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Electrophysiological and behavioral responses *Dendroctonus frontalis* and *D. terebrans* (Coleoptera: Curculionidae) to resin odors of host pines (*Pinus* spp.)

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Abstract
Southern pine beetle (*Dendroctonus frontalis* Zimmermann) and black turpentine beetle (*Dendroctonus terebrans* Olivier) are two sympatric bark beetle pests of the southeastern United States of America that adversely affect pine (*Pinus* spp.) health. Successful host tree colonization and reproduction is dependent on a chemical communication system that includes compounds produced by both the beetles and their host trees. To better understand the role of host volatiles in the ecology of these species, we (1) used coupled gas chromatography-electroantennographic detection (GC-EAD) to analyze olfactory sensitivity of *D. frontalis* and *D. terebrans* to volatile constituents of host resin, and (2) investigated olfactory stimulants for behavioral effects on both pest species and a major predator, *Thanasimus dubius* Fabricius (Coleoptera: Cleridae) in field trapping studies. In GC-EAD analyses of the headspace of fresh host resin, antenna of both *D. frontalis* and *D. terebrans* produced strongest responses to *alpha*-pinene, *beta*-pinene, myrcene, and 4-allylanisole. Field tests indicated that *alpha*-pinene, *beta*-pinene, and 4-allylanisole significantly enhanced attraction of *D. frontalis*, *D. terebrans,* and *T. dubius* to traps baited with attractive pheromone components of both bark beetle species, and myrcene diminished this response for *D. frontalis.* The observed attractive synergism of 4-allylanisole contrasts with previously reported repellency of this compound for *D. frontalis* and instead suggests this semiochemical may have multiple ecological roles for this species. Lures used for monitoring *D. frontalis* may be enhanced in sensitivity by adjusting the composition of their host odor components.

Keywords Bark beetles · Cleridae · Monoterpenes · *Pinus* · Semiochemicals

Introduction

The genus *Dendroctonus* is a group of economically and ecologically important bark beetles (Coleoptera: Curculionidae) characterized by a life-history spent primarily in the phloem and cambium of host pines, where they excavate feeding and reproductive galleries (Six and Bracewell 2015). Colonization can result in tree death due to the mining activity of the beetle (which causes girdling of the phloem) and possibly also the effects of weakly pathogenic fungi introduced by the beetles (Nebeker et al. 1993; Six and Wingfield 2011). Conifers have evolved an elaborate chemical defense against biotic invaders that includes the production of toxic oleoresin components and phenolics (Brignolas et al. 1995; Raffa and Smalley 1995; Tisdale et al. 2003; Franceschi et al. 2005; Chiu et al. 2017; Huang et al. 2020). Nevertheless, host trees have strongly co-evolved with *Dendroctonus* and other bark beetles, and monoterpenes present in host oleoresin for defensive purposes can also be attractive and aid in host location and selection (Byers 1992; Seybold et al. 2006; Miller and Rabaglia 2009).

Within the *Dendroctonus* genus, *D. frontalis* and *Dendroctonus terebrans* are the only two species present in the southeastern United States of America (U.S.), and *D.*

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frontalis also occurs in parts of northeastern and southwestern U.S., Mexico, and Central America (Cognato 2011; Dodds et al. 2018). Both beetles colonize all species of pines within their range, but in the southeastern U.S., *D. frontalis* infests predominantly loblolly (*Pinus taeda* L.) and shortleaf (*P. echinata* Mill) pines (Thatcher and Barry 1997; Clarke and Nowak 2009). Typically, these beetle species colonize trees weakened by other agencies such as lightning strikes, other insects, or diseases (Paine et al. 1981; Coulson et al. 1986; Flamm et al. 1993; Sullivan et al. 2003a). Both species may colonize the same host trees and often occur in combination with other bark beetles (Thatcher and Pickard 1966; Flamm et al. 1993; Nebeker 2011). *Dendroctonus frontalis* is considered a more aggressive and destructive pest of pines than *D. terebrans,* although both are capable of being primary agents of tree mortality at high population densities (Hopkins 1909; Smith and Lee 1972; Merkel 1981; Thatcher and Barry 1997; Gan 2004). *D. frontalis* must mass attack to deplete host defenses sufficiently to colonize healthy trees, but *D. terebrans* can colonize trees that continue to produce a defensive response. Both species use semiochemicals in host finding, mate location, and aggregation, and these may include host odors as well as pheromones of conspecific and heterospecific bark beetles [topics reviewed in Sullivan (2016) and Munro et al. (2019)].

Dendroctonus frontalis releases an aggregation pheromone attractive to both sexes that is strongly synergized by odors from the host tree, and this semiochemical combination stimulates and sustains mass attacks on individual trees (Sullivan 2016). The aggregation pheromone of *D. frontalis* consists primarily of the female-produced component frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) and male-produced *endo*-brevicomin (*endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane) (Renwick and Vité 1969; Vité et al. 1985; Sullivan et al. 2007). Frontalin is the only semiochemical attractive to flying *D. frontalis* in the absence of other semiochemicals (Payne et al. 1978), although this attractiveness is weak and dramatically increased by the presence of other pheromone components and host odors (Billings 1985; Sullivan et al. 2007). Although host odors (including odors arising from damaged pine tissues, pine resin, and resin distillates) can strongly increase the attractiveness of the aggregation pheromone, host odors alone have not demonstrated attractiveness to flying *D. frontalis* (Sullivan 2016), and it is unclear how "pioneer" (those first to attack) female beetles locate suitable hosts. At least three non-exclusive mechanisms have been proposed to mediate host selection by pioneer *D. frontalis*: (1) chance landing of beetles on suitable hosts, (2) pheromones of sympatric pine bark beetles, and (3) visual or olfactory stimuli from host trees (Person 1931; Gara et al. 1965; Heikkenen 1977; Payne 1986; Payne et al. 1987; Hain et al. 2011). Although there is no evidence of long-range attraction to uninfested trees by host odors in *D. frontalis*, some evidence suggests host odors could influence host selection at short range by stimulating arrestment (Payne 1986; Payne et al. 1987).

In contrast to *D. frontalis*, *D. terebrans* is strongly attracted to host odors in the absence of pheromone components (Fatzinger 1985; Siegfried et al. 1986), and they apparently use these to locate suitable hosts prior to bark beetle attacks. Hence, *D. terebrans* utilizes a "primary" host attractant, a trait that is typical of *Dendroctonus* species with parasitic relationships with their hosts (Six and Bracewell 2015). As in *D. frontalis,* female *D. terebrans* produce the pheromone components frontalin, and males produce brevicomin (the *exo*- rather than the *endo*-isomer). It is hypothesized that *D. terebrans* pheromone components function primarily in mediating interactions between the sexes rather than in host finding or overcoming host defenses (Payne et al. 1987; Phillips et al. 1989).

Limited study has been devoted to the chemical composition of the host-generated semiochemicals for these two species. Numerous studies have demonstrated that distillates of the volatile components of pine resin (i.e., turpentine) are either attractive or synergize attraction for both species [discussed in Sullivan (2016) and Munro et al. (2019)]. However, the volatile composition of pine resin is highly complex, and can differ by tree species, geographic location, and site characteristics (Mirov 1961; Zavarin et al. 1969; Smith 1977, 2000). The volatile components of the resin of preferred host species for both *D. frontalis* and *D. terebrans* are similar and dominated by monoterpenes (in particular, *alpha*-pinene, *beta*-pinene, *beta*-phellandrene, camphene, myrcene, and limonene) and the phenylpropanoid 4-allylanisole (Mirov 1961). Lower levels (typically <1%) of *alpha*-humulene, *alpha*-phellandrene, *alpha*-terpinene, *beta*-caryophyllene, *gamma*-terpinene, *para*-cymene, sabinene, terpinolene, and tricyclene may also occur (Hodges et al. 1979; Smith 2000; Sullivan et al. 2003b; Turner et al. 2018; Bookwalter et al. 2019). The enantiomeric composition of chiral host monoterpenes may also vary (Mirov 1961; Phillips et al. 1999; Marques et al. 2012). To date, *alpha*-pinene (typically the dominant volatile constituent of resin of host pines) is the only component demonstrated to be an attractant synergist for *D. frontalis* (Staeben et al. 2015), whereas 4-allylanisole can reduce attraction (Hayes et al. 1994a). *D. frontalis* has previously shown a preference for (+)-*alpha*-pinene over (−)-*alpha*-pinene but shows no preference for (+)-*alpha*-pinene over racemic mixtures (Staeben et al. 2015). Although turpentine is attractive to flying *D. terebrans,* none of its major constituents (*alpha*-pinene, *beta*-pinene, camphene, myrcene, limonene, and *beta*-phellandrene) were significantly attractive alone (Siegfried et al. 1986). Further, when combined in proportions matching those in the turpentine, these monoterpenes were less attractive than the turpentine itself, implying that the

turpentine contained additional attractive constituents. Electroantennogram studies of olfactory sensitivity have indicated that antennae of both species can respond to several individual host odor components (Payne 1975; Dickens and Payne 1977; Delorme and Payne 1990; Niño-Domínguez et al. 2015, 2018).

