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Field Response of Black Turpentine Beetle to Pine Resin Oxidation and Pheromone Displacement

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Abstract

The black turpentine beetle, *Dendroctonus terebrans,* is an economically important pest of pines in the Southeastern U.S., with a high potential for invasion to other pine-rich regions. *Dendroctonus terebrans* attraction to an injured host tree lessens over time as the host material degrades. Likewise, kairomonal volatiles emitted from the host change as constituents of the defensive resin oxidize. Therefore we hypothesized that volatiles associated with a fresh host would be more attractive to *D. terebrans* than those associated with a dead or dying host. We replicated the natural oxidation process of turpentine, fractionated the distilled products to isolate the oxidized products, and deployed the complex mixtures to measure field attraction based on the amount of oxidation performed. Contrasting with previous studies, our results suggest that *D. terebrans* attraction is not primarily based on host tree degradation. In a second experiment incorporating *Dendroctonus* pheromones, we demonstrate *D. terebrans* has a displacement-dependent response to *endo*-brevicomin, a pheromone associated with the sympatric southern pine beetle, *D. frontalis*. This has implications not only for possible interspecific signaling, but also for the role of *endo*-brevicomin in *D. terebrans* colonization behavior. The results from this study broaden the understanding of *D. terebrans* chemical ecology and directly contribute to the development of an effective lure-based monitoring system that will benefit future research and management efforts. This may become important if the species is established outside its native range, as in the closely related red turpentine beetle, *Dendroctonus valens*, which caused mass pine tree mortality following its introduction to Asia.

Keywords *Dendroctonus terebrans* · Semiochemical · Lure · Host volatiles · Kairomone · α-pinene · Frontalin · Endobrevicomin

Introduction

The black turpentine beetle, *Dendroctonus terebrans* (Oliver), is a bark beetle (Coleoptera: Curculionidae) native to the United States. It is considered a secondary pest of pines and red spruce (Staeben et al. 2010), typically targeting weakened or stressed trees for colonization (Smith and Lee 1972; Wood 1982). *D. terebrans* is also often found cohabiting host trees with other bark beetle species, including *Ips* spp. and most notably, the southern pine beetle, *Dendroctonus frontalis* (Zimmermann), a congener and a primary pest known to cause widespread tree mortality during outbreaks in pine forests (Coulson and Klepzig 2011; Payne et al. 1987).

Dendroctonus terebrans is currently distributed throughout the eastern U.S., ranging from eastern Texas into Florida, stretching northward to reach southern Maine. Its main hosts include pitch pine (*Pinus rigida*), loblolly pine (*P. taeda*), shortleaf pine (*P. echinata*), slash pine (*P. elliottii*), and red spruce (*Picea rubens*)(Staeben et al. 2010), though infestations are most severe in stands of loblolly and slash pine (Smith and Lee 1972). Previously a pest mainly affecting the naval stores industry, black turpentine beetle is currently seen as a threat to high value urban trees such as Japanese black pine (*Pinus thunbergii*)(Lambert 1997; Staeben et al. 2010). Perhaps the greatest concern, and a reason for continued studies of this beetle, is the possibility of the black turpentine beetle becoming invasive outside its current range. This is the case in the closely related red turpentine beetle,

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D. valens (LeConte), which has caused unprecedented pine mortality in China (Yan et al. 2005). Recent reviews on *D. terebrans* have emphasized a need for effective lure-based trapping systems that can be utilized for research and management purposes (Munro et al. 2019).

Black turpentine beetle is consistently found only in the basal portions of trees, particularly in freshly cut stumps greater than 10 cm in diameter. Females will begin colonization by boring into the trunk or exposed roots. This typically elicits a defensive response from the tree in the form of pressurized resin extrusion, which when released can form a pitch tube (Franceschi et al. 2005; Mayfield et al. 2018; Wood 1982). If the female can overcome the tree's defenses, she begins gallery formation in the phloem and is soon joined by a male. After mating, the female lays approximately 100 eggs which hatch after 10–14 days. Larvae then feed gregariously on the phloem, which can girdle the tree if multiple galleries are established. After feeding, larvae will pupate, eclose, and exit the tree to find a new host (Smith and Lee 1972; Staeben et al. 2010). In northern Florida, black turpentine beetle can be active year-round, but most dispersal occurs from February to October, with a spike of activity in March and April (Smith 1957).

