Olfactory Stimulants for *Sirex nigricornis* (Hymenoptera: Siricidae) and its Parasitoid, *Ibalia leucospoides* (Hymenoptera: Ibaliidae), in Odors of Stressed and Bark Beetle–Colonized Pines[1]

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J. Entomol. Sci. 58(1): 1–15 (January 2023)
DOI: 10.18474/JES22-03

Abstract We conducted studies with the native woodwasp *Sirex nigricornis* F. (Hymenoptera: Siricidae) in Louisiana to identify host-associated olfactory stimulants that may include attractive semiochemicals for this species as well as *Sirex noctilio* F., an invasive species that can attack healthy trees. Loblolly pines (*Pinus taeda* L.) treated with a stressing agent were felled and chipped 1–2 mo after treatment, and the chips were steam–water distilled in a Clevenger apparatus to extract volatile compounds. Using coupled gas chromatography–electroantennographic detection, we analyzed the distillates with antennae of both *S. nigricornis* and the *Sirex* parasitoid *Ibalia leucospoides* (Hochenwarth) (Hymenoptera: Ibaliidae) to identify olfactory stimulants. In addition, we assayed *S. nigricornis* antennae with synthetic mixtures containing 23 volatile compounds associated with stressed and dying pines, including pheromones of bark beetles (Coleoptera: Curculionidae: Scolytinae) that also use these hosts. Antennae of both male and female *S. nigricornis* responded to 29 identifiable volatiles in the distillates, whereas *I. leucospoides* responded to 23 volatiles. Eighteen compounds in the synthetic mixtures were olfactory stimulants for *S. nigricornis*. Olfactory stimulants in the woodchip distillates were predominantly hydrocarbon and oxygenated monoterpenes, and the strongest antennal stimulants among compounds in the synthetic mixtures were oxygenated monoterpenes associated with pine death and early decay (e.g., verbenone, fenchone, and terpinen-4-ol). Bark beetle pheromones (frontalin, *endo*-brevicomin, ipsenol, and ipsdienol) also stimulated *S. nigricornis* antennae. The very large number of host-associated olfactory stimulants for *S. nigricornis* implies that our data may offer limited assistance in targeting individual compounds for investigation as possible attractants and components for a *Sirex* lure.

Key Words GC-EAD, *Sirex noctilio*, woodwasp, host volatiles

Sirex nigricornis F. (Hymenoptera: Siricidae) is native to southern Canada, the Great Lakes region, and New York through the southeastern United States (Schiff et al. 2012). It infests at least a dozen pine (*Pinus* L.) species as well as spruce (*Picea* Miller) (Hartshorn 2021, Hartshorn et al. 2016, Schiff et al. 2012). Like other

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woodwasps in the subfamily Siricinae, it oviposits into weakened and dead conifers, and the larvae mine and complete development in the xylem (Morgan 1968; Schiff et al. 2006, 2012). It is dependent on symbiotic fungi in the genus *Amylostereum* that provide nutrients for the developing larvae (Coyle et al. 2012, Schiff et al. 2006). Because it does not infest healthy trees, it is not considered a significant pest (Hartshorn et al. 2016, Schiff et al. 2012) and has received limited study.

By contrast, the Palearctic native *Sirex noctilio* F. can kill healthy trees and is a major forestry pest in portions of the southern hemisphere where it has become established (Hurley et al. 2007, Krivak-Tetley and Hajek 2021, Ryan and Hurley 2012, Schiff et al. 2012, Spradbery 1973). Tree mortality is caused by a phytotoxic venom injected during oviposition and growth of the symbiotic fungus *Amylostereum areolatum* (Fries) Boidin (Krivak-Tetley and Hajek 2021). *Sirex noctilio* was discovered in the United States (New York state) in 2004 (Hoebeke et al. 2005) and subsequently has spread to nine U.S. states and two Canadian provinces (Liebhold and Hajek 2021). Although tree mortality in North American forests invaded by *S. noctilio* has been substantially less than that observed in the southern hemisphere (Dodds et al. 2010, Haavik et al 2018), there remain concerns about this species' broader ecological impacts and the potential economic harm it may cause as its range expands and it encounters new host species and different forest management practices (Haavik and Foelker 2021, Liebhold and Hajek 2021).