Our study investigated the semiochemistry of host resin volatiles with *D. frontalis* and *D. terebrans* to a depth not addressed in previous research. To detect candidate semiochemicals possibly not identified in prior studies, we applied coupled gas chromatography-electroantennographic detection (GC-EAD) to (1) screen for olfactory stimulants in fresh host resin and (2) assess beetles' relative olfactory sensitivities to these volatiles, others reported in the resin of host species, and two suspected degradation products of resin monoterpenes encountered by the authors in association with aging resin, distillates, and pine tissue. Semiochemical status for olfactory stimulants was then investigated with trapping assays that released these compounds in combination with pheromone components. Our hypothesis was that, since *D. terebrans* displays strong attraction to host odors in the absence of their pheromone whereas *D. frontalis* does not, this disparity could be reflected in differences in both olfactory and behavioral responses to volatile constituents of resin. In-depth knowledge of host-produced semiochemicals for these species may be used to (1) enhance lures for pest detection and monitoring, (2) identify semiochemicals for use in tree and stand protection (e.g., as repellants or attraction disruptants), (3) understand the role of semiochemistry in host discrimination and selection by bark beetles, and (4) elucidate semiochemical interactions between these two species during joint colonization of hosts. Additionally, we examined behavioral responses of the common bark beetle predator, *Thanasimus dubius* Fabricius (Coleoptera: Cleridae), to the experimental lures, since the same resin-associated semiochemicals may influence predator efficiency in locating prey and thereby affect predation rates for pest management (Erbilgin and Raffa 2001a).

Methods

Electrophysiological response

Dendroctonus frontalis and *D. terebrans* used for GC-EAD analyses were reared from logs cut from naturally infested pines in the Homochitto National Forest, southwestern Mississippi, U.S. (approximately 31.4°N, 91.0°W). Additionally, due to difficulties in obtaining sufficient numbers of *D. terebrans* by this method alone, some experimental insects were obtained from pine logs artificially infested in the laboratory (with parents from the aforementioned field-collected logs), and others were collected as callow adults directly under the bark of infested trees at the same location and allowed to melanize in Petri dishes (with moistened filter paper held at room temperature). Beetles used for antennal preparations had emerged or melanized less than 3 weeks earlier, and during this interval were housed at 5 °C in plastic containers with moistened paper wipes. *D. frontalis* and *D. terebrans* were sexed using characters in Wood (1982) and Godbee and Franklin (1978), respectively.

GC-EAD apparatus and antennal preparation methods (Asaro et al. 2004, Shepherd and Sullivan 2013) have been described in detail previously. Briefly, the effluent from the GC (Hewlett-Packard model 5890, Palo Alto, California, U.S.) was split with half delivered to a flame ionization detector (FID) and the remainder conveyed to an antennal preparation via a stream of charcoal-filtered, humidified air. The antennal preparation consisted of a pair of glass capillary electrodes (containing Beadle–Ephrussi ringer's solution and $AgCl_2$-coated silver wires) either inserted into the insect's excised head or placed in contact with one side of the antennal club. Voltage changes across the electrodes were conditioned with a Syntech (Buchenbach, Germany) Auto-Spike 2/3 IDAC and recorded with an SRI Instruments (Torrance, California, U.S.) model 202 analog–digital converter interfaced with a PC operating with Peak Simple software (SRI Instruments). The GC had an HP-INNOWax column (60 m × 0.25 mm × 0.25 µm film; Agilent Technologies, Wilmington, Delaware, U.S.), and used helium as the carrier gas (30.5 PSI constant pressure). The temperature program was 40 °C for 1 min, ramped 16 °C/min to 80 °C, then 7 °C/min to 230 °C held 8 min. GC-EAD analyses were performed with (1) headspace of fresh resin combined from three major host species (*P. taeda*, *P. echinata*, and *Pinus elliottii* Engelm.) of *D. frontalis* and *D. terebrans* and (2) a dose-series (three concentrations) of commercially obtained compounds associated with host pine resin and its distillates.

For resin headspace GC-EAD analysis, resin was tapped from mature trees in Pineville, Louisiana, U.S. (31.36°N, 92.43°W). Glass capillaries (3–5 cm × 1.5 mm i.d.) were inserted into nail-produced holes at 1.5–2 m height on the bole; these penetrated the bark to sapwood depth. Capillaries (one per tree) were left in place until they were at least partially filled with resin (typically 1–2 h). Afterwards, they were removed and immediately placed together (3–6 capillaries) into a 20 ml-capacity amber glass vial with a Teflon-lined septum closure. Although a general effort was made to balance the representation of pine species in the vial headspace, variability in resin production by the individual trees caused disproportions among species. Since the purpose of the test was a broad screening for olfactory stimulants in fresh host resin, we considered this sampling scheme to be adequate. A new headspace vial was prepared fresh each day. The capped vial was shaken to cause resin to exit the capillaries and then left undisturbed for >0.5 h at

room temperature prior to headspace sampling. Headspace air (500 µl) from the interior of the vial was sampled at room temperature with a clean gas-tight syringe and after three syringe pumps was injected directly into the GC inlet (200 °C) in split mode (1:20 split ratio). Six males and six females were analyzed for each species. Composition of the headspace samples was analyzed by coupled gas chromatography–mass spectrometry (GC–MS) (model 6890 GC coupled to a 5973-mass selective detector; electron impact mode and a quadrupole ion trap; Hewlett Packard, Palo Alto, California, U.S.) running with identical oven program and column as the GC-EAD; carrier gas was helium at a constant 1 ml/min. Headspace odors were identified by mass spectral data and retention time matches to commercial standards (*beta*-phellandrene was identified from dipentene, Millenium, Hunt Valley, Maryland, U.S.). Quantitative proportions of compounds in the headspace mixtures were calculated relative to the dominant compound (*alpha*-pinene) from relative FID integration areas corrected with response factors calculated from commercially obtained standards. EAD spikes were considered evidence of an olfactory response if the voltage amplitude at the given retention time was greater than the 90th percentile of the background noise amplitude for 4 or more of the 12 sampled beetles of each species (sexes were pooled to provide better sensitivity; binomial probabilities test, $\alpha = 0.05$).

For GC-EAD dose-series analyses, two synthetic mixtures each with nine different compounds identified from resin of hosts of *D. frontalis* and *D. terebrans* (Mirov 1961; Sullivan 1997; Sullivan et al. 1997; Bookwalter et al. 2019) were prepared as sources of antennal stimuli (Table 1). Monoterpene alcohols, ketones, and aldehydes (which typically appear in minor quantities in pine resin and extracts) were not included since these compounds are often produced by the beetles (i.e., they may have additional origins than the host) and include pheromone components. *beta*-Phellandrene was not available to us in sufficient purity to be included in the tests. Although two monoterpene ethers, 4-cineole and eucalyptol, have only occasionally been detected by the authors in odors of pines of the southeastern U.S., they were included in the GC-EAD test mixtures because of their presence in substantial quantities in some commercial turpentines as well as commercial host odor lures for *D. frontalis* (BTS,

Table 1 Composition of standard mixtures utilized in GC-EAD analyses of antenna of *Dendroctonus terebrans* and *D. frontalis*