Dendroctonus terebrans chemical ecology is thought to be primarily driven by attraction to host plant volatiles. Pine turpentine is a strong attractant for *D. terebrans*, its attractive properties being enhanced further by the presence of ethanol (Phillips et al. 1988; Smith et al. 1993). Further studies have measured the attractiveness of various other chemicals to *D. terebrans*. Some have used specific compounds within turpentine, such as (−)-α-pinene, to successfully attract *D. terebrans* (Miller and Rabaglia 2009). The congeneric *D. valens* is attracted to other constituents of turpentine, including (+)-3-carene and (-)-ß-pinene, effects which are also synergized by ethanol (Kelsey and Westlind 2022).

Individual components of turpentine however, as well as synthetic mixtures intended to imitate turpentine, are all less attractive to *D. terebrans* than naturally derived turpentine (Siegfried et al. 1986). In a study using electroantennograms (EAG) to measure antennal response of *D. terebrans*, Delorme and Payne (1990) demonstrated that *D. terebrans* requires a much greater threshold for response to both α-pinene and ß-pinene individually than it does for whole turpentine. They suggest this higher sensitivity to whole turpentine may be due to the cumulative response to multiple components within a complex mixture. Recent studies have detected strong electrophysiological responses of *D. terebrans* to individual pine-associated volatiles, as well as increased attraction in the field when isolated host volatiles were combined with sympatric beetle pheromones (Munro et al. 2020).

In nature, terpenes exposed to air oxidize via both abiotic autoxidation (Hunt et al. 1989) and via metabolism in aerobic organisms. Terpenes are detoxified via cytochrome oxidation in bark beetles and their symbionts (Hunt and Borden 1990). Verbenone and verbenols are examples of oxidized α-pinene products with known behavioral activity in bark beetles. Lindgren and Miller (2002) reported two of five species of pine infesting bark beetles were not sensitive to verbenone, a major α-pinene oxidation product, while other species are inhibited with relatively low doses of verbenone. They suggested that species which do not have a requirement for fresh host tissue will be more tolerant of the presence of verbenone. Volatiles emitted from loblolly pine billets change as the resin terpenes oxidize over time. Flechtmann et al. (1999) showed that beetle species colonizing the tree at different time points, from fresh to decaying, vary in their attraction to pine billets over time, including *D. terebrans*.

As opposed to testing beetle response to individual compounds, this study took a broader approach, using mixtures of oxidized turpentine products. We mimicked natural terpene autooxidation processes by bubbling oxygen into warm turpentine. The oxidation products are complex (Moore et al. 1956), but can be grouped by their appearance at different stages of the oxidation process. Early in the process more alcohols and epoxide products are formed, while later oxidation mixtures contain more highly oxidized carbonyl products like verbenone. We hypothesized that the early oxidation products containing a higher concentration of alcohols are more likely to be attractive to *D. terebrans*, as they could reflect the chemistry of a stressed or weakened host. Later oxidation products containing more carbonyls, such as verbenone, could signify a dead host or at least a host in a more advanced stage of deterioration and thus are hypothesized to be less attractive.

In addition to plant volatiles, there are multiple pheromones associated with *D. terebrans* host colonization. Female black turpentine beetles produce a bicyclic ketal, frontalin, which attracts males to the newly formed gallery (Godbee and Franklin 1976). Males produce a different bicyclic ketal upon finding the female, *exo*-brevicomin, which negates the attractive effects of frontalin in other males, leading to the description of these two chemicals as sex pheromones (Delorme and Payne 1990; Phillips et al. 1989).

Endo-brevicomin is another semiochemical that has received increased attention in studies of pine bark beetle chemical ecology and colonization behavior (Sullivan and Brownie 2021). While trace amounts have been detected in male *D. terebrans* hindguts (Payne et al. 1987), other studies have been unable to corroborate this finding (Munro et al. 2019; Phillips et al. 1989), despite *D. terebrans* also being shown to be very olfactorily sensitive to *endo*-brevicomin (Delorme and Payne 1990).