The semiochemistry of host location has been studied in both *S. noctilio* and *S. nigricornis*. Both species are attracted to trees that have been artificially stressed with biocide or girdling (Barnes et al. 2014, Madden 1971, Madden and Irvine 1971, Zylstra et al. 2010), but artificial trap lures formulated from analyses of volatiles from stressed trees have so far not proven as attractive as stressed trees or cut billets (Barnes et al. 2014, Bashford and Madden 2012, Crook et al. 2012, but see Johnson et al. 2013). The undesirable sacrifice of trap trees commonly used for monitoring pest populations of *S. noctilio* (Dodds and de Groot 2012), and the resource investment in creating and monitoring these trees, could be eliminated by a potent, synthetic trap lure. Currently, the operational, artificial lure for trapping *S. noctilio* consists of a 70:30 blend of two major monoterpenes in pine resin, α- and β-pinene, respectively (Bashford and Madden 2012, Hartshorn et al. 2015). However, electroantennogram studies have revealed that *S. noctilio* can sense many additional odors from suitable hosts (Crook et al. 2012) and that it can discriminate among pine species and chemotypes that differ in their volatile profiles (Bookwalter et al. 2019, Böröczky et al. 2012, Xu et al. 2019). This suggests that a more complex blend of semiochemicals might be a superior lure for *S. noctilio*. Numerous compounds have been tested as possible adjuvants to the pinene lure, but none have produced a significant improvement in trap catches for either *S. noctilio* or *S. nigricornis* (Allison 2021, Barnes et al. 2014, Crook et al. 2012, Johnson et al. 2013).

Although *S. nigricornis* is not a significant pest, identification of attractants for *S. nigricornis* could be informative for improving lures for *S. noctilio*, because the biological similarities between the congeners suggest that they share cues and strategies for locating hosts. Furthermore, more attractive lures and better trapping protocols for *S. nigricornis* may help researchers in monitoring this native siricid and determining the ecological impacts of *S. noctilio* as the latter species invades new areas (Barnes et al. 2014, Hartshorn 2021). The original plan of this study was to

identify *S. nigricornis* attractants by (a) extracting gram quantities of essential oil by steam–water distillation of woodchips from trees in a condition attractive to *S. nigricornis*, (b) testing the distillates and fractions as possibly superior trapping lures for *S. nigricornis*, and (c) analyzing the composition of the distillates and fractions and identifying olfactory stimulants (and thus potential semiochemicals) for *S. nigricornis* within both the chip extracts and mixtures of chemical standards. Our approaches in components a and b were based on studies by author BTS in which steam–water distillation was used to extract host-infested bark to obtain gram quantities of the host location attractant for two species of bark beetle parasitoid (Sullivan et al. 1997, 2003). These extracts were fractionated in sufficient quantities for trapping experiments, and their composition was copied to produce a candidate synthetic attractant. Synthetic mixtures tested in olfactory studies of component c included compounds already known to be associated with hosts for *S. noctilio* and *S. nigricornis*: those of healthy host pines (e.g., resin monoterpene hydrocarbons), stressed and dying pines, and pheromones of bark beetles (Coleoptera: Curculionidae: Scolytinae) that prefer similar hosts and whose presence may indicate weakened trees suitable for *S. nigricornis*. Oppositely, these pheromones may signal host unsuitability, as previous studies have shown that bark beetle associates may negatively affect *S. noctilio* (Haavik et al. 2020, Ryan et al. 2012).

However, trapping assays for *S. nigricornis* conducted in 2007 with the woodchip distillates and in 2008 with a combination of three olfactory stimulants, as well as the commercial *Sirex* lure, captured insufficient numbers of *S. nigricornis* to permit statistical inferences. Herein, we report the results of the electrophysiological and analytical chemistry components of the larger study. These data are cited as unpublished in Crook et al. (2012). We also report some data for olfactory sensitivity of *Ibalia leucospoides* (Hochenwarth) (Hymenoptera: Ibaliidae), a parasitoid of *Sirex* eggs and early instars (Smith and Schiff 2002), to compounds in the woodchip extracts. This parasitoid is an efficacious biological control agent against *S. noctilio* (Coyle and Gandhi 2012, Fischbein and Corley 2015).

Materials and Methods

Experiment 1: olfactory responses to compounds in steam–water distillates of stressed and dying pines. At a single site in Grant Parish, LA (31.594°N, 92.416°W), loblolly pines (*Pinus taeda* L.; 15–20 cm in diameter at 1.4-m height) were treated with the wood fumigant sodium *N*-methyl-dithiocarbamate (WoodFume®, Osmose, Inc., Buffalo, NY) and dimethyl sulfoxide in a 4:1 ratio. Cotton wicks soaked with 10 ml of the solution were inserted into hatchet frill cuts (spaced 5 cm around the bole and 31 cm above the soil line) that penetrated into the sapwood (Johnson et al. 2013). This artificial stress treatment has been shown to make trees attractive to *S. nigricornis* and reduce tree resin yield, thereby making them more susceptible to attack by subcortical insects generally (Strom et al. 2004, Johnson et al. 2013). Two trees were treated in September 2007. One tree was cut in October at the first sign of bark beetle attack (appearance of entrance holes and frass of *Ips* species), and the other tree was cut in November when the bark was thoroughly colonized and had begun to detach from the bole. This felling schedule corresponded with peak flight times for *S. nigricornis* (Hartshorn et al. 2015,

Johnson et al. 2013). In addition, four trees were treated in April 2008: two of these trees were cut in May and the other two trees were cut in June according to the same criteria used for trees cut in 2007. More trees were treated and cut in 2008 than in 2007, with the intent of producing larger volumes of distillates for use in trapping assays. Although we did not cut the trees in spring 2008 when adult *S. nigricornis* were flying, we wanted to evaluate *S. nigricornis* responses to potentially different wood volatile components collected during the fall and spring sampling events. The timing of these events was intended to span the interval between early tree decline and the loss of host suitability (coincident with heavy bark beetle colonization) and thus permit us to obtain extracts with elevated concentrations of *S. nigricornis* attractants.