Standard mixture[a]	Compound name	CAS number	Source[a]	Concentration of standards injected into GC (µg/µl)[b]		
1	Tricyclene	508-32-7	Aldrich	3.6	0.40	0.040
	(±)-Camphene	79-92-5	Aldrich	3.1	0.34	0.034
	(+)-Sabinene	3387-41-5	Aldrich	2.8	0.32	0.032
	Myrcene	123-35-3	Aldrich	2.7	0.30	0.030
	1,4-Cineole	470-67-7	Fluka	2.9	0.32	0.032
	(S)-(±)-Limonene	138-86-3	Aldrich	2.9	0.33	0.033
	gamma-Terpinene	99-85-4	Aldrich	3.0	0.33	0.033
	Terpinolene	586-62-9	Aldrich	2.8	0.31	0.031
	(−)-Bornyl acetate	5655-61-8	Aldrich	3.4	0.37	0.037
	4-Allylanisole	140-67-0	Aldrich	3.4	0.38	0.038
2	(±)-*alpha*-Pinene	7785-26-4	Aldrich	3.0	0.34	0.034
	(−)-*beta*-Pinene	18172-67-3	Aldrich	3.1	0.34	0.034
	(+) 3-Carene	13466-78-9	Aldrich	3.1	0.34	0.034
	(−)-*alpha*-Phellandrene	99-83-2	Fluka	2.9	0.33	0.033
	alpha-Terpinene	99-86-5	Fluka	2.9	0.32	0.032
	Eucalyptol	470-82-6	Aldrich	3.3	0.36	0.036
	para-Cymene	99-87-6	Aldrich	3.6	0.40	0.040
	para, *alpha*-Dimethylstyrene	1195-32-0	Aldrich	3.1	0.34	0.034
	(−)-*beta*-Caryophyllene	87-44-5	Fluka	2.8	0.32	0.032
	alpha-Humulene	6753-98-6	Fluka	2.7	0.30	0.030

[a]Supplier of the chemicals used as standards were either Sigma-Aldrich (St. Louis, Missouri, United States of America) or Fluka (Buchs, Switzerland)

[b]Calculated from mass or volume present in the original, undiluted mixture corrected for density (liquids), contamination, and further dilution. Two microliters were injected into the GC with a 1/40 split ratio and a 1/1 split between the GC and EAD. Therefore, a rough approximation of quantities of each compound delivered to the antenna was 0.2, 0.02, and 0.002 µg

unpublished data). Several of the compounds were chiral, and the included enantiomeric composition varied among compounds (Table 1). The enantiomeric ratios (in samples from tissues, volatiles, and resin) reported for *alpha*-pinene, camphene, and limonene, vary considerably among major host species for the two bark beetles (Mirov 1961; Phillips et al. 1999; Marques et al. 2012), and they were therefore included as 1:1 blends of manufacturer-labeled (+) and (−)-products. [Enantiomeric excess (EE) was indicated only on the *alpha*-pinene products, with both being >96%.] *beta*-Pinene in hosts is reported in these same references to be >80% (−); hence, only (−)-labelled commercial product was used in the dilutions. For the remaining chiral compounds, no enantiomeric composition data were available for host species, and enantiomeric composition of the included component was that of the commercial sources available to us.

Compounds were assigned to each mixture to maximize antenna recovery time between exposure to consecutively eluting compounds (minimum 13 s retention time difference). Mixtures were tenfold diluted in solvent (hexane) twice to produce three concentrations, and an identical amount of an olfactory stimulant (*endo*-brevicomin) was added to each dilution as an internal standard. Mixtures were prepared by adding standards at a fixed volume using calibrated microcapillaries, and approximate (i.e., uncorrected for density or purity) concentrations of compounds in tested dilutions were 0.04, 0.4, and 4 mg/ml (0.04 mg/ml *endo*-brevicomin in all). Since analyses were run with a 1:1 split ratio between the FID and EAD, a 1:20 split ratio at the GC inlet, and an injection volume of 2 µl, rough approximations of quantities of each compound delivered to the antenna were 0.002, 0.02, and 0.2 µg (all with 0.002 µg *endo*-brevicomin). At the beginning and end of each GC-EAD run, the antenna was exposed to a standard odor stimulus puffed from a Pasteur pipette (10 µl of a 0.5 mg/ml mineral oil solution of frontalin on a strip of filter paper) into the airstream flowing over the antennal preparation. Three to four each of the male and female beetles of each species were assayed with each concentration of either standard. Olfactory response amplitude to test compounds was normalized relative to response amplitude to the *endo*-brevicomin internal standard. This latter value was adjusted to compensate for a decline in antenna responsiveness during the run (due to loss in preparation vigor over time) by presuming a linear trend in antennal response voltage with an x-intercept calculated from the change in response amplitudes to the odor puffs at the beginning and end of the run (Sullivan et al. 2007). A genuine olfactory response was recognized at a given retention time if in three or more runs the EAD peak amplitude exceeded the 90th percentile of background amplitude (binomial probabilities test, $\alpha = 0.05$).

Beetle behavioral response

Compounds eliciting an exceptionally strong olfactory response in *D. frontalis* and *D. terebrans* were field tested in two consecutive trapping studies to determine if these compounds could influence beetle attraction when pheromone components were also present. Experiment 1 assessed the capacity of each selected olfactory stimulant alone to influence responses of both beetle species to their attractive pheromone components. Test compounds that did not enhance beetle attraction in Experiment 1 were further assessed in Experiment 2, in which their effects were observed when released simultaneously with the pheromone and a demonstrated host-produced attractant synergist (*alpha*-pinene). In the absence of a host odor synergist, attraction of both species to their pheromone components is minimal (Phillips et al. 1989; Sullivan 2016); hence detection of possible inhibitory effects required that the lure common to all traps include an attractive host odor component.

Lines of traps were established at four sites in the Oconee Ranger District (ORD), Chattahoochee National Forest, Greene County, Georgia, U.S (center coordinate; site 1: 33.41°N, 83.21°W; site 2: 33.40°N, 83.21°W; site 3: 33.40°N, 83.20°W; site 4: 33.40°N, 83.19°W). The sites were at a sufficient distance from each other to ensure that traps at any two sites were >350 m apart. *D. frontalis* populations were at endemic (i.e., non-outbreak) levels during experiments, as no infestations had been detected that year in this portion of ORD. Sites were comprised of mixed pines (primarily mature *P. taeda* and *P. echinata* Miller) and hardwoods [principally flowering dogwood (*Cornus florida* L.), hickory (*Carya* spp.), oaks (*Quercus* spp.), red maple (*Acer rubrum* L.), sweetgum (*Liquidambar styraciflua* L.), and tulip poplar (*Liriodendron tulipifera* L.)].

Both experiments employed black cross-vane panel traps (IPM Technologies, Portland, Oregon, U.S.) the tops of which were suspended from the metal poles at approximately 2.5 m above the ground. Traps within sites were located >150 m apart to minimize interactions among semiochemical lures and >9 m from the nearest pine to reduce the risk of inducing a beetle attack. Each site had traps equal in number to the treatments, and treatments were assigned randomly to each trap at each site. Treatment positions were re-randomized without replacement every time catches were collected, with collections equal in number to treatments. Hence, our design was a multiple Latin square (to control both temporal and spatial variation within site) that treated sites as squares, and, within each square, traps as rows and collection dates as columns. Trap collection cups were partially filled with dilute propylene glycol (Prestone® Low Tox® Antifreeze/Coolant, Prestone Products Corporation, Danbury, Connecticut, U.S.) to preserve captured insects, and catches

were collected every 4 days. Traps in both experiments were consistently baited with frontalin and both *endo*- and *exo*-brevicomin, as this ternary combination included the major attractive pheromone components of each respective species [frontalin for both species; *endo*-brevicomin for *D. frontalis* and *exo*-brevicomin for *D. terebrans* (Phillips et al. 1989; Phillips et al. 1990; Sullivan 2016)]. At low release rates (similar to those produced by the devices chosen for this study) both isomers of brevicomin have demonstrated attraction-enhancing effects for both species (Phillips et al. 1990; Pureswaran et al. 2008). Relative release rates and enantiomeric ratios of individual host-associated compounds in lures broadly reflected the relative concentrations in the odor blends associated with host resin or commercial turpentine, with the major components (*alpha*-pinene, *beta*-pinene, and myrcene) released at 3–4 g/day @ ~23 °C, and minor components (4-allylanisole, 1,4-cineole, and eucalyptol) tested at 1–2 orders of magnitude lower release rates (Table 2). The rates used for the major monoterpenes reflected those in host-odor component baits used operationally for monitoring *D. frontalis* population levels (Sullivan 2016). 1,4-Cineole was tested at two different rates (approximately 0.01 and 0.1 g/day) to detect possible dose-dependent variation in the behavioral activity of this compound, as suggested by the very low olfactory response thresholds of both species to it. Release devices were all attached adjacent to one another at the center of each panel trap.

