere may be limited to the herbivorous insect. Since *D. frontalis* is considered ecologically and economically the most important bark beetle species in the southeastern USA, studies assessing their responses to chirality of other abundant pine monoterpenes, and their concentration and dose-levels in lures may be warranted in the future.

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