All felled trees were chipped with a woodchipper (chips typically $<$300 cm^2) and then chips were steam–water distilled with a large-scale Clevenger apparatus to extract water-insoluble volatile oils (Sullivan et al. 2003). Distillates were designated first cut, fall 2007 (Fa1); second cut, fall 2007 (Fa2); first cut, spring 2008 (Sp1); and second cut, spring 2008 (Sp2). The Sp1 and Sp2 samples each contained the combined distillates from two trees. The bark chip distillates as well as steam-distilled, commercially obtained *P. taeda* turpentine (Hercules, Inc., Wilmington, DE) were diluted 1/100 by volume in redistilled hexane.

We live collected female *S. nigricornis* (both color morphs; Schiff et al. 2012) and female *I. leucospoides* in Lindgren 12-unit multiple-funnel traps (Lindgren 1983) in Grant Parish (31.594°N, 92.416°W). Woodwasps were trapped between November and December 2007 and 2008 during their peak flight in the southern United States (Hartshorn et al. 2015, Johnson et al. 2013), and female *I. leucospoides* were trapped in December 2007. Traps were baited with host odors 75% (+)-α-pinene, (−)-β-pinene, and ethanol and bark beetle pheromone components (±)-ipsdienol and (±)-ipsenol (all known or suspected attractants for these species), and they were located in an undisturbed, mixed pine–hardwood stand approximately 0.1 km from pine log piles awaiting processing at a plywood mill. Insects were collected twice per week from trap collection cups that contained several wadded paper towels as a substrate for walking or hiding. Emerging male *S. nigricornis* were obtained in fall 2009 from naturally infested *Pinus echinata* Miller, *Pinus palustris* Miller, and *P. taeda* logging slash from an active sawtimber thinning site in Grant Parish (31.673°N, 92.467°W). Slash was placed into outdoor metal rearing drums, and emerging wasps were collected daily. Collected insects were stored in a refrigerator (~4°C) in ventilated plastic specimen cups containing moistened paper wipers and were tested within 5 d.

Using coupled gas chromatography–electroantennographic detection (GC-EAD), we assayed olfactory responses of male and female *S. nigricornis* and female *I. leucospoides* to constituents of the steam–water distillates. The Fa1 and Fa2 distillates were each tested on eight female *S. nigricornis* and two female *I. leucospoides*; Sp1 and Sp2 were tested on nine female and eight male *S. nigricornis*. Turpentine was tested with eight female and eight male *S. nigricornis* and two female *I. leucospoides*. Numbers of insects tested were limited by the availability of each species or sex collected from traps or rearing drums. Identical instrumentation and similar methods as in Asaro et al. (2004) were used for GC-EAD analyses. A single excised antenna was positioned between two glass electrodes (filled with Beadle-Ephrussi saline and 0.5% polyvinylpyrrolidone, with

Ag–AgCl wires making contact with the saline) in a charcoal-filtered and humidified airstream (400 ml/min). The antenna was clipped with Vannas scissors midway through both the scape and the distal segment of the flagellum. The tip of each electrode was cut to make the opening slightly larger than the diameter of the antenna, and the ends of the antenna were inserted into the openings. One microliter of each sample was injected splitless into the GC of the GC-EAD (model 5890, Hewlett–Packard, Palo Alto, CA) fitted with an HP-INNOWax column (Agilent Technologies, Wilmington, DE); the temperature program was 35°C for 1 min, 16°C/min to 80°C, 8°C/min to 230°C, and then held for a final 8 min, with helium as the carrier gas. Half of the column effluent was directed to the flame ionization detector (FID) and half into the airstream passing over the antennal preparation.

If an EAD trace deflection at a particular retention time exceeded the 90th percentile of the background noise amplitude in at least 3 of 8, 4 of 9, or 4 of 10 runs (or both runs for *I. leucospoides*), we inferred that a genuine olfactory stimulant eluted at that retention time (binomial probabilities test, $\alpha = 0.05$). Peaks in the FID trace were identified by analyzing the same samples by coupled gas chromatography–mass spectrometry (GC-MS) on a Hewlett–Packard 6890-5973 instrument by using the same column and GC-operating parameters as used for the GC-EAD analyses. Peak identities were determined from mass spectral and retention time matches to identified standards (Sigma-Aldrich Corp., St. Louis, MO; Fluka Chemical Corp., Buchs, Germany; Acros Organics, Geel, Belgium; Fisher Scientific, Hampton, NH). Isopinocamphone was obtained by the oxidation of isopinocampheol (