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Alternative Formulations of Trap Lures for Operational Detection, Population Monitoring, and Outbreak Forecasting of Southern Pine Beetle in the United States

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Abstract

The southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae) is a major destructive pest of *Pinus* L. In the southeastern United States, numbers of this species and a major predator, *Thanasimus dubius* (Fabricius) (Coleoptera: Cleridae), captured during an annual springtime trapping survey are used to make forecasts of the likelihood and severity of an outbreak during the following summer. We investigated responses by both species to six lure formulations to evaluate their suitability for the survey and allow integration of historical data sets produced with differing lure compositions. Trapping trials were performed at four locations across three states (Louisiana, Mississippi, and Alabama) during spring, and at these and one additional location (North Carolina) in fall 2016. All lures included the pheromone component frontalin. Southern pine beetle preferred lures that additionally included the pheromone component *endo*-brevicomin and turpentine as a source of host odors (rather than a 7:3 mixture of monoterpenes *alpha*- and *beta*-pinene). *Thanasimus dubius* displayed little discrimination among lure compositions. Lure preferences by southern pine beetle did not differ significantly among locations in spring but were influenced by season. Gas chromatography (GC)-electroantennographic detection analyses with southern pine beetle and GC-mass spectrometry identified numerous known and potential semiochemicals that distinguished volatiles released by the tested host odor devices. The lure combination that included *endo*-brevicomin and *alpha/beta*-pinene is recommended for the trapping survey because of its high sensitivity for southern pine beetle and potential for greater data integrity resulting from its reproducible composition.

Keywords: bark beetle, forecast, host odor, monoterpene, pheromone

The southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae) is among the most significant biotic mortality agents of *Pinus* spp. L. (Pinales: Pinaceae) across its range (Clarke and Nowak 2010), which currently spans from New England southward to Florida, westward to eastern Texas, and from Arizona and New Mexico south to Nicaragua (Birt 2011b, Dodds et al. 2018). They feed and reproduce within the phloem tissue of the stems of mature pines, and can disperse by flight more than 1 km between host trees (Gara 1967, Turchin and Thoeny 1993). The southern pine beetle is typical of tree-killing bark beetles in that invasion of healthy hosts requires a simultaneous attack by hundreds or thousands of individuals that results in rapid

depletion of host defenses (Lindgren and Raffa 2013, Raffa et al. 2015). Such 'mass attacks' by southern pine beetle are mediated by an aggregation attractant that is composed of pheromone components (released by both beetle sexes after landing and entering the bark), as well as volatile constituents of the host's defensive resin released from beetle-damaged tissues (Pureswaran and Sullivan 2012, Sullivan 2016). Synthetic compounds and pine extracts (and their constituents) have been incorporated into lures used operationally for detecting southern pine beetle populations and forecasting outbreaks (Billings 2011a, Strom and Clarke 2011, Dodds et al. 2018).

Population densities of southern pine beetle vary dramatically over time and across the landscape, ranging from undetectable up to severe outbreak levels that result in extensive mortality of healthy trees (Clarke 2012, Martinson et al. 2013). Outbreaks in the southeastern United States may encompass multiple states; they typically persist for 1–3 years and may recur at 5- to 15-year intervals (Price et al. 1998, Birt 2011a, Clarke et al. 2016). Despite the periodic nature of outbreaks, conditions that trigger them are not well understood and their timing is largely unpredictable (Turchin et al. 1991, Weed et al. 2017). Pest management has focused on use of silvicultural practices (in particular, the reduction of stand density) that minimize stand risk, as well as suppression of localized infestations ('spots') through cutting of infested and adjacent, threatened trees (Billings 1980, 2011b; Clarke 2001; Nowak et al. 2015; Asaro et al. 2017).

For the past three decades, forecasting of the current-year likelihood of an outbreak has been accomplished with a coordinated trapping survey conducted annually during spring across the southeastern United States (Billings 1988, 2011a; Billings and Upton 2010). Multiple-funnel traps (Lindgren 1983) baited with a uniform lure are deployed for 4 wk, with up to three traps per county or U.S. National Forest Ranger District. Trap deployment is timed to the flowering of redbud trees (*Cercis canadensis* L.; until 2015, flowering dogwood, *Cornus florida* L., was used) in order to approximately synchronize data collection with beetle phenology and the occurrence of southern pine beetle's springtime dispersal flight (Hedden and Billings 1977, Sullivan et al. 2016, Lombardo et al. 2018, Thomason et al. 2020). Counts of southern pine beetle as well as a major predator [*Thanasimus dubius* (Fabricius); Coleoptera: Cleridae] that is also attracted to the lure (Vité and Williamson 1970, Billings and Cameron 1984) are entered into a formula or model with other data (i.e., infestation abundance during previous years). The survey historically provided a local (at the scale of a single county or National Forest Ranger District) assessment of 1) beetle population trends (increasing, static, or declining) and 2) expected spot abundances (low, moderate, high, or outbreak level) for the coming summer (Billings and Upton 2010, Billings 2011a). The current, revised southern pine beetle prediction system (available at www.spbpredict.com) utilizes a statistical model to provide an expected range of spot numbers per county or Ranger District. Predictions are disseminated within weeks following completion of trapping, and these aid pest management by providing foresters additional time to prepare for an outbreak, for example, by scheduling detection flights for infestations and securing the necessary financial and human resources needed for suppressing them.

The operational lure has changed twice since inception of the springtime survey in 1987. The original, two-component lure consisted of the female-produced southern pine beetle aggregation pheromone component frontalin (racemic; released at ~10 mg/d from two closed polyethylene microcentrifuge tubes) and turpentine (a distillate of pine resin) derived from southern pine species and released from a wick-and-reservoir device (hereafter 'wick device') at a rate of several grams per day (Billings 1988). Attractiveness of the frontalin lure is strongly synergized (1–2 orders of magnitude) by the host odors present in turpentine released at a high rate (Billings 1985, Sullivan 2016). In the mid-2000s, turpentine from southern pine beetle host species became unavailable commercially. Due to the uncertain effects of using turpentine from alternative commercial sources and the likelihood of varying compositions, the turpentine wick device was replaced by a plastic enclosure (hereafter, 'sachet') releasing a 7:3 mixture of *alpha*:*beta*-pinene at a rate of several grams per day. These monoterpenes are the predominant volatile constituents of the resin of hosts for southern pine beetle (Mirov 1961, Bookwalter et al. 2019), and they are attractive pheromone synergists both individually and in combination (Staeben et al. 2015, Munro et al. 2020). In 2017, the male-produced pheromone component *endo*-brevicomin was added to the lure (racemic; released at 0.1–0.2 mg/d; device positioned 4–6 m from the trap) and substantially increased southern pine beetle attraction (Vité et al. 1985, Sullivan et al. 2007, Sullivan and Mori 2009).

As implemented until the middle 2010s, the springtime trapping survey generated forecasts often with undesirable levels of error. For example, increases in infestation abundances of more than 25% from the previous year were correctly predicted only 62% of the time (Billings and Upton 2010); however, accurate prediction is most critical for resource protection during rapid population buildup. Researchers are investigating the possibility that revisions to the survey procedures and data analysis might increase the information content of the resulting data and improve reliability of forecasts. Modifications that are proposed, under development, or already implemented include those made to 1) the composition of the trap lure, 2) the timing and duration of trap deployment, 3) the predictive model and the format of model output, and 4) the kinds of data included in the predictive model.