Experiment 1: Response to host volatiles plus frontalin and brevicomin

Eight panel traps were established at each site (32 traps total; 256 samples) in April 2018. Treatments (i.e., additions to the ternary pheromone lure) were (1) no additional semiochemical (control), (2) *alpha*-pinene, (3) *beta*-pinene, (4) myrcene, (5) 1,4-cineole (lower rate), (6) 1,4-cineole (higher rate), (7) eucalyptol, and (8) 4-allylanisole (Table 2).

Experiment 2: Response to host volatiles plus frontalin, brevicomin, and alpha-pinene

Five panel traps were established at each site (20 traps total; 100 samples) in May 2018. In addition to the ternary pheromone blend, the standard lure for experiment 2 included the *alpha*-pinene device utilized in experiment 1. Treatments (i.e., additions to the standard lure) were: (1) no additional semiochemical (control), (2) myrcene, (3) 1,4-cineole (lower rate), (4) 1,4-cineole (higher rate), and (5) eucalyptol. For both experiments, collected adult *D. frontalis*, *D. terebrans*, and *T. dubius* predators were identified, and voucher specimens were deposited at the Insect Collection at the Natural History Museum, University of Georgia, Athens, Georgia, U.S.

Statistical analyses

Trap catch data were non-normal (Shapiro–Wilk's test) and over-dispersed (Cameron and Trivedi 1990); therefore, the main effects of the lure treatment on insect captures (i.e.,

Table 2 Composition, construction, and release rates of lures used in trapping study

Compound	Source[a]	Purity (%)[b]	Release device	Device load	Release rate @ mean 21 °C
(±)-*alpha*-Pinene	Sigma-Aldrich	≥97	120-ml brown glass bottle with 9.5-cm-diam. dental wick protruding through cap	85 g	4 g/day
(−)-*beta*-Pinene	Sigma-Aldrich	≥97	120-ml brown glass bottle with 9.5-cm-diam. dental wick protruding through cap	85 g	4 g/day
(±)-Frontalin	Synergy semiochemicals		LDPE microcentrifuge tube (×2)	275 mg	5–6 mg/day
(±)-*endo*-Brevicomin	Synergy semiochemicals		Flexlure (polymer matrix)	11.2 mg	0.12 µg/day
(±)-*exo*-Brevicomin	Synergy semiochemicals		Flexlure (polymer matrix)	11.2 mg	0.12 µg/day
Myrcene			4 oz amber glass Boston round bottles with black ribbed cap and 3/8″ dental wick	71 g	3–4 g/day
1,4-Cineole	Sigma-Aldrich	≥95	LDPE microcentrifuge tube (low release rate) Sealed LDPE transfer pipette (high release rate) (×2)	260 mg 2.6 g	7 mg/day 105 mg/day
Eucalyptol	Sigma-Aldrich	99	Sealed LDPE transfer pipette	2.8 g	45 mg/day
4-Allylanisole	Sigma-Aldrich	98	Sealed LDPE transfer pipette	2.9 g	48 mg/day

[a]Sigma-Aldrich Corporation, St. Louis Missouri, United States of America. Synergy Semiochemicals, Delta, British Columbia, Canada

[b]As indicated by the manufacturer

response variable) were determined using negative binomial generalized linear models (GLMs) with fixed effects:

$$Y_{ijkl} = \exp\left(\ln(\mu) + \tau_i + \delta_j + \gamma_k + \tau_i\delta_j + \tau_i\gamma_k + \gamma_k\rho_l\right), \quad (1)$$

where τ represents the ith treatment, δ represents the jth site, γ represents the kth date, and ρ represents the lth trap. Sum-to-zero contrasts were used for site and trap, so these terms represent differences from the grand mean for each parameter. For all three species, interactions were found to be non-significant and were removed from the final model.

Post hoc Dunn's tests (i.e., nonparametric pairwise multiple-comparison procedure) with a Holm stepwise adjustment were performed to detect significant differences between lure treatments. Due to low trap catches for *D. terebrans* in experiment 1, trap catches were summed within site (32 samples; four per treatment), and the log-transformed data (which met parametric assumptions) were analyzed assuming normally distributed errors as a complete block design with site as a block (i.e., model with main effects treatment and site) (Eq. 2).

$$\log(Y_{ij}) = \mu + \tau_i + \delta_j. \quad (2)$$

Since *D. terebrans* in experiment 1 met parametric assumptions, Post hoc Tukey's HSD range tests were performed when a significant main effect for treatment was detected. All analyses were completed using R statistical software version 3.6.2 (R Core Team 2019) and RStudio (RStudio Team 2016) using the packages FSA (Ogle et al. 2019), ggplot2 (Wickham 2009), lattice (Sarkar 2008), MASS (Venables and Ripley 2002), multcomp (Hothorn et al. 2008), plyr (Wickham 2011), and rcompanion (Mangiafico 2018). All tests used an alpha level of 0.05.

Results

Electrophysiological response

In the GC-EAD tests with headspace of fresh pine resin (Fig. 1), significant olfactory responses were registered from both species at the retention times of five FID peaks (followed by their quantitative contribution to the odor composition in the same headspace): *alpha*-pinene (66–73%), *beta*-pinene (22–28%), myrcene (0.90–2.25%), limonene (0.72–4.7%), and 4-allyanisole (0.10–0.72%). Significant responses also were recorded at the retention times of tricyclene (0.33–0.43%) in *D. terebrans* and *beta*-phellandrene (0.30–1.2%) in *D. frontalis.* For both species, strong responses were observed consistently only at the retention times of *alpha*-pinene, *beta*-pinene, myrcene, and 4-allylanisole (Fig. 1).

All compounds in the two synthetic blends elicited an electrophysiological response in *D. frontalis* and/or *D. terebrans* at least with the highest concentration of exposure (Figs. 2, 3). *D. frontalis* and *D. terebrans* both displayed particular sensitivity (indicated by a low response threshold and generally higher response amplitudes) to *alpha*-pinene, *beta*-pinene, myrcene, 1,4-cineole, eucalyptol, *alpha*-terpinene, and 4-allylanisole. The two species differed conspicuously only in sensitivity to the sesquiterpene *alpha*-humulene which produced a response in *D. frontalis* at all three concentrations but did not generate a response in *D. terebrans*.

Beetle behavioral response

Experiment 1: response to host resin volatiles plus frontalin and brevicomin

D. frontalis was the most abundant beetle (12,541 adults) trapped followed by *T. dubius* (7679), and *D. terebrans* (168). Presence of either *alpha*-pinene ($z = 16.1$; $p < 0.001$), *beta*-pinene ($z = 10.2$; $p < 0.001$), or 4-allylanisole ($z = 14.5$; $p < 0.001$) increased catches of *D. frontalis* by the pheromone blend alone (Fig. 4a). Both *alpha*-pinene and 4-allylanisole increased average catches approximately 50-fold (albeit with a high degree of variance). The two treatments did not differ in their effect, and both produced greater enhancement than the *beta*-pinene treatment. In contrast, a high-rate of 1,4-cineole ($z = -2.52$; $p = 0.016$) and myrcene ($z = -2.17$; $p = 0.040$) reduced *D. frontalis* captures by threefold, while eucalyptol and low 1,4-cineole had no effect on catches. Although *D. terebrans* was trapped in low numbers, their catches were increased by all lure treatments other than eucalyptol ($F_{8,23} = 5.74$; $p < 0.001$) (Fig. 4b). Mean catches of *T. dubius* by the pheromone blend were increased greater than fivefold by *alpha*-pinene ($z = 11.4$; $p < 0.001$), *beta*-pinene ($z = 11.8$; $p < 0.001$), and 4-allylanisole ($z = 7.39$; $p < 0.001$) (Fig. 4c). The increase caused by 4-allylanisole was significantly less than that of either *alpha*- or *beta*-pinene. Low 1,4-cineole also increased catches of *T. dubius* ($z = 11.4$; $p < 0.001$); however, we saw less than a doubling in numbers. High 1,4-cineole, eucalyptol, and myrcene did not affect trap catches of *T. dubius* (Fig. 4c).