Endo-brevicomin is produced by the sympatric *D. frontalis*, which relies on it in addition to frontalin to perform coordinated attacks used to overwhelm a target tree's

defenses (Sullivan and Clarke 2021). In *D. frontalis*, *endo*-brevicomin has proximity-dependent effects, functioning as a dose-dependent synergist on an area-wide scale by enhancing arrestment of beetles to nearby sources of frontalin and host odors when placed in the vicinity, an effect termed "displacement-enhanced synergism" (Sullivan and Mori 2009). The increase in beetle capture is so significant that *endo*-brevicomin is now used in government-sponsored programs such as the Florida Forest Service southern pine beetle spring trapping survey (Pearce 2021). It is currently unknown whether the displacement-enhanced synergism only functions in *D. frontalis*, or whether it is a more widespread phenomenon in other bark beetle species.

As part of our broader objective of developing an effective lure-based *D. terebrans* trapping system, we tested whether displacement-enhanced synergism of *endo*-brevicomin also occurs in *D. terebrans*. While *D. terebrans* and *D. frontalis* exhibit different ecological habits (Godefroid et al. 2019), they are sympatric in the southeast United States and have been hypothesized to experience interspecific cross-attraction (Payne et al. 1991).

In this study, we perform two experiments to measure attraction of different combinations of lures placed on standard bark beetle traps. The first experiment compares the attraction of *D. terebrans* to different oxidized turpentine products. The second experiment compares the attraction of *D. terebrans* to paired pheromone and host product lure combinations, as well as the attraction when the pheromone component is physically separated from the trap and host products.

Methods and Materials

Field Site Trapping experiments occurred near Gainesville, Florida, at the Austin Cary Forest (ACF), from Feb 18 to Mar 25, 2021 and May 23 to Jun 27, 2021. ACF is a research forest maintained by the University of Florida. The study area consists predominantly of mixed pines, the most common tree species being loblolly pine, *Pinus taeda*, which is one of the major hosts of *D. terebrans*. Traps were placed in a sprawling area that spanned multiple stands within ACF that experienced varied management styles. Some traps in the second experiment were placed in positions closer to a recently logged area of the forest to benefit from edge effects (Peltonen et al. 1997), showing increased *D. terebrans* abundance.

Traps were of the eight-unit Lindgren funnel trap design (Synergy Semiochemicals, Delta, BC, Canada). Each was suspended from metal shepherd hooks or taut line with the top of the trap approximately two meters from the ground. Traps were placed as far from nearby trees as forest density would allow, approximately three meters. Traps were separated from one another by a minimum distance of fifty meters. With the exception of the spatially displaced *endo*-brevicomin treatment, all lures were attached at the same position on the trap exterior underneath the trap canopy. Collection cups were filled with water and an unscented detergent solution. Cups were emptied and refilled weekly to prevent decomposition of specimens and attraction of scavengers.

Fifty traps and five treatments were used in each experiment. Treatments were distributed in a repeating pattern across trap locations. At the end of every week, captured specimens were collected and traps with their associated lure treatment were rotated to the neighboring location, such that after five weeks every location experienced each treatment. This experimental design creates multiple Latin squares with identical temporal effects, resulting in what is known as a Latin rectangle (Giesbrecht and Gumpertz 2004). This provides spatial and temporal replication while controlling for both variability in trap location and weekly variation mainly due to weather conditions.

Chemical Lures All chemical lures were supplied by Synergy Semiochemicals Corp. The turpentine used was a natural product derived from *Pinus* spp. wood which had undergone the kraft pulping process (Tran and Vakkilainnen 2012). The composition was primarily of α-pinene with smaller amounts of ß-pinene, limonene, 3-carene, and other naturally occurring terpenes. Turpentine was packaged using permeable plastic pouches, with a release rate of 1.125 ± 0.125 g/day at 25 °C. Ethanol lures were also packaged using permeable plastic pouches, with a release rate of 0.3 g/day at 25 °C. Oxidates of turpentine were packaged using permeable plastic bubble caps. Early-stage oxidate lures had a release rate of 7.5 ± 2.5 mg/day at 25 °C and late-stage oxidate lures had a release rate of 2 mg/day at 25 °C. These relatively lower release rates better reflect the natural environment, where turpentine oxidates make up a small proportion of emitted host volatiles. Beetle pheromones were formulated in flexible polymer rods loaded with volatile chemical. Frontalin and *exo*-brevicomin lures had release rates of 0.125 mg/day at 20 °C. Racemic *endo*-brevicomin lures had a release rate of 0.175 mg/day at 20 °C.

Preparation of Oxidation Mixtures A 3-L three neck flask was fitted with a flow meter connected to a gas sparging tube, thermocouple, and efficient double surface condenser. The flask was charged with 1000 g turpentine and a magnetic spin bar. Oxygen flow was started at approximately 3 L/min and the flask was warmed in a heating mantle to 50 °C. After a brief induction period, the reaction became mildly exothermic and oxygen flow and temperature were adjusted to sustain stable conditions for 5 h. After 5 h the exotherm ceased and flow of oxygen was stopped. The mixture then cooled and the hydroperoxides were reduced by stirring overnight

with 1 L of saturated sodium sulfite solution. The phases were then separated. The aqueous phase was extracted with 500 ml of diethyl ether and pooled with the organic layer. The combined organic phase was washed with brine and dried over sodium sulfate. The solvent was evaporated, and the residue was then simple distilled *in vacuo* (40–50 mm Hg, 60–70 °C) to remove the polymeric materials. This distillate was fractionated through 30 cm Vigreux column (5–7 mm Hg, 50–70 °C) to separate the terpenes from oxidized products. The carbonyl fraction was removed from the distillate using 2 cycles of sodium metabisulfite extraction using Blumann and Zeitschel's (1913) method.

The oxidation mixtures were complex and not all compounds could be identified. The composition of the turpentine and the two oxidation fractions were determined using an Agilent 6890 GC/5872 MS fitted with a DB5 MS column with guard column (see Table 1). Compound identities were determined via mass spectra screened against the NIST 8 mass spectral library and by comparisons with authentic standards. The approximate composition of major components of the whole turpentine and the two oxidation fractions is given in Table 2.

Treatments The first experiment had five treatments. The first treatment was a positive control that used basic host compounds previously known to be attractive to *D. terebrans*: unoxidized whole turpentine and ethanol. The second treatment combined these same host volatiles with the *D. terebrans* female sex pheromone, frontalin, which has also been shown to be attractive in conjunction with host volatiles. The third and fourth treatments included complex mixtures isolated by oxidizing turpentine. The products were formed from the principal components of turpentine, primarily from α- and ß-pinene. The two treatments differed in the total amount of oxidation performed. While it is difficult to determine the exact composition of the oxidation mixtures, many individual products included are well-known compounds. The first mixture contained a blend of

Table 1 Agilent 6890 GC/5872 MS parameters

Column	Agilent DB-5MS + DG. 30 m × 0.25 mm (I.D), df = 0.25 µm, with 10 m Duraguard column
Inlet Temperature	275 °C
Injection mode	Manual injection. 20:1 split ratio
Injection volume	1 µL
Carrier gas Flow rate	1.0 ml/min
Oven Program	50 °C, 1 min hold. 25 °C/min to 250 °C, 2 min hold

Table 2 Major components of whole turpentine and oxidate fractions

Turpentine	GC %	Carbonyl fraction	GC %	Alcohol fraction	GC %
α-pinene	82.1	verbenone	50.6	trans-verbenol	27.4
3-carene	4.9	α-campholenal	13.3	longifolene	7.8
limonene	2.7	fencholenal	8.8	cis-3-pinen-2-ol	6.9
ß-pinene	2.1	verbenone epoxide	5.8	trans-carveol	6
verbenone	2	3-carene-5-one	3.2	p-cymene-8-ol	4.9
camphene	1.3	myrtenal	2.1	myrtenol	4.3
longifolene	1	carvone	2	benzene	3.8
Total Identified	*96.1*	*Total Identified*	*85.8*	α-pinene epoxide	3.1
				linalool	2.5
				verbenene	1.7
				pinocamphene	1.4
				methanazulene	1
				habanene	0.8
				1,3,8 menthatriene	0.8
				limonene	0.6
				eucalyptol	0.5
				cycloisosativene	0.5
				α-pinene	0.3
				Total Identified	*74.3*

products rich in alcohols (*trans*-verbenol, *cis*-3-pinen-2-ol, *trans*-carveol, and myrtenol), α-pinene epoxide, and neutral terpenes (longifolene and verbenene). The carbonyl fraction treatment was a mixture of aldehydes and ketones, including verbenone, 3-caren-5-one, myrtenal, carvone, α-campholenal and fencholenal. The alcohol enriched fraction and the carbonyl fraction were packaged separately and emitted alongside unmodified turpentine and ethanol lures. In addition to the positive control, a negative control treatment was included to detect any baseline collection of *D. terebrans* unmediated by lure attraction. This consisted of a blank unbaited trap.

The second experiment also had five treatments. The positive control treatment used host volatiles (basic turpentine and ethanol) as well as frontalin. These components were included in the other treatments as well. For the second treatment these three components were also combined with *exo*-brevicomin, the pheromone associated with male *D. terebrans*. In the third treatment, they were combined with *endo*-brevicomin, the pheromone typically associated with *D. frontalis* aggregation behavior. The fourth treatment aimed to detect the displacement-enhanced synergism of *endo*-brevicomin by attaching the turpentine, ethanol, and frontalin to the trap and attaching *endo*-brevicomin to a wooden stake placed in the ground four meters away. The second experiment also included a negative control treatment consisting of an unbaited trap.

Statistical Analysis In the first experiment, the numbers of collected specimens were non-normal according to a Shapiro–Wilk test. A generalized linear mixed model (GLMM) using a Poisson distribution was therefore chosen to interpret the effects of lure attractiveness in the first experiment. Collection data for the second experiment was non-normal as well as over-dispersed, as judged by observation of a high residual deviance relative to the residual degrees of freedom. A GLMM using a negative binomial distribution was chosen to interpret lure effects in the second experiment.

For both models, the response variable was the number of *D. terebrans* collected per trap per week. Lure treatment and weeklong trapping period were treated as fixed effects, while individual trap location was treated as a random effect. Traps were not spatially grouped by Latin square, so square effects were not designated in the model as they are included under locational effects. Post-hoc tests to determine significant differences in lure attraction were performed by applying pairwise Tukey's HSD tests ($\alpha = 0.05$) to the estimated marginal means predicted by each model. Negative control treatments were eliminated from this post-hoc analysis, as blank unbaited blank treatments can increase the experimentwise error rate (Reeve and Strom 2004). Instead, Fisher's exact tests ($\alpha = 0.001$) were used to confirm a significant absence of attraction in blank treatments compared to baited treatments, based on whether individual traps caught ≥ 1 *D. terebrans* per week.

Analysis was conducted in R statistical software (R Core Team 2021) using the packages ggplot2 (Wickham 2016), multcomp (Hothorn et al. 2008), glmmTMB (Brooks et al. 2017), MASS (Venables and Ripley 2002), and emmeans (Lenth et al. 2021).

Results

Turpentine Oxidation Weeklong trapping period had a significant effect on attraction ($\chi 2 = 206.47$, $df = 4$, $P < 0.001$) as did baited lure treatment ($\chi 2 = 182.98$, $df = 3$, $P < 0.001$). Treatments which included increased amounts of oxidized terpenes, either the early or late stage oxidate lures, did not result in a significant difference in captured *D. terebrans* compared to the positive control which contained only unmodified turpentine (early oxidates: $\chi 2 = 0.378$, $df = 191$, $P = 0.982$; late oxidates: $\chi 2 = 0.037$, $df = 191$, $P = 1.0$). Mean daily catch rates of *D. terebrans* were highest in traps baited with turpentine and frontalin. Over the course of the 5-week experiment, this treatment captured significantly higher numbers of *D. terebrans* per day than all other lure combinations tested (Fig. 1). Unbaited traps caught zero individuals and the blank treatment exhibited significant absence of attraction compared to baited treatments ($P < 0.001$).

Paired Pheromone and Tree Host Volatiles Weeklong trapping period had a significant effect on attraction ($\chi 2 = 438.72$, $df = 4$, $P < 0.001$) as did baited lure treatment ($\chi 2 = 11.12$, $df = 3$, $P = 0.011$). Neither the addition of either *exo*-brevicomin or *endo*-brevicomin directly to the frontalin with turpentine trap showed a statistically significant difference in catch rates ($t = 0.891$, $df = 190$, $P = 0.809$; $t = 2.029$, $df = 190$, $P = 0.181$, respectively).

The only significant difference in capture rates between baited treatments was between the two treatments utilizing *endo*-brevicomin. The spatially displaced *endo*-brevicomin treatment showed higher capture rates than the treatment in which the *endo*-brevicomin was placed on the trap with the other lures ($t = 3.22$, $df = 190$, $P = 0.008$), indicating that a displacement of four meters increased the attractiveness of the trap. Across the 5-week study, the treatment which attracted the highest number of *D. terebrans* per day was the *endo*-brevicomin spatially displaced treatment (Fig. 2). The blank treatment exhibited a significant absence of attraction compared to baited treatments ($P < 0.001$), indicating that the incidence of capture unmediated by lure attraction was negligible.

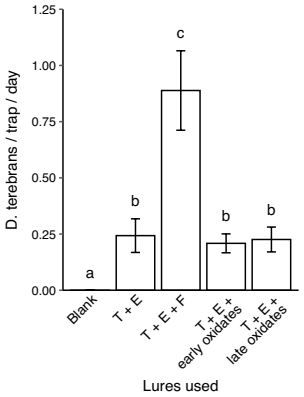

Fig. 1 Mean daily catch rate (±SE) of *Dendroctonus terebrans* with lure treatments used in the turpentine oxidation experiment (treatment abbreviations: T = unmodified turpentine, E = ethanol, F = frontalin, early oxidates = products isolated early in the turpentine oxidation process, late oxidates = products isolated late in the turpentine oxidation process); columns including the same letters were not statistically different (among baited treatments: Tukey's HSD, $\alpha = 0.05$; between blank and baited treatments: Fisher's exact test, $\alpha = 0.001$)

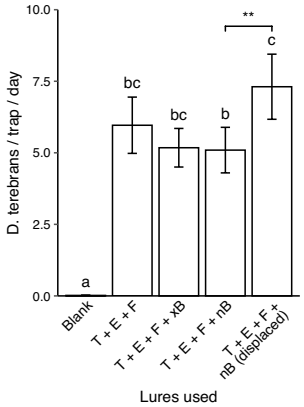

Fig. 2 Mean daily catch rate (±SE) of *Dendroctonus terebrans* with lure treatments used in the experiment pairing pheromones and tree host volatiles (treatment abbreviations: T = unmodified turpentine, E = ethanol, F = frontalin, xB = exo-brevicomin, nB = endo-brevicomin); columns including the same letters were not statistically different (among baited treatments: Tukey's HSD, $\alpha = 0.05$; between blank and baited treatments: Fisher's exact test, $\alpha = 0.001$)

Capture rates were higher across all treatments for the second experiment compared to the first. This is potentially due to the rearrangement of some individual trap locations in between experiments. Capture rates varied between individual trap locations, as well as between weeklong trapping periods (Fig. 3).

Discussion

Results from the first experiment suggest that turpentine oxidation products do not affect attraction in *Dendroctonus terebrans* when presented with unmodified turpentine. Our hypothesis that early oxidation products of turpentine will be more attractive was not supported. Our results contrast with the suggestion made by Flechtmann et al. (1999) that reduced attraction of *D. terebrans* to loblolly billets over time was due to resin oxidation products emitted from the billets. Table 2 shows verbenone was approximately 2% of our initial turpentine sample indicating some oxidation had already occurred. Moreover, the turpentine devices used were clearly attractive with this level of verbenone. Verbenone also composed nearly half of the carbonyl fraction tested in the first experiment. In striking contrast to several other *Dendroctonus* species, our observations suggest *D. terebrans* is insensitive to low doses of verbenone in a background of host attractant. *Dendroctonus terebrans* primary attraction thus appears to be governed by a different mechanism than just host product degradation.

This is not to say that individual chemicals within turpentine are not more or less attractive, which has been shown in previous studies (Munro et al. 2020), only that our results indicate the artificial enrichment of products grouped by oxidation stage has little effect on overall attraction. Nor does this indicate that host quality is ignored by *D. terebrans*. Amounts of constituents within the controlled turpentine oxidation mixture used in this experiment may differ from those found in the natural colonization environment due to metabolization of certain products by beetles.

The most attractive lure, and the only one statistically significantly more attractive than pure turpentine, was the combination of turpentine and the sex pheromone frontalin. This corroborates results by previous studies (Phillips et al. 1989) which demonstrate that this mixture is particularly attractive to males.

Results indicate *D. terebrans* behavioral activity is influenced by the spatial positioning of *endo*-brevicomin. It remains unclear however, whether the difference between *endo*-brevicomin treatments is due to removal of potential *endo*-brevicomin inhibitory effects at high doses, the addition of potential low-dose attractive effects, or a combination of both. Thus, the hypothesis of displacement-dependent *endo*-brevicomin attractiveness in *D. terebrans* is supported, but not its synergistic effect with other known attractants.

The biological rationale for the *endo*-brevicomin activity in *D. terebrans* is unclear, particularly because it has not been conclusively shown that the species produces the chemical. One possible explanation assumes that *D. terebrans* does produce this compound and uses it for intraspecific communication, but perhaps in smaller doses or shorter time-windows than in other *Dendroctonus* and therefore the compound production has not yet been confirmed. Trace production of *endo*-brevicomin by *D.*

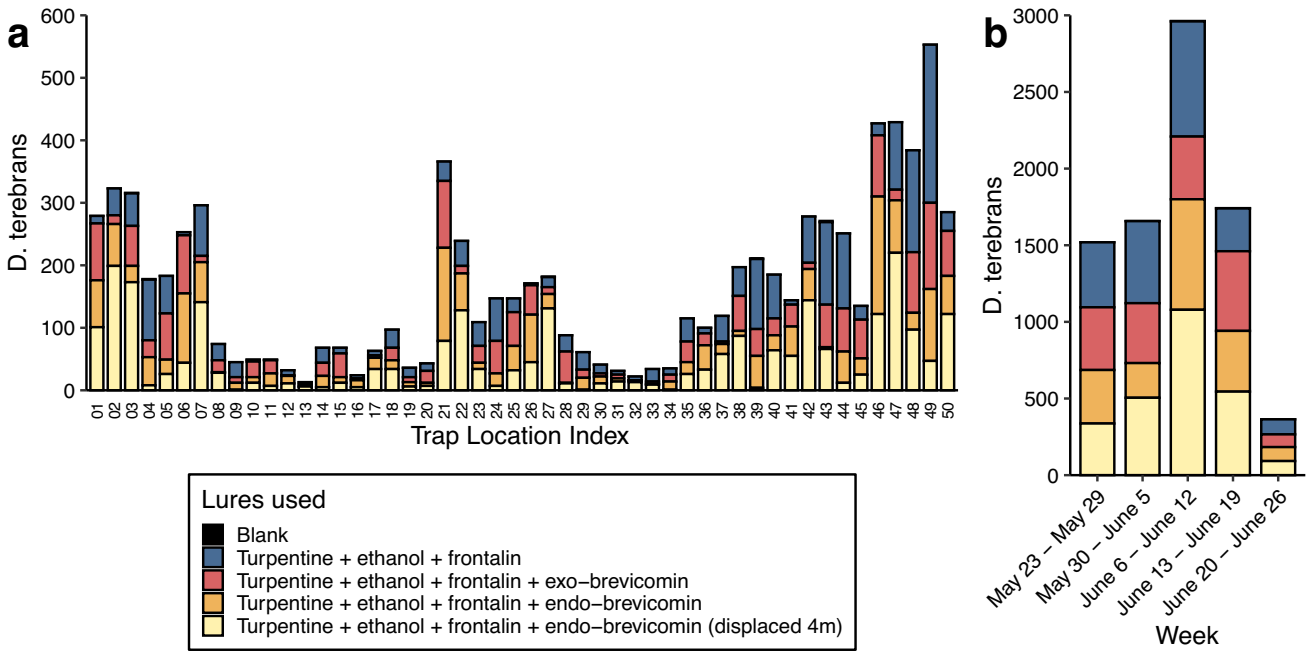

Fig. 3 Total captures of *Dendroctonus terebrans* in the second experiment comparing variation between trapping location (**a**) and between trapping periods (**b**), anticipated fixed and random effects which were addressed by the experimental design outlined in the methods

terebrans females has been reported (Payne et al. 1987), but the study has not yet been corroborated.

Another hypothesis assumes that *D. terebrans* does not produce *endo*-brevicomin and that a behavioral response to the semiochemical constitutes interspecific signaling (Payne et al. 1987, 1991). *Endo*-brevicomin is produced abundantly by the southern pine beetle *D. frontalis*, as well as multiple other species. Considering the ubiquity of similar pheromones in bark beetle chemical ecology (Symonds and Elgar 2004a), *endo*-brevicomin serving roles in multiple species could be a result of semiochemical parsimony (Blum 1996). In this scenario a semiochemical may achieve widespread usage among taxa due to its low biosynthetic cost or particularly communicative properties (Huber et al. 1999).

Though *D. terebrans* and *D. frontalis* diverged *ca.* 14 Mya (Godefroid et al. 2019), it is also possible that a shared similar response to *endo*-brevicomin is an ancestral trait within the genus. While pheromone usage in the genus *Dendroctonus* often evolves via sudden shifts, which may result in closely related species producing different pheromonal blends (Symonds and Elgar 2004b), *endo*-brevicomin production is unique as being strongly correlated with phylogeny (Symonds and Gitau-Clarke 2016).

If sensitivity to endo-brevicomin represents interspecific signaling, the compound may serve a similar density-modulating role in multiple species. In *D. frontalis,* dose-dependent responses may be adaptive in that individuals may locate aggregations of conspecifics while avoiding trees in which colonization capacity has been exceeded (Sullivan et al. 2011). *Dendroctonus terebrans* may benefit from having the same response during episodic *D. frontalis* outbreaks. While these outbreaks are rare at any given place due to suitable stand age and density requirements, the opportunistic tendencies of *D. terebrans* to routinely accumulate on weakened pines or stumps (Merkel 1981) suggest that the species has a more uniform background presence wherever pines are present. Further studies are needed to determine if this phenomenon is common to other bark beetle species and if the mechanism functions in a similar manner.

Phillips et al. (1989) suggested that *endo*- and *exo*-brevicomin are not distinguished from one another by *D. terebrans* when emitted alongside turpentine and frontalin, due to their elicitation of apparently equal behavioral activity, though the study has been critiqued for the unnaturally high release rates of turpentine used. The addition of *exo*-brevicomin or *endo*-brevicomin to the frontalin-turpentine lure does not significantly alter overall attractiveness, but displacement dependent effects have not been tested for *exo*-brevicomin. One strength of the Phillips et al. (1989) study is the separate measurement of male and female attractiveness, a methodological oversight of the current study that if included in future experiments may reveal stronger responses to the spatial positioning of pheromonal components.

Our results represent a step in the understanding of the semiochemical ecology of the black turpentine beetle,

Dendroctonus terebrans, as well as a step towards the development of a lure for its monitoring. This may become important in the future. Should the turpentine extraction industry resume its importance in the Southeast U.S., or should the beetle become established and damaging in a region with susceptible pines, knowledge on optimal lure components and spatial deployment may be urgently needed.

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Declarations

Competing Interests LeMay and Hulcr declare no competing interests. O'Loughlin and Wakarchuk are members of a semiochemical production company Synergy Semiochemicals which supplied the experimental lures.

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