This paper reports investigations of alternative formulations of the trapping lure (item 1 above) with regard to effects on catch variables potentially relevant to southern pine beetle population forecasting. In this study we investigated the relative attractiveness of different candidate lure combinations to both target species, as well as possible geographic and seasonal variability in beetle responses to these lures. We examined these variables within the context of parallel lure comparison experiments performed at several widely spaced locations in the southeastern United States in spring (four sites) and fall (five sites) of 2016. To increase the immediate practical utility of the information, we limited our research to lure components and release devices that are currently commercially available or have been used operationally in the past for southern pine beetle monitoring and detection. Additionally, we analyzed the composition of volatiles arising from host odor release devices to identify differences in chemical composition that might contribute to observed differences in behavioral responses. This analysis was done both by means of coupled gas chromatography-electroantennographic detection analysis (GC-EAD) to detect olfactory stimulants for southern pine beetle, and coupled gas chromatography-mass spectrometry (GC-MS) to identify and quantify known or possible semiochemicals released by the lures.

Materials and Methods

Trapping Experiments

At five locations (Table 1), two lines of five traps were established, with traps within lines located 150–400 m apart and lines positioned 2–8 km apart. Traps were 12-unit multiple-funnel-type (as used for

Table 1. Locations of trapping experiments

Site	Coordinates[a]	Spring experiment	Fall experiment	No. spots[b] 2016	No. spots 2017
Sicily Island State Park, Louisiana	31.84N, 91.76W	16 Feb. to 26 Apr. 2016	4 Oct. to 13 Dec. 2016	0	0
Homochitto National Forest, Mississippi	31.43N, 91.16W			471	1,056
Bienville National Forest, Mississippi	32.22N, 89.42W			368	2,446
Oakmulgee National Forest, Alabama	32.87N, 87.19W	17 Feb. to 27 Apr. 2016	5 Oct. to 14 Dec. 2016	272	839
Nantahala National Forest[c], North Carolina	35.41N, 83.69W	not executed		17	75

[a]Averaged among trap locations.
[b]Number of southern pine beetle infestations detected at the site.
[c]Cheoah District.

Table 2. Lures used in trapping experiments

Treatment number	Abbreviation[a]	Variable lure[b]	Release rate[c] (g/d)	Source and part number[d]
1)	1 Pin. sachet	Single sealed plastic bag (8 × 19 cm) releasing a 70:30 mixture of *alpha*- and *beta*-pinene (~150 ml loading).	3.16 ± 0.06	Synergy 3077
2)	2 Pin. sachets	Two devices as #1 above.	6.32 ± 0.12	Synergy 3077
3)	Turp. sachet	Single sealed plastic bag as #1 releasing turpentine (distilled from resin of *Pinus oocarpa* Schiede ex Schltdl. (Pinales: Pinaceae); ~150 ml loading).	2.79 ± 0.09	Synergy 3403
4)	Turp. wick	250-ml brown glass bottle with 3/8″ (0.375 cm) diam. cotton dental wick extending 3.5–4.0 cm through hole in cap. Filled with ~200 ml turpentine of #3.	7.82 ± 0.14	N/A
5)	1 Pin. sachet & brev.	One device #1 plus an *endo*-brevicomin release device[e].	3.16 ± 0.06	Synergy 3077
6)	Turp. sachet & brev.	One device #3 plus an *endo*-brevicomin release device[e].	2.79 ± 0.09	Synergy 3403

[a]Used in figures.
[b]All traps were uniformly baited with a frontalin device (Synergy #3065; two LDPE microcentrifuge tubes loaded with 250 µl racemic frontalin; 12 mg/d release at 24°C).
[c]Measurements in a fume hood at an average 23°C. Host odor devices only.
[d]Synergy Semiochemicals Corp., Delta, BC, Canada.
[e]*endo*-Brevicomin was released from a 'flexlure' device (Synergy #3188, a fiber containing a polymer matrix infused with active compound; 0.15 mg/d release at 21°C) that was attached to the branch of a nearby nonhost tree and positioned approximately 6 m from the trap.

the survey) and suspended from a branch or a cord between hardwood trees. The trap's collection cup was positioned 1–2 m above the ground and filled with a mixture of water and automotive antifreeze for preservation of captured insects. Lines were established within mature forests containing mixed pine and hardwoods. Traps were located >15 m from the nearest pine. Within each line, five lure combinations (treatments) were assigned randomly to the five traps. Catches were collected at approximately 1-wk intervals. At the time of collection, lure combinations were re-randomized without replacement to different traps within the line until every lure combination had been at every trap position once (five catch collections; one complete rotation). In both spring and fall of 2016, two consecutive, complete rotations of lure combinations (hereafter 'rotation') were conducted with the same trap positions but with a different randomization scheme and a fresh set of lures for each rotation. Trapping was conducted at the Nantahala National Forest site (North Carolina) only in fall. Lure release rates were determined by re-weighing the lures after 2 wk in a fume hood at room temperature (Table 2; otherwise, they were obtained from the manufacturer).

Six lure combinations were compared (Table 2), and these differed in the composition of the host odor component (turpentine or a 7:3 mixture of *alpha*- and *beta*-pinene [hereafter, simply 'pinene']), the release device for turpentine (wick or sachet), and the presence of *endo*-brevicomin. The first spring rotation tested lure combinations 1–5, whereas lure combination 2 was removed and 6 added in the second spring rotation and in fall. Based on preliminary examination of results, it was concluded that the latter lure combination would be more informative.

Counts of both southern pine beetle and *T. dubius* were calculated as catches per trap per day and were log-transformed to meet parametric test assumptions; suitability of transformations was determined through examination of residuals plots. Due to differences between the lure combinations and the sites used in different rotations and seasons, several analyses were conducted on different subsets of the data. Data collected at the four sites in the first and second spring rotations were analyzed separately by rotation because of the substitution of lure combination 6 for lure combination 2. A third analysis was conducted on the data collected from both fall rotations at all five sites (including Nantahala). A fourth tested for an interaction between season and lure response on trap catch for each species; it included catches from the second spring and both fall rotations (which had identical lure combinations) but omitted the Nantahala site (which was added in the fall).

To detect site-dependent variation in lure response and compare lures averaged across sites for each spring rotation, the mixed-model ANOVA included as explanatory factors the five tested lure combinations ('lure'), the forests where the experiment was performed ('site'), the two lines of five traps deployed at each site ('line'), the five traps within each line ('trap'), and the date on which the catch was collected ('date'). Fixed effects were lure, site, and their interaction; random effects were line(site), lure * line(site), trap(line * site), and date(site). Additionally, ANOVAs were performed for sites separately with fixed factor lure and random effects line, trap(line), and date. For the fall trials, the last two dates of the second complete rotation of lures among traps (variable 'rotation') caught very few insects across lure combinations (more than half the traps

caught no southern pine beetle), and removal of these dates was necessary to meet distributional assumptions and caused minimal loss of information. For both beetle species in fall, we performed a mixed-model ANOVA with fixed factors lure, site, rotation, and all possible interactions; random effects were line(site), lure * line(site), trap(line * site), and date(site * rotation). For separate analyses by site for the fall data, the ANOVA had fixed effects lure, rotation, and their interaction, and random effects line, lure * line trap(line), and date(rotation).

Analyses also included a dependent variable equal to southern pine beetle catch divided by the sum of southern pine beetle catch and *T. dubius* catches (variable 'percent southern pine beetle'). This calculation has been a data component used in a forecasting table available to practitioners (Billings 1988, 2011a; Billings and Upton 2010). These data were arcsin √x-transformed and were analyzed similarly as southern pine beetle and *T. dubius* counts. As with the analyses of counts, data for the last two dates of the second rotation in fall were omitted, and the Homochitto National Forest (Mississippi) and Nantahala sites were eliminated entirely from the fall analysis due to insufficient catches.