Experiment 2: response to host resin volatiles plus frontalin, brevicomin, and alpha-pinene

Dendroctonus frontalis was the most abundant beetle species trapped (5976 adults) followed by *T. dubius* (3083), and *D. terebrans* (166). Only myrcene altered (reduced) trap catches by the standard attractant composed of pheromone components plus *alpha*-pinene ($z = -2.08$; $p = 0.037$) for *D. frontalis* (Fig. 5a). All other host odor treatments did not alter responses: low 1,4-cineole, high 1,4-cineole, and

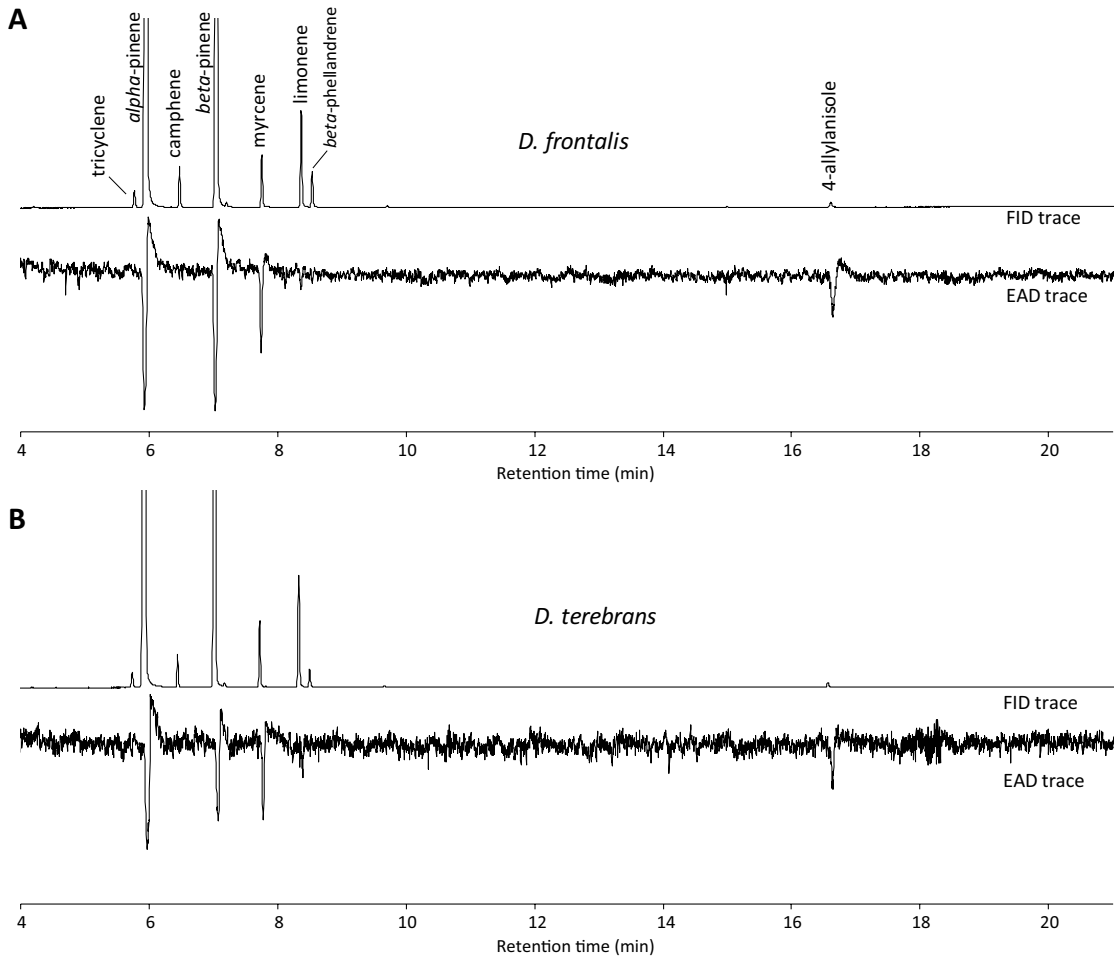

Fig. 1 Coupled gas chromatograph-electroantennographic detector (GC-EAD) recordings of the antenna of bark beetles *Dendroctonus frontalis* (**a**) and *D. terebrans* (**b**) responding to the headspace of freshly tapped resin of host pines. Both the flame ionization detector (FID) and electroantennographic detector (EAD) traces represent composites (averaging of voltages at identical retention times) of multiple analyses that had low EAD noise levels (for *D. frontalis*, four analyses of each sex; for *D. terebrans*, analyses of two males and four females). Responses for males and females were similar within species and thus combined. Peak labels are applicable to both traces

eucalyptol. For *D. terebrans*, catches by the pheromone and *alpha*-pinene combination were altered by low ($z = 1.74$; $p = 0.04$) and high ($z = 2.88$; $p = 0.004$) 1,4-cineole and eucalyptol ($z = 2.68$; $p = 0.007$) (Fig. 5b). All three lure treatments increased mean catches by around threefold. Addition of myrcene did not affect trap catches of *D. terebrans*. Lure treatment did not have a significant effect on catches of *T. dubius* (Fig. 5c).

Discussion

In our GC-EAD studies, *D. frontalis* and *D. terebrans* exhibited similar olfactory response profiles to volatiles associated with resin of their hosts. The odors in the direct headspace samples of fresh resin presumably reflected the same compounds and relative proportions that are encountered in the air by a bark beetle responding to constitutive resin released from a potential host tree as a result of penetration of the bark (as might result from a beetle attack or mechanical damage to the tree). Thus, olfactory stimulants detected in these analyses are candidates as mediators of host finding, selection, and acceptance. The five compounds in resin headspace that generated an olfactory response in both beetle species (*alpha*-pinene, *beta*-pinene, myrcene, limonene, and 4-allylanisole) are the predominant components of resin of their shared host species (Mirov 1961; Cook and Hain 1986; Strom et al. 2002; Bookwalter et al. 2019). These compounds were not the only olfactory stimulants in the resin volatiles, as some stimulants identified in the dose–response study such as sabinene were found in very small amounts by GC–MS in the resin headspace; however, the concentrations were likely beneath the threshold of antennal