To identify seasonal effects on lure responses, a mixed-model ANOVA was applied to log-transformed catches for both species. Only rotations and sites with the same lure combinations in both spring and fall were included (i.e., Nantahala data and the first rotation of spring were excluded). Fixed effects were season, lure combination, site, and all interactions; also, rotation nested in season and the interaction of lure and rotation nested in season. Random effects were line nested within site, trap nested within line and site, and date nested within site, rotation, and season. Analyses were performed with SAS 9.4 (SAS Institute, Cary, NC). All pairwise contrasts among lure combinations were performed with a Tukey adjustment and $\alpha = 0.05$.

We estimated the average ratio of change in spring catches that were produced by either of the two historical modifications of the monitoring lure [i.e., 1) change of the host odor component from turpentine wick to a pinene sachet (2007), and 2) addition of *endo*-brevicomin (2017)]. For each trap line within site (with both rotations averaged, $N = 8$), we calculated the ratio of catches between the lure combinations and averaged the values.

Analyses of Volatiles From Host Odor Devices

Volatiles arising from each of the three tested host odor release devices were sampled and analyzed 1) for their chemical composition with GC-MS, and 2) for olfactory sensitivity of southern pine beetle to constituents with GC-EAD.

Release devices were placed singly into 7.5-liter capacity, glass desiccators with a ~4-cm-diameter opening through the lid. Corrugated polytetrafluoroethylene (PTFE) tubing connected to a vacuum pump was inserted ~20 cm into the desiccator interior through a plug in the opening consisting of a strip of activated charcoal mesh (Universal Replacement Prefilter HRF-AP1; Honeywell, Southborough, MA) rolled to form a cylinder. This plug provided ventilation of the desiccator while restricting incursion of outside volatiles; it was replaced for each sampling. An adsorbent cartridge (a 3.2 mm i.d., 5 cm length of perfluoroalkoxy alkane (PFA) tubing containing 0.1 g conditioned Porapak Q [50–80 mesh; Grace Chromatography, Columbia, MD]) was inserted into the opening of the corrugated tubing inside the desiccator and positioned to prevent contact with the devices and be near the floor of the desiccator. For a subset of samplings, an identical cartridge was additionally placed within the vacuum tubing leading to the pump to check for breakthrough in the first (sampling) cartridge; these indicated >98% retention of the major analytes by the sampling cartridge. Release devices were placed into the closed desiccators, and, following a 15-min equilibration period, air was drawn by the vacuum pump into the cartridges for 1 h at 30 ml/min at room temperature (20–25°C). Three replicate devices of each type (pinene sachet, turpentine sachet, turpentine wick) were sampled, with one of each (plus an empty, control desiccator) sampled at any one time. Devices were sampled twice: at 1–3 d and again 15–17 d following being suspended within an outdoor covered space (shaded and protected from rain) at the Pineville, LA facility. Devices were weighed to within 0.01 g at each sampling. Climate data from the covered space were inadvertently lost; however, data from an airport located 11 km away indicated an average daily temperature of 27.9°C (± 1.0° SD) during the interval between lure placement outdoors and final sampling. Cartridges were extracted once with 1.5 ml redistilled pentane; each extract was spiked with 3.5 µg heptyl acetate (dissolved in 5 µl hexane) as an internal standard and then stored at −75°C prior to analysis.

Cartridge extracts were analyzed with a GC-MS (Hewlett-Packard 6890-5973, Palo Alto, CA). The GC was operated in split mode (1:30 split ratio) with an HP-INNOWax (Agilent Technologies, Torrence, CA) polyethylene glycol capillary column (60 m length, 0.25 mm i.d., 0.25 µm film thickness) and helium as the carrier gas. The temperature program was 40°C for 1 min; 16°C/min to 80°C; 7°C/min to 230°C held 10 min. The mass spectral detector (MSD) operated in electron impact mode. Peaks in the total ion chromatograms were identified with mass spectral and retention time matches to commercially obtained standards (Sigma-Aldrich, St. Louis, MO) and quantified against a standard curve constructed from dilutions of the analytes which contained an internal standard at approximately the same concentration as the samples. Descriptive statistics were performed on the resulting quantities. Additionally, the enantiomeric composition of *alpha*-pinene in both the turpentine and pinene lures was analyzed for a subset of lure samples by using the previously described GC-MS fitted with an enantiomerically selective capillary column (CyclodexB; J&W Scientific, Folsom, CA; 30 m length, 0.25 mm i.d., 0.25 µm film thickness) and operating with a temperature program of 40°C for 1 min, 5°C/min to 150°C, then 15°/min to 230° held 10 min. Enantiomeric ratios were calculated directly from integration areas of the total ion chromatograms.

GC-EAD analyses were performed on southern pine beetle with apparatus and general procedures described previously (Asaro et al. 2004, Sullivan 2005). In summary, antennal preparations were placed within a humidified and charcoal-filtered air stream (400 ml/min) into which half of the effluent of a GC (Hewlett-Packard 5890) was diverted; the other half entered a flame ionization detector (FID). The antennal preparation was a reference electrode ($Ag/AgCl_2$ wire inserted into a sharpened glass microcapillary filled with Beadle-Ephrussi ringer solution with 0.5% polyvinylpyrrolidone) inserted into the foramen of a beetle's removed head. The tip of an identical recording electrode was placed against one side of the antennal club with the saline at the electrode tip in contact with the club cuticle. Voltage changes across the antenna were amplified with a 10× high-impedance preamplifier (Universal AC/DC Probe; Syntech, Buchenbach, Germany), conditioned with a Syntech Autospike IDAC 2/3, converted to a digital signal with an SRI Instruments (Torrance, CA) model 202 computer interface, and analyzed with Peak Simple software (SRI Instruments). The GC was operated with the same column and settings as used for the nonchiral GC-MS analyses. One-microliter undiluted cartridge extract (pooled by lure type and sampling week) was injected into the GC inlet running in split mode (1:20 split ratio). If a voltage spike exceeding the amplitude

of 90% of background noise was observed at the retention time of an FID peak for four or more of six total beetles (three of each sex) tested with each sample, the compound corresponding to the FID peak was classified as an olfactory stimulant. Southern pine beetles in GC-EAD tests had been reared from infested logs or bark from the Homochitto National Forest, MS, and were housed in vented plastic containers with moistened paper wipers at room temperature or 4°C.

Results

Trapping Experiments

We remind the reader that a frontalin device was present in every lure combination mentioned. Across all sites (Table 3), there was a significant effect of lure composition on southern pine beetle catches for both rotations in spring (first, $F_{4, 127} = 79.5$, $P < 0.001$; second, $F_{4, 16} = 57.7$, $P < 0.001$) and in fall ($F_{4, 21.7} = 96.1$, $P < 0.001$). Presence of *endo*-brevicomin in the lure combination caused a several-fold increase in southern pine beetle catches (Fig. 1), and *endo*-brevicomin with the turpentine sachet was the most attractive lure (Fig. 1B and C). Lure composition had a significant effect at each individual site and for both seasons except at the Nantahala site during fall ($F_{4, 4.24} = 5.13$, $P = 0.066$), likely due to low catches (Table 4; Fig. 2). There was not a significant interaction between lure composition and site for either spring rotation (first, $F_{12, 127} = 1.73$, $P = 0.068$; second, $F_{12, 16} = 1.16$, $P = 0.380$), but an interaction occurred in fall ($F_{16, 21.7} = 2.46$, $P = 0.026$) (Table 3). However, the pattern of lure preferences did not differ conspicuously among the five sites during fall (Fig. 2). Catches of southern pine beetle were higher in spring than fall ($F_{1, 43} = 119.4$, $P < 0.001$), and there was a marginally significant interaction between lure composition and season ($F_{4, 399} = 2.37$, $P = 0.052$). There was a three-way interaction among lure composition, season, and site ($F_{12, 397} = 2.76$, $P = 0.001$). During spring, substitution of the turpentine wick with the pinene sachet device (the 2007 modification to the southern pine beetle monitoring lure) caused a mean 5.11 (± 0.92 SE)-fold decrease in southern pine beetle catches, whereas addition of an *endo*-brevicomin device to the pinene sachet/frontalin combination (the 2017 modification) caused a 9.45 (± 1.56 SE)-fold increase.

For *T. dubius* catches across sites (Table 3; Fig. 3), there was a significant effect of lure composition for the first spring rotation ($F_{4, 127} = 5.66$, $P < 0.001$) and fall ($F_{4, 22} = 15.4$, $P < 0.001$), and interactions between lure composition and site were detected for both the

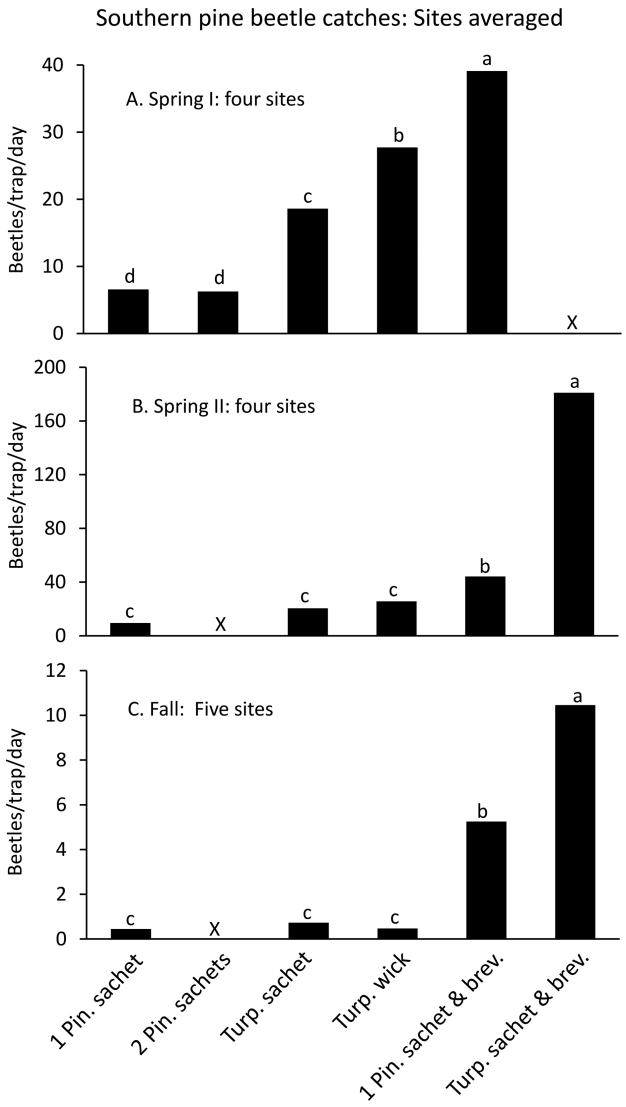

Fig. 1. Trap catches (averaged among study sites) of southern pine beetle with different lure combinations. Lure abbreviations are given in Table 2; all lures had a frontalin device. An 'X' indicates that the lure combination was not in that trial. Means with the same lower-case letter were not significantly different (Tukey test on transformed data; $\alpha = 0.05$).

Table 3. Results of ANOVA tests of the effect of lure composition and site on responses by southern pine beetle and clerid predator *T. dubius* to traps

		Spring I			Sping II			Fall		
Dependent variable	Factor	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Southern pine beetle trapped per day	Lure	79.5	4, 127	<0.001	57.7	4, 16	<0.001	96.1	4, 21.7	<0.001
	Site	10.6	3, 12.3	0.001	2.33	3, 11.9	0.126	2.35	4, 9.1	0.131
	Lure × site	1.73	12, 127	0.068	1.16	12, 16	0.380	2.46	16, 21.7	0.026
T. dubius trapped per day	Lure	5.66	4, 127	<0.001	0.71	4, 16	0.597	15.4	4, 22	<0.001
	Site	8.5	3, 6.5	0.012	11.5	3, 12.3	<0.001	2.02	4, 8.2	0.182
	Lure × site	1.9	12, 127	0.040	1.40	12, 16	0.260	2.41	16, 21.9	0.029
Southern pine beetle/(*T. dubius* + southern pine beetle)	Lure	48.5	4, 128	<0.001	31.2	4, 16.3	<0.001	104.5[a]	4, 13.7	<0.001
	Site	10.2	3, 11.3	0.002	0.85	3, 5.8	0.517	0.91	2, 3.9	0.475
	Lure × site	1.14	12, 127	0.335	1.14	12, 16.2	0.398	2.91	8, 13.7	0.040

[a]For southern pine beetle /(*T. dubius* + southern pine beetle) data in fall, the Nantahala and Homochitto sites were removed because these data did not sufficiently meet distributional assumptions for analysis due to exceedingly low catches.

Table 4. Results of ANOVA tests of the effect of lure composition on responses by southern pine beetle and clerid predator *T. dubius* to traps for each individual site where experiments were conducted

		Spring I			Spring II			Fall		
Dependent variable	Trapping site	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Southern pine beetle trapped per day	Sicily Island WMA, Louisiana	11.3	4, 32	<0.001	48.3	4, 32	<0.001	36.1	4, 57.2	<0.001
	Bienville NF, Mississippi	60.3	4, 32	<0.001	16.8	4, 32	<0.001	65.5	4, 5.3	<0.001
	Homochitto NF, Mississippi	29.3	4, 32	<0.001	28.0	4, 32	<0.001	15.8	4, 4.3	0.008
	Oakmulgee NF, Alabama	10.8	4, 31.4	<0.001	27.5	4, 32	<0.001	18.8	4, 4.4	0.005
	Nantahala NF, North Carolina	—[a]	—	—	—	—	—	5.13	4, 4.24	0.066
T. dubius trapped per day	Sicily Island WMA, Louisiana	1.39	4, 32	0.259	1.20	4, 32	0.328	8.99	4, 56.7	<0.001
	Bienville NF, Mississippi	8.85	4, 32	<0.001	1.59	4, 32	0.201	6.50	4, 4.4	0.042
	Homochitto NF, Mississippi	2.11	4, 32	0.102	2.03	4, 32	0.114	7.67	4, 55.4	<0.001
	Oakmulgee NF, Alabama	2.68	4, 31.3	0.050	0.59	4, 32	0.673	1.54	4, 56	0.202
	Nantahala NF, North Carolina	—	—	—	—	—	—	2.14	4, 4.3	0.231
Southern pine beetle/(*T. dubius* + southern pine beetle)	Sicily Island WMA, Louisiana	7.08	4, 4	0.042	17.0	4, 4	0.009	18.4	4, 4	0.008
	Bienville NF, Mississippi	62.3	4, 4	<0.001	1.79	4, 4	0.293	67.9	4, 4	<0.001
	Homochitto NF, Mississippi	7.30	4, 4	0.040	5.63	4, 4	0.061	i[b]	i	i
	Oakmulgee NF, Alabama	13.8	4, 4	0.013	29.9	4, 4	0.003	22.7	4, 4	0.005
	Nantahala NF, North Carolina	—	—	—	—	—	—	i	i	i

[a]No spring data for this location.