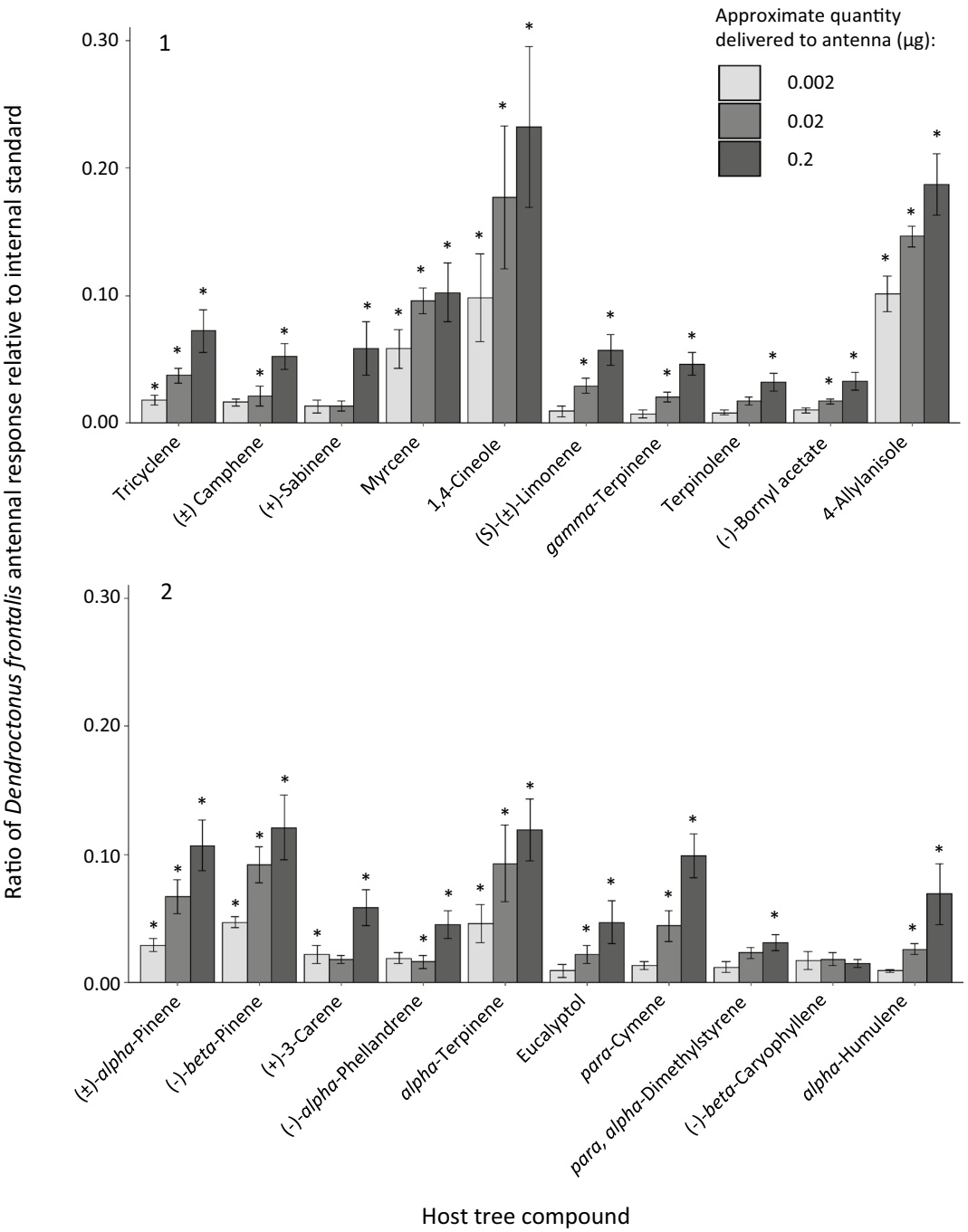

Fig. 2 Responses (±SE) of *Dendroctonus frontalis* antenna exposed to three dose levels of two different mixtures (**1** and **2**; Table 1) of selected pine-associated volatiles in a GC-EAD. Data are the combined responses of three females and three males tested at each concentration. *Y* axis values are amplitude of voltage response to the indicated compound divided by the response to an olfactory stimulant internal standard (*endo*-brevicomin) included in identical concentrations in both mixtures. An *asterisk* above a *bar* indicates that a genuine olfactory response was detected (i.e., the signal amplitude was consistently greater than background noise; see text)

response at the assayed concentration of the headspace. In general, both beetle species exhibited greater sensitivity (Figs. 2, 3) to the more abundant hydrocarbon monoterpenes (e.g., *alpha*-pinene, *beta*-pinene) associated with the resin of their hosts. Evident exceptions to this trend were camphene (often an abundant host resin component but not a strong olfactory stimulant) and *alpha*-terpinene (a strong olfactory stimulant but typically present in minute quantities in resin). This general resemblance of olfactory response profile with the odor composition of the

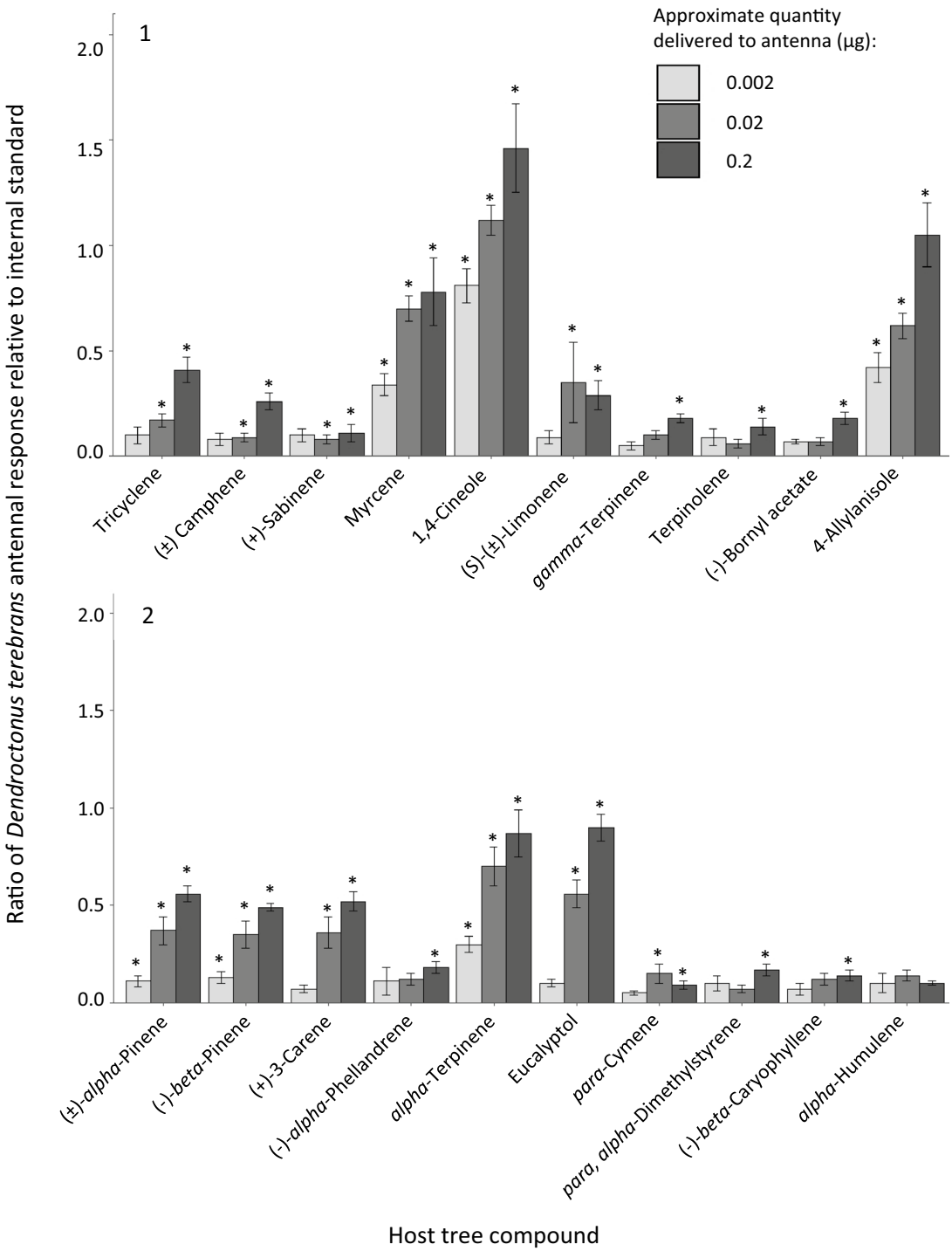

Fig. 3 Responses (±SE) of *Dendroctonus terebrans* antenna exposed to three dose levels of two different mixtures (**1** and **2**; Table 1) of selected pine-associated volatiles in a GC-EAD. Data are the combined responses of four females and four males tested at each concentration. Otherwise, as Fig. 2 legend

host's constitutive resin is consistent with adaptations by these species to their host taxa (Becerra 1997; Bruce et al. 2005). Further, the similarity of the olfactory response profiles between these two bark beetle species suggests that their semiochemical-mediated host interactions are governed by many of the same compounds, and that both species—despite conspicuous differences in life-history strategies and host use—are using olfaction to derive similar information about their hosts. Olfactory sensitivity by *D. frontalis* to *alpha*- and *beta*-pinene, and 3-carene

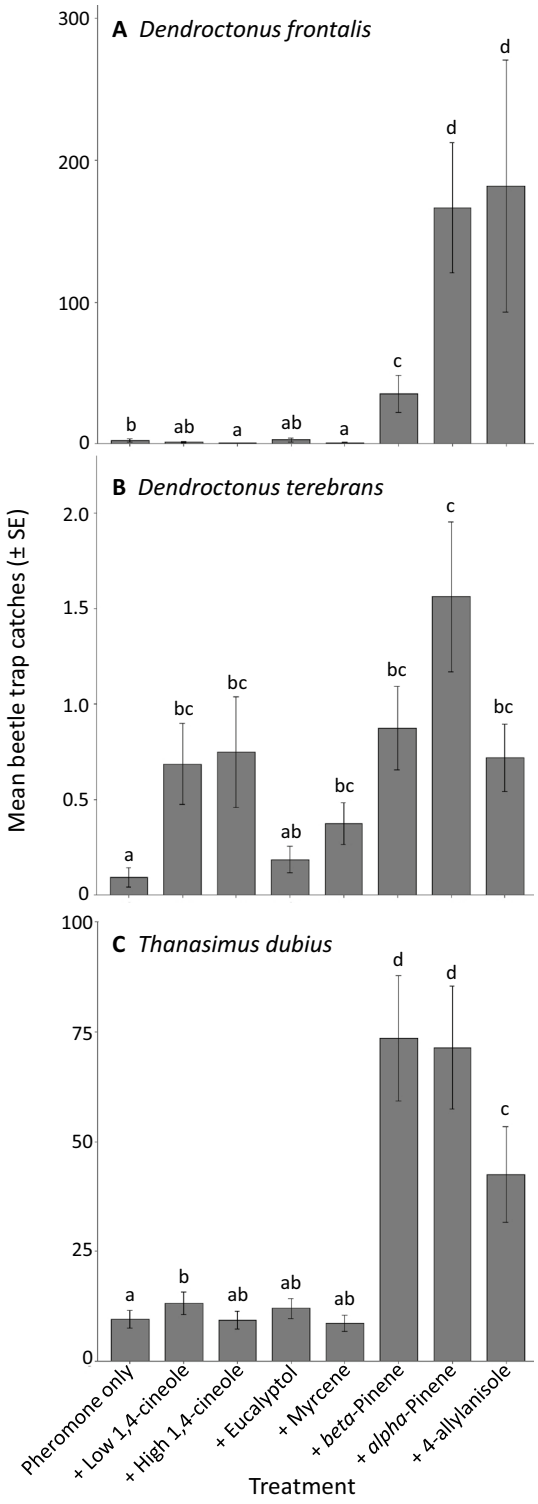

Fig. 4 Mean number (±SE) of bark beetles **a** *Dendroctonus frontalis*, **b** *D. terebrans*, and **c** clerid predator *Thanasimus dubius* trapped with different host-associated olfactory stimulants for both species **a** and **b** in combination with a blend of pheromone components for both species (frontalin, *endo*-brevicomin, and *exo*-brevicomin). Lure composition and release rates are in Table 2. The control had pheromone but lacked a host odor lure. *Bars* with the *same letters* were not significantly different [Dunn's test; for *D. terebrans*, Tukey HSD; ($\alpha = 0.05$)]

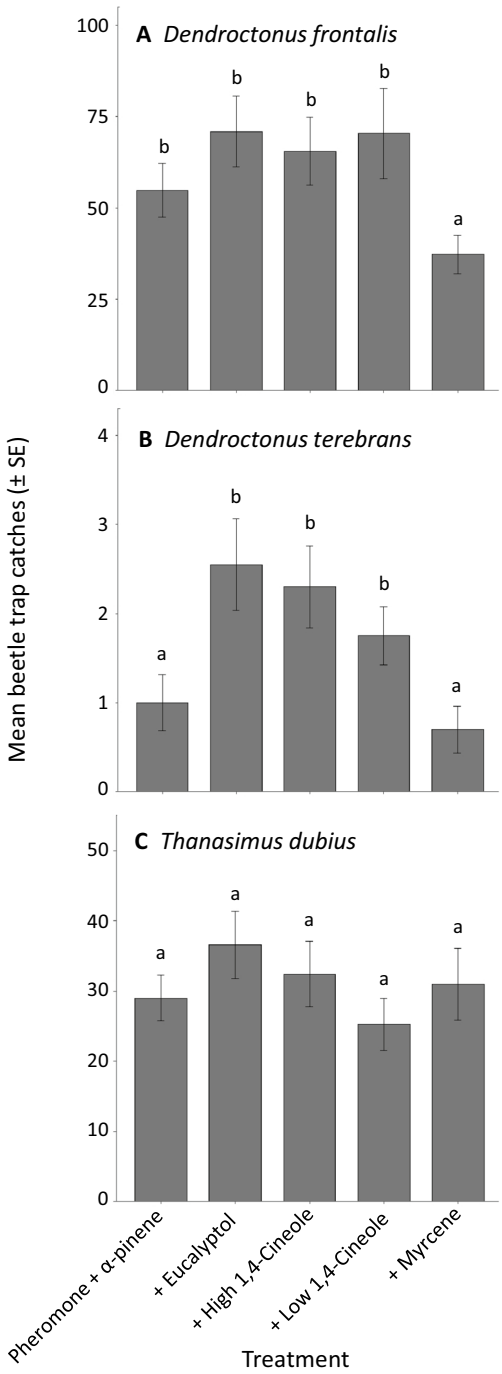

Fig. 5 Mean number (±SE) of bark beetles **a** *Dendroctonus frontalis*, **b** *D. terebrans,* and **c** clerid predator *Thanasimus dubius* trapped with different host-associated olfactory stimulants for both species **a** and **b** in combination with a blend of pheromone components for both species (frontalin, *endo*-brevicomin, and *exo*-brevicomin) and the attractive synergist *alpha*-pinene. Lure composition and release rates are in Table 2. The control had pheromone and *alpha*-pinene. *Bars* with the *same letters* were not significantly different (Dunn's test; $\alpha = 0.05$)

(Smith et al. 1993; Niño-Domínguez et al. 2015), and by *D. terebrans* to *alpha*- and *beta*-pinene (Delorme and Payne 1990) has been demonstrated in previous research.

Furthermore, the four compounds in the headspace of host resin coinciding with the largest EAD response—*alpha*-pinene, *beta*-pinene, myrcene, and 4-allylanisole—were shown to influence the attraction of both *D. frontalis* and *D. terebrans* to traps baited with their pheromone components (Fig. 4a, b). *alpha*-Pinene and *beta*-pinene are the predominant two components of the volatile fraction of oleoresin in the major host species of both *D. frontalis* and *D. terebrans* (Mirov 1961; Wood 1982; Turner et al. 2018; Bookwalter et al. 2019), typically composing >75% of this fraction. Both *alpha*- and *beta*-pinene significantly enhanced attraction of both bark beetle species to their combined pheromone components. Attractant synergism or attraction by *alpha*-pinene has been demonstrated previously for both *D. frontalis* and *D. terebrans* (Renwick and Vité 1969; Miller and Rabaglia 2009; Staeben 2014), although the limited published research on the behavioral activity of *beta*-pinene with these species failed to discover a response (Renwick and Vité 1969; Siegfried et al. 1986). Additionally, both *alpha*-pinene and *beta*-pinene have been identified as attractants or attractant synergists for numerous conifer-infesting beetles (Schroeder 1988; Volz 1988; Schroeder and Lindelow 1989; Hofstetter et al. 2008; Miller and Rabaglia 2009) and are hypothesized to function as a general indicator of host suitability and susceptibility for these insects.

The phenylpropanoid 4-allylanisole was a particularly potent olfactory stimulant and significantly enhanced attraction of both *D. frontalis* and *D. terebrans* to pheromone components. This increase was similar to that generated by an approximately 50-fold higher release rate of (racemic) *alpha*-pinene, previously the only verified host-produced synergist for *D. frontalis* attraction. Our result is surprising since 4-allylanisole had been shown to be an attraction inhibitor of *D. frontalis* (Hayes et al. 1994a; Strom et al. 1999), and as such it was explored considerably for use as a tree protectant (Hayes et al. 1996; Strom et al. 2004). It was registered with the Environmental Protection Agency, U.S. as a biorational pesticide against this and other conifer pests (PC Code 062150). However, in the only fully replicated study examining 4-allylanisole as a tree protectant against *D. frontalis* (Strom et al. 2004), the release of 4-allylanisole from "challenged" trees (i.e., treated with *D. frontalis* aggregation attractant or weakened with microbiocide) neither decreased nor increased their rate of mortality. Evidence had suggested that 4-allylanisole might serve as an indicator of host susceptibility for *D. frontalis*, as its quantity declined in pines whose susceptibility was increased artificially with herbicide treatment (Hayes et al. 1994b), with similar results observed in other bark beetle–host systems (Hobson 1995). Avoidance of 4-allylanisole could potentially be beneficial to *D. frontalis*, as it has been shown to deter the growth of this species' fungal symbionts and thus potentially interfere with brood development (Bridges 1987). However, other research has not found an association between elevated 4-allylanisole and low host susceptibility: progeny of *D. frontalis* "escape" trees (pines that survived within infestations and thus, were ostensibly less susceptible to attack) had a lower 4-allylanisole content in their resin than trees in the general population (Strom et al. 2002). In other Scolytinae, 4-allylanisole has been shown either to be an attractant (Rappaport et al. 2000; Joseph et al. 2001) or inhibitor of attraction (Werner 1995; Hobson 1995; Joseph et al. 2001). To our knowledge, there are no prior reports of behavioral activity of 4-allylanisole with *D. terebrans*; however, Joseph et al. (2001) found it to be an attractant at low release rates (19.2 mg/day at 21 °C) for *D. valens*, a sibling species to *D. terebrans*.

There are several conceivable explanations for the contrasting results between our study and previous work regarding response of *D. frontalis* to 4-allylanisole. It is possible that 4-allylanisole is a "multifunctional"-type semiochemical that enhances attraction at low release rates but inhibits attraction at high rates (Rudinsky 1973; Borden 1997). Bark beetles have displayed multifunctional-type dose responses to certain host odors (Bakke 1983; Erbilgin et al. 2003; Gallego et al. 2008). The earlier research which showed that 4-allylanisole reduced response by *D. frontalis* to attract-baited traps utilized higher release rates of this compound (160 mg/day) (Hayes et al. 1994a, b) than our study (~50 mg/day). Other differences between the current and past experiments that presumably could have influenced the outcome include (1) differences in the composition of the lures used for the standard attractant (i.e., the earlier studies did not include brevicomin in the lure), (2) trap design (funnel versus panel traps), and (3) positioning of traps within active *D. frontalis* infestations (earlier studies) or not (our study).

Myrcene was the only olfactory stimulant in host resin found to inhibit attraction of *D. frontalis* to its aggregation pheromone, either alone or with an attraction-enhancing host odor component present (Figs. 4a, 5a). This diminished response was not observed in either *D. terebrans* or the predator, *T. dubius*. Myrcene has been found associated with pines within *D. frontalis* infestations that had escaped mortality, and thus it was hypothesized to be an indicator of an unsuitable host (Gollob 1980).

Although the monoterpene ethers 1,4-cineole and eucalyptol have rarely been reported as pine-associated odors (Pettersson et al. 2000; Amin et al. 2013; Kiliç and Koçak 2014), they were included in the GC-EAD dose–response study because they have been detected in association with host odor lures used for trapping conifer-infesting beetles (BTS, unpublished data). Although, neither was detected either by GC-EAD or GC–MS in our headspace of pine resin, both compounds generated particularly strong

olfactory responses in the dose–response GC-EAD study. Both produced some behavioral activity at the relatively low release rates used in our trapping tests (7–105 mg/day), including enhancement of attraction of *D. terebrans* by both compounds (Figs. 4b, 5b) and reduction of *D. frontalis* response to the pheromone component lure by the higher release rate of 1,4-cineole (Fig. 4a). Eucalyptol previously was identified by GC-EAD as a *D. frontalis* olfactory stimulant present in volatiles of leaves and bark of non-host *Carya alba* (L.) (Shepherd and Sullivan 2013). Neither compound has previously been reported to have behavioral activity with *Dendroctonus*; however, eucalyptol has been shown to strongly inhibit response by spruce bark beetle, *Ips typographus* L., to its pheromone (Andersson et al. 2010). There may be semiochemicals produced by tissues of the host pine but absent in fresh host resin (Sullivan et al. 2000); additionally, semiochemicals produced by non-hosts may play a role in host discrimination (Zhang and Schlyter 2004). Our research did not consider such odors, and it is possible that these also may influence host location and selection in both *Dendroctonus* species.

Thanasimus dubius is considered a generalist predator of conifer-infesting bark beetles and is possibly an important mortality agent of *D. frontalis* (Turchin et al. 1999; Erbilgin and Raffa 2001b). It has displayed attraction to a range of semiochemicals produced by both their prey and their prey's host trees (Herms et al. 1991; Costa and Reeve 2011). Natural selection should favor predators whose attractive cues closely resemble those of their prey (Greenstone and Dickens 2005), and this may be reflected in the general similarities in behavioral responses by *T. dubius* and *D. frontalis* to the bioassayed host-associated odors. Strongest attraction enhancement was observed to the same three compounds (*alpha*-pinene, *beta*-pinene, and 4-allylanisole); however, preferences among these compounds differed between predator and prey. Unlike *D. frontalis*, *T. dubius* was less responsive to 4-allylanisole than *alpha*-pinene at the tested release rates and showed a similar level of response to both *alpha*- and *beta*-pinene (*D. frontalis* was much more attracted to *alpha*- than *beta*-pinene). Prior studies showed similar *T. dubius* responses to *alpha*-pinene and *beta*-pinene (Mizell et al. 1984). Conversely, although the present study found 4-allylanisole to be an attractant synergist for *T. dubius*, previous research found that 4-allylanisole had no effect on the attraction of *T. dubius* (Hayes et al. 1994a, b).

Due to a shared pheromone component (frontalin) and cross-attractiveness of non-shared pheromone components (*endo*- and *exo*-brevicomin), a significant amount of cross-attraction between *D. frontalis* and *D. terebrans* can be presumed to occur during host colonization (Sullivan 2016; Munro et al. 2019). Our data indicate that similar olfactory sensitivities and behavioral responses to host-associated odors should additionally enhance cross-attraction during host colonization. This phenomenon is particularly interesting since it has been hypothesized that *D. frontalis* may exploit the pheromones from *D. terebrans* attacks as a means of locating suitable hosts (Payne et al. 1987; Munro et al. 2019), and thus there may be positive feedback between the two species for optimal host location.

Three important considerations should be made in interpreting results of this study: (1) It was not intended as an exhaustive or comprehensive survey of host-generated volatiles that might influence insect–host interactions by the two bark beetles; rather, our studies focused on volatiles associated with constitutive host resin. As previously discussed, numerous field experiments have demonstrated that host resin contains semiochemicals for both species (see reviews Sullivan 2016 and Munro et al. 2019), and, therefore, volatiles associated with constitutive resin are an appropriate initial focus for studies of host-produced semiochemicals for these two pine bark beetles. However, additional odors or differing proportions may be associated with the whole undamaged tree, tree tissues, or induced defensive responses to insect or fungal colonization (Paine et al. 1987, Delorme and Lieutier 1990, Harley et al. 1998, Sullivan et al. 2000, Semiz et al. 2012). Such odors may have distinct influences on beetle behavior and play their own role in insect–host semiochemical interactions, and thus deserve future, additional study. (2) We examined behavioral responses of *D. frontalis* and *D. terebrans* to host compounds in the presence of the bark beetles' pheromones, and thus our behavioral experiments may be relevant to semiochemistry of resin odors only in the context of location of a host tree after beetles have released secondary attractants. Both behavioral tests included pheromone lures because our primary interest was understanding the role that host odors play in cross-attraction between species. It is possible that behavioral responses of *D. terebrans* to odors present in host resin might be different in the absence of con- or heterospecific pheromones, since in this instance host odors would mediate a different biological function, namely, initial location of an uninfested host. We would not expect an attractive response by *D. frontalis* to any of the tested host odors in the absence of pheromone since no such response has been reported to resin or distillates (Sullivan 2016). (3) Electroantennogram and trapping experiments included just a single enantiomeric blend of chiral compounds, and this could have influenced the observed activity levels. *D. frontalis* displays a small but statistically significant difference in both its olfactory and behavioral responses to the enantiomers of *alpha*-pinene (Staeben 2014), although the racemate does not differ significantly in attractive synergism from the more active (+)-enantiomer. Enantiomeric discrimination may occur also with the other tested chiral compounds of the present study.

The demonstrated significance of semiochemicals in the ecology of bark beetles has inspired extensive research into

the use of semiochemicals in monitoring and management of pest species, and our results are particularly relevant to semiochemical management tools for *D. frontalis*. The host odor component in the lure currently deployed for *D. frontalis* population monitoring and forecasting consists of a polyethylene enclosure releasing approximately 70% *alpha*-pinene and 30% *beta*-pinene (Billings 2011). Our study provides the first experimental evidence indicating *beta*-pinene to be a synergist for *D. frontalis* pheromone components, although the effect of combining *alpha*- and *beta*-pinene requires investigation. Further, our results indicate that 4-allylanisole should be investigated as a possible lure adjuvant for use in *D. frontalis* monitoring and management. A more potent attractive lure may aid in the early detection of invasive *D. frontalis* populations, such as those evidently expanding their range northward in the eastern U.S. in response to climate change (Lesk et al. 2017; Dodds et al. 2018). Our results illustrate how a semiochemical that shows potential as a tree protectant (4-allylanisole) may possibly produce unanticipated and undesirable outcomes. Since 4-allylanisole applications have the potential to increase the attraction of *D. frontalis,* they presumably could have counter-productive effects such as increased risk of attack on treated trees or enhanced growth of treated infestations. Future research may examine this host volatile more thoroughly to understand the variability of its activity and further investigate the possibly complex role of host volatiles in general in the chemical ecology of *D. frontalis.*

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Author contributions All authors contributed to the study conceptualization. GC-EAD methodology and analyses were performed by Holly L. Munro, Brian T. Sullivan, and William P. Shepherd. Trapping surveys were performed by Holly L. Munro and Brittany F. Barnes. Statistical analyses were performed by Holly L. Munro and checked by Cristian R. Montes. The first draft of the manuscript was written by Holly L. Munro and Brian T. Sullivan, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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