[b]Not analyzed due to catches being insufficient for data to meet distributional assumptions of tests.

first spring rotation ($F_{12, 127}$ = 1.9, P = 0.040) and fall ($F_{16, 21.9}$ = 2.41, P = 0.029) but not the second spring rotation ($F_{12, 16}$ = 1.40, P = 0.26). With sites considered individually (Table 4; Fig. 4), lure composition effects were significant only for the Bienville National Forest ($F_{4, 32}$ = 8.85, P < 0.001) and the Oakmulgee National Forest ($F_{4, 31.3}$ = 2.68, P = 0.050) in the first spring rotation, and at Sicily Island Wildlife Management Area ($F_{4, 56.7}$ = 8.99, P < 0.001), Bienville National Forest ($F_{4, 4.4}$ = 6.50, P = 0.042), and the Homochitto National Forest ($F_{4, 55.4}$ = 7.67, P < 0.001) during fall. *Thanasimus dubius* catches did not differ significantly between seasons ($F_{1, 43}$ = 3.52, P = 0.067), but there was a significant interaction between lure composition and season ($F_{4, 397}$ = 4.52, P = 0.001) as well as a three-way interaction among lure composition, season, and site ($F_{12, 397}$ = 2.50, P = 0.004). At no location or season did inclusion of the *endo*-brevicomin lure produce a significant change in *T. dubius* catches. In general, discrimination of lure compositions was more evident in fall than spring. Across sites in fall, lure combinations that included the turpentine sachet trapped fewer *T. dubius* than the other combinations (Fig. 3).

Across sites (Table 3), there was a significant effect of lure composition on the percent capture of southern pine beetle (i.e., southern pine beetle catches divided by the sum of southern pine beetle and *T. dubius* catches) for both rotations in spring (first, $F_{4, 128}$ = 48.5, P < 0.001; second, $F_{4, 16.3}$ = 31.2, P < 0.001) and in fall ($F_{4, 13.7}$ = 104.5, P < 0.001), and an interaction between lure composition and site was detected in fall ($F_{8, 13.7}$ = 2.91, P = 0.040). For both spring rotations and in fall (Fig. 5), percent capture of southern pine beetle was significantly increased by inclusion of *endo*-brevicomin in the lure combination. Data for sites individually are displayed in Supp Material 1 (online only). Overall, percent capture of southern pine beetle was much lower in fall than spring ($F_{1, 42.6}$ = 211.3, P < 0.001), and there was an interaction between lure composition and season ($F_{4, 393}$ = 2.77, P < 0.027) and a three-way interaction among lure composition, season, and site ($F_{12, 391}$ = 2.03, P = 0.021)

Analyses of Volatiles From Host Odor Devices

In GC-EAD analyses of host odor lures, southern pine beetle antenna responded to 15 compounds (Table 5; Supp Material 2 [online only]). For both the turpentine and pinene devices, responses were detected to three hydrocarbon monoterpenes (*alpha*-pinene, *beta*-pinene, and myrcene) and three oxygenated monoterpenes (*alpha*-pinene oxide, *trans*-pinocarveol, *trans*-verbenol, and verbenone). Antenna responded to four additional compounds in the turpentine devices: the hydrocarbon monoterpene 3-carene, the sesquiterpene longifolene, the phenylpropanoid 4-allylanisole, and the oxygenated monoterpene linalool. Antenna responded to three additional compounds in the pinene devices: 6-methyl-5-hepten-2-one, and the green leaf volatiles 1-hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol. The dominant compounds (>1% composition) in volatiles of the pinene lures were *alpha*-pinene (77%) and *beta*-pinene (22%), and in the turpentine lures were *alpha*-pinene (91–94%), camphene (1%), *beta*-pinene (1%), and 3-carene (2%). There was a general trend for antenna-stimulating hydrocarbon monoterpenes to decrease in headspace concentration between week 1 and 3 and oxygenated monoterpenes to remain unchanged or increase. Enantiomeric ratios of *alpha*-pinene for the turpentine lures was 12.9:1 (+):(−), and 3.1:1 for the pinene lures.

Discussion

Attractiveness of the Lures

Southern pine beetle were more attracted to lure combinations that included *endo*-brevicomin, and combinations with *endo*-brevicomin were more attractive if they included a sachet containing turpentine rather than turpentine components *alpha*- and *beta*-pinene alone. The preference for turpentine likely is due to the greater chemical complexity in its blend of host odors (discussed below). *endo*-Brevicomin has been shown to be a potent attractive synergist for combinations of host odors and frontalin (Sullivan et al. 2007, Sullivan 2016). The predator *T. dubius* displayed far less discrimination among lures than did southern pine beetle. In particular, *endo*-brevicomin did not influence *T. dubius* attraction, in agreement with previous studies (Sullivan et al. 2016, B. T. Sullivan, unpublished data). Additionally, *T. dubius* did not strongly distinguish host odor lures, although the data suggested some preference for the *alpha*/*beta*-pinene combination over turpentine (Fig. 4). Host odors greatly increase responses

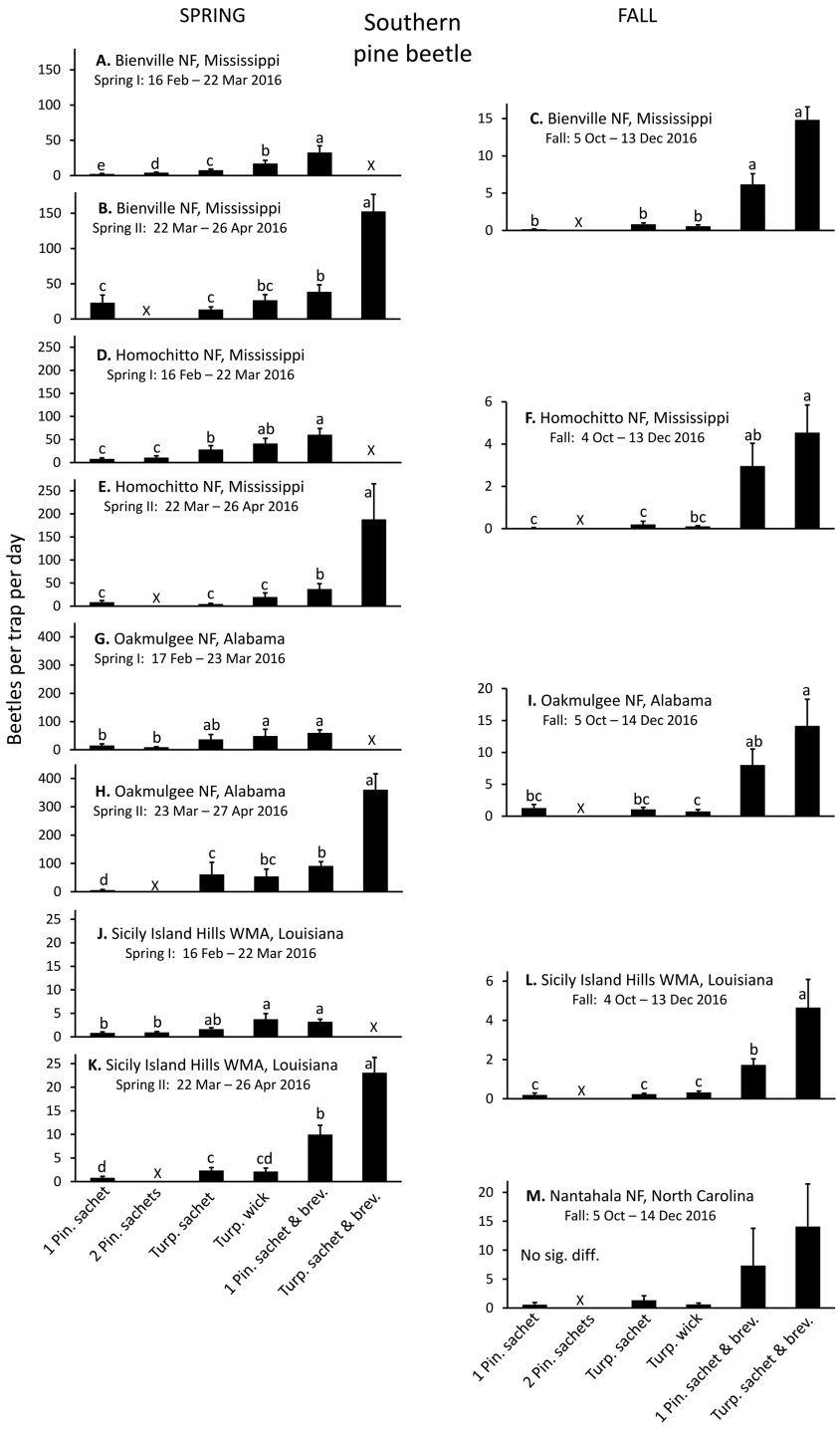

Fig. 2. Trap catches of southern pine beetle with different lure combinations at individual study sites. Other information in Fig. 1 legend.

of *T. dubius* to southern pine beetle pheromone components (Billings 1985, Shepherd and Sullivan 2018), and this species can discriminate among individual host compounds (Staeben et al. 2015, Munro et al. 2020).

Regional Variation

For southern pine beetle, there was not a statistically significant interaction between site and lure response during the spring trials, and this suggests that the springtime survey results are not affected by regional variation. However, the geographic area covered by our spring tests represented only a portion of the range of southern pine beetle in the United States, as the mid-South and East Coast were not included. Regional variation in southern pine beetle production of, and behavioral response to, semiochemicals has been reported for populations in the southeastern United States (Berisford et al. 1990, Grosman et al. 1997). However, production differences were small (Grosman et al. 1997), and behavioral assays were performed in the laboratory (Berisford et al. 1990) and as such may not have reflected responses of flying insects (Niño-Domínguez et al. 2015, 2016). Largely unhindered

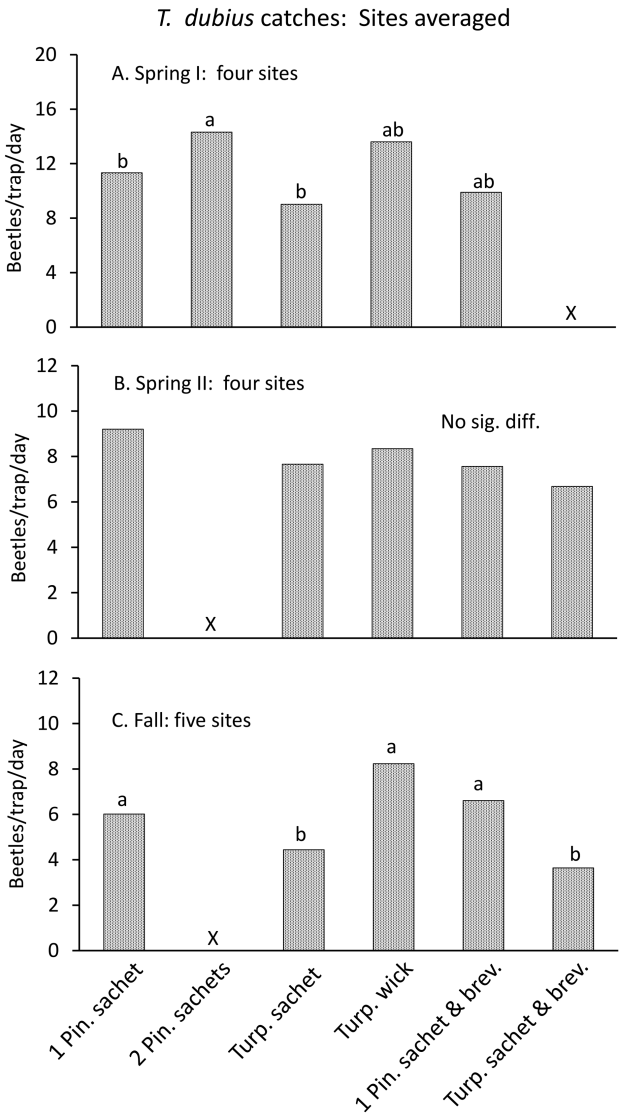

Fig. 3. Trap catches (averaged among study sites) of the predator *Thanasimus dubius* with different lure combinations. Other information in Fig. 1 legend.

gene flow among populations of southern pine beetle within the southeastern and mid-Atlantic United States (Schrey et al. 2008, Havill et al. 2019) should promote uniformity in semiochemical responses across this portion of the range of this species. A weak statistical interaction between site and lure during the fall trials suggests some regional variation in lure responses; however, the preferences observed for lures at all five sampled sites were similar and the variation seems unlikely to have either biological or practical significance.

Seasonal Variation

Research by the authors is underway to determine whether monitoring traps established in fall may have value in forecasting outbreaks during the following summer and thereby provide more advanced warning. In our study, responses of both southern pine beetle and *T. dubius* differed significantly between fall and spring, indicating that selection of operational lures should consider season. This agrees with previous evidence indicating that semiochemical responses by southern pine beetle are influenced by the time of year (Roberts et al. 1982, Sullivan et al. 2016). The size of southern pine beetle catches across all lure types was much greater in spring (with a mean 18-fold difference in catches between spring rotation 2 and fall) at all four locations where trapping occurred in both seasons. Evidence indicates that southern pine beetle may have both a fall and spring dispersal flight (Hedden and Billings 1977) (also J. J. Riggins and S. R. Clarke, unpublished data; however, see Sullivan et al. 2016); however, the far lower catches in fall than spring at the four sites where data for both seasons were available (Fig. 2) suggest that a fall dispersal flight was very small or absent. It is noteworthy that our fall southern pine beetle catch numbers displayed no correspondence with spot abundances in either the previous or following summers at the respective sites (Table 1; Fig. 2), and three sites with far lower fall than spring catches were in outbreak status (Table 1). There was no such seasonal difference in overall catches apparent for *T. dubius*. Strong statistical interactions among lure combination, season, and site were detected both for southern pine beetle and *T. dubius*, indicating that preferences among the lure combinations varied between seasons for at least some sites. For southern pine beetle, season by lure interactions could be explained in part by typically stronger synergistic effects of *endo*-brevicomin in fall than spring (Figs. 1 and 2). However, the major trends of preferences among lure combinations in our study (e.g., strong enhancing effects of *endo*-brevicomin and preference of turpentine over pinene lures for southern pine beetle; absence of strong lure discrimination by *T. dubius*) appeared to be unchanged by season (Figs. 1–4).

Composition of the Host Odor Component

The turpentine sachet was superior to the pinene sachet in enhancing attraction of the southern pine beetle when *endo*-brevicomin was a component of the lure combination (Figs. 1 and 2). Since the release rates of these two lures were very similar (Table 2), the difference in behavioral responses should be attributed to differing semiochemical compositions. Both devices released compounds noted in previous studies to be either attraction enhancers or reducers for southern pine beetle (Sullivan 2016, Munro et al. 2020), and the majority of antennal responses were to such semiochemicals. Attraction-enhancing semiochemicals shared by both lures included *alpha*-pinene, *beta*-pinene, and *trans*-verbenol (Payne et al. 1978, Staeben et al. 2015, Shepherd and Sullivan 2018, Munro et al. 2020); both also contained attraction reducers myrcene, *trans*-pinocarveol, and verbenone (Salom et al. 1992, Sullivan 2005, Munro et al. 2020). The turpentine devices additionally released small amounts of 4-allylanisole which has been reported alternatively to reduce or enhance southern pine beetle response to components of the aggregation attractant (Hayes et al. 1994, Munro et al. 2020). An attraction-reducing semiochemical for southern pine beetle (the green leaf volatile 1-hexanol; Dickens et al. 1992) was detected in trace amounts from the pinene sachet in week 1. The presence of both attractive and inhibitory semiochemicals in different concentrations in both host odor lures precludes a simple interpretation of the differences in southern pine beetle responses. It should be noted that all compounds in Table 5 except *alpha*-pinene oxide have previously been identified as olfactory stimulants for southern pine beetle (Payne 1975; Sullivan 2005; Shepherd and Sullivan 2013; Niño-Domínguez et al. 2015, 2018; Munro et al. 2020), although several have not been studied behaviorally.

Both the pinene and turpentine lure compositions were dominated by *alpha*-pinene. The *alpha*-pinene in both was predominantly the (+)-enantiomer; however, the turpentine lures had a substantially higher proportion of the (+)-enantiomer than the pinene lure. In

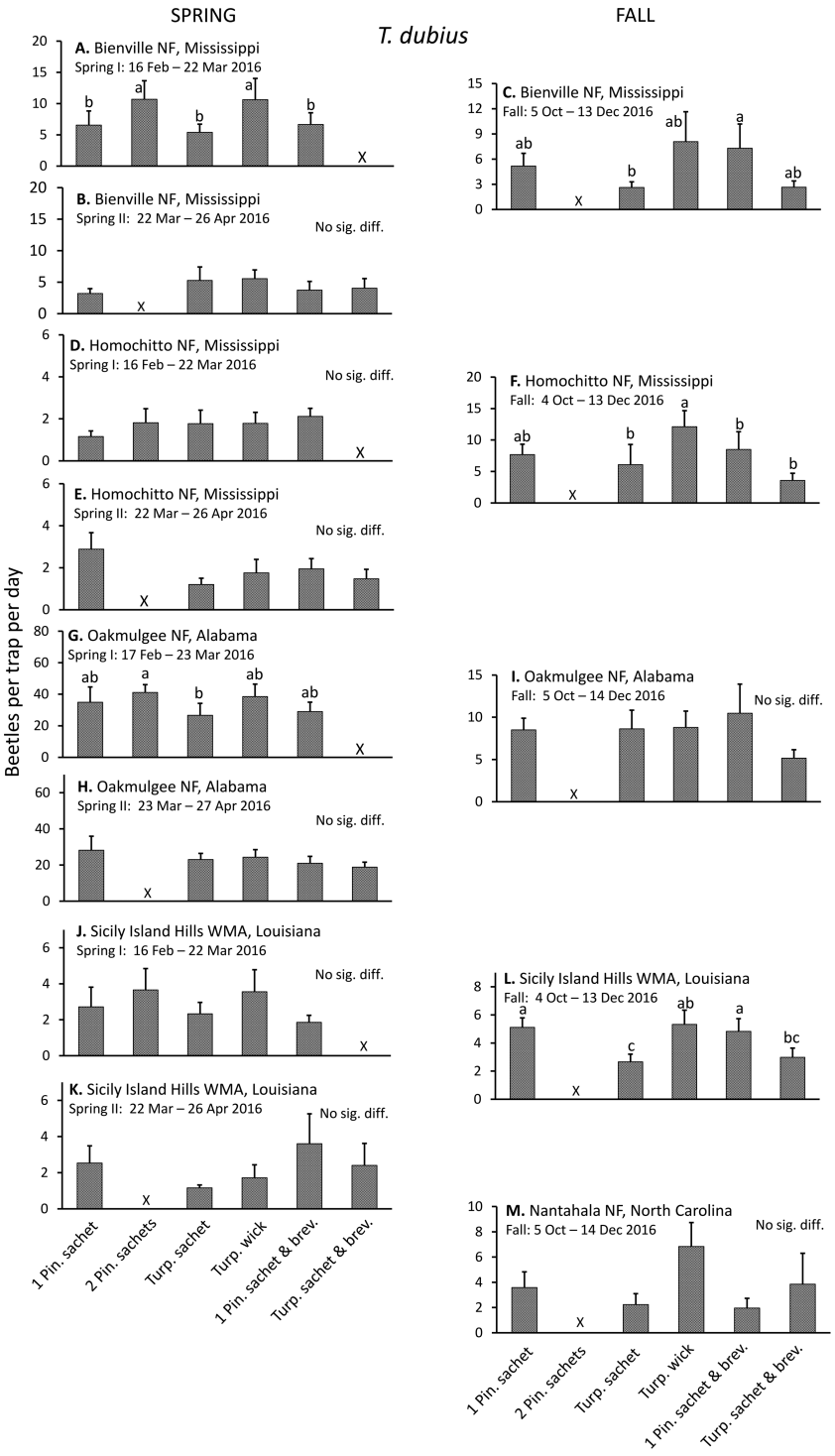

Fig. 4. Trap catches of the predator *Thanasimus dubius* with different lure combinations at individual study sites. Other information in Fig. 1 legend.

trapping trials with southern pine beetle (Staeben et al. 2015), the (+)-enantiomer of *alpha*-pinene was a more potent attractant synergist than the (−)-enantiomer, but the (+)-enantiomer did not differ from racemic *alpha*-pinene. Hence, the difference in the enantiomeric ratio of *alpha*-pinene between the turpentine and pinene lures likely did not contribute significantly to the stronger responses by southern pine beetle to the turpentine lures in the present study.

Our data imply that modifications to the composition of the pinene lure should allow significant enhancement of its attractiveness. The addition of a 4-allylanisole device significantly increased southern pine beetle attraction to the lure combination that included frontalin, *endo*-brevicomin, and the terpene sachet (i.e., lure 5; authors' unpublished data). A major drawback to using a commercial turpentine as a lure component is that consistency of the composition, and thus activity of the lure, cannot be guaranteed due to difficulties in assuring uniformity in resin collection methods, turpentine processing, and source tree species and lineage. Complex but compositionally fixed, synthetic host odor lures assembled from commercially produced, single compounds

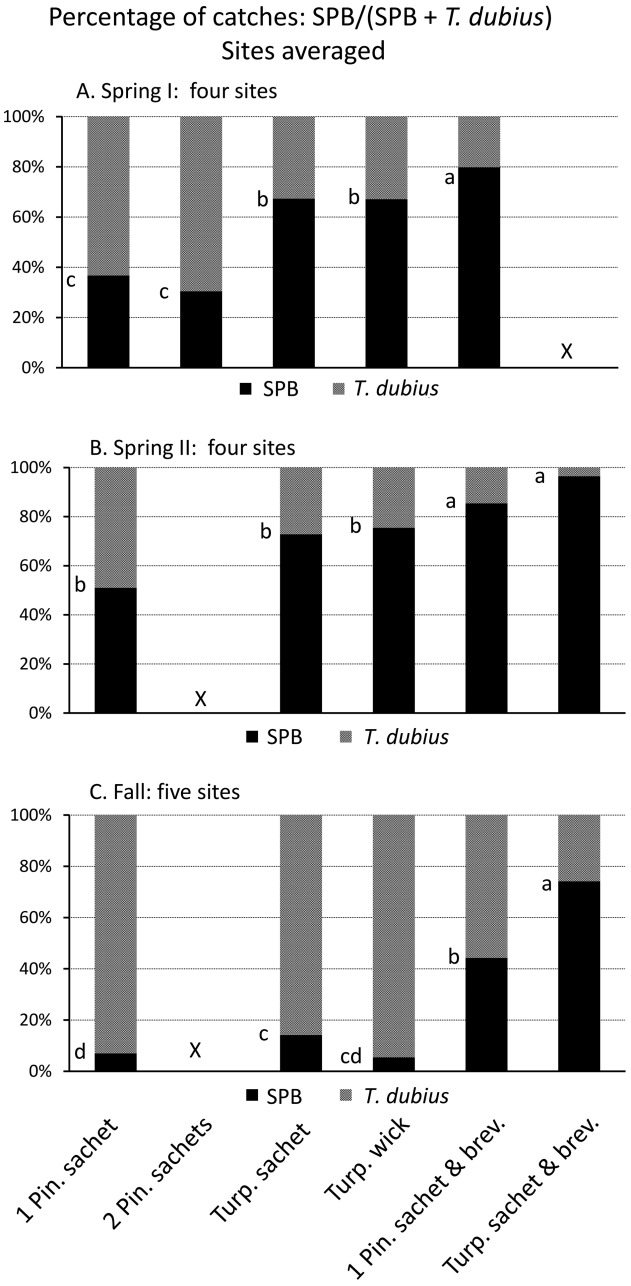

Fig. 5. Percent capture of southern pine beetle (southern pine beetle catches divided by the sum of southern pine beetle and *T. dubius* catches) with different lure combinations averaged among study sites. This particular calculation has been used historically as a variable for forecasting southern pine beetle outbreaks. Other information in Fig. 1 legend.

of known purity may offer enhanced lure sensitivity while assuring reproducibility and compatibility of data.

Adjustments to Data Collected With Different Lures

We estimate that replacement of the turpentine wick device with the pinene sachet in the springtime survey lure in 2007 decreased southern pine beetle attraction by a factor of 0.2, whereas addition of *endo*-brevicomin to the lure in 2017 caused a 9- to 10-fold increase. These factors provide an empirical basis for adjusting southern pine beetle catch numbers and integrating historical data sets produced with the different lure combinations. Caution is advised since these suggested correction factors assume no differences in release rates of devices between the present study and past surveys (the present study used devices being utilized for the springtime survey at the time of the tests). These factors also assume that the attractiveness of the turpentine in this study was similar to the turpentine used in the pre-2007 lure formulation; however, these turpentines were derived from different tree species (*P. oocarpa* vs *P. taeda* L., respectively). Since the springtime survey's inception, release rates and dynamics for commercial release devices have varied somewhat due to modifications in construction and materials used in the devices (B. T. Sullivan, unpublished data). Such variability may be difficult to prevent in future given inevitable changes in cost and availability of the raw materials to the manufacturers (D. Wakarchuk, personal communication). As the three historically implemented lure combinations did not differ significantly in catches of *T. dubius* (Fig. 3), no adjustment is recommended to reconcile data sets in raw numbers of this species. This implies that lure-dependent variation in percentage of southern pine beetle trapped is influenced only by the lure preferences of southern pine beetle.

Conclusions

Our overall assessment of lure composition for southern pine beetle is that the frontalin/*endo*-brevicomin/turpentine sachet combination is superior for detecting cryptic beetle populations such as within the northern limits of the expanding range of southern pine beetle. At present, we believe the frontalin/*endo*-brevicomin/pinene sachet combination is the appropriate lure for the springtime survey due to its combination of high sensitivity and defined, reproducible composition.

Caution is recommended during use of any southern pine beetle lure, particularly those that are highly attractive. Baited traps can stimulate lethal mass attacks on adjacent pines and potentially initiate infestations (Vité 1970), and the probability increases when southern pine beetle population densities are high. The current recommendation is that baited traps be placed in mixed pine/hardwood stands and be at least 23 m (75 ft) from the nearest pine in order to prevent such 'spillover' attacks. Infestations are less likely to be initiated in stands with a lower pine content, and these stands are less able to sustain infestation growth. Traps should be visited frequently and surrounding trees inspected for beetle attacks, particularly if catches of southern pine beetle are large. Cessation of trapping at a location where attacks are observed can often terminate further attacks and prevent tree mortality and spot initiation.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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Table 5. Compounds (mg/h) present in dynamic headspace samples of host odor lures that stimulated responses[a] in antenna of southern pine beetle

	Turpentine sachet		Turpentine wick		Pinene sachet	
	Week 1	Week 3	Week 1	Week 3	Week 1	Week 3
Compound	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
alpha-Pinene	**25.1 (2.1)**	**17.5 (6.3)**	**11.6 (4.0)**	**6.8 (1.1)**	**19.3 (2.80)**	**15.1 (2.3)**
beta-Pinene	**0.27 (0.02)**	**0.24 (0.03)**	**0.18 (0.03)**	**0.13 (0.01)**	**5.38 (0.58)**	**4.16 (0.25)**
3-Carene	**0.688 (0.064)**	**0.393 (0.073)**	**0.460 (0.076)**	0.259 (0.022)	nd[b]	nd
Myrcene	**0.088 (0.009)**	**0.037 (0.008)**	**0.051 (0.008)**	**0.029 (0.002)**	**0.024 (0.003)**	0.008 (0.001)
6-Methyl-5-hepten-2-one	nd	nd	nd	nd	**0.003 (0.001)**	trace[c]
1-Hexanol	nd	nd	nd	nd	**trace**	nd
(*Z*)-3-Hexen-1-ol	nd	nd	nd	nd	**trace**	nd
alpha-Pinene oxide	**0.022 (0.002)**	**0.048 (0.005)**	0.014 (0.004)	**0.021 (0.001)**	0.021 (0.002)	**0.053 (0.008)**
(*E*)-2-Hexen-1-ol	nd	nd	nd	nd	**trace**	nd
Linalool	**0.016 (0.001)**	**0.015 (0.003)**	**0.010 (0.003)**	**0.007 (0.001)**	nd	nd
Longifolene	**0.001 (<0.001)**	**0.005 (0.001)**	**0.007 (0.004)**	**0.012 (0.002)**	nd	**nd**
trans-Pinocarveol	**0.003 (<0.001)**	**0.005 (0.001)**	0.003 (0.001)	**0.002 (<0.001)**	0.005 (0.001)	**0.009 (<0.001)**
4-Allylanisole	**0.009 (0.001)**	**0.004 (0.001)**	**0.014 (0.003)**	**0.009 (0.001)**	nd	nd
trans-Verbenol	**0.005 (0.001)**	**0.007 (0.001)**	**0.003 (0.001)**	**0.003 (<0.001)**	0.003 (0.001)	**0.006 (<0.001)**
Verbenone	0.002 (<0.001)	**0.002 (<0.001)**	0.001 (<0.001)	0.001 (<0.001)	0.001 (<0.001)	**0.002 (0.001)**

[a]In coupled gas chromatography-electroantennographic detection (GC-EAD) analyses. A genuine olfactory response was recorded when an EAD trace deflection occurred at the retention time of the compound in at least four out of six antenna tested. Bold type indicates samples for which an olfactory response was recorded.

[b]'nd' indicates that the compound was not detected.

[c]'trace' indicates that the compound was detected in the aeration sample but occurred beneath the threshold for quantitation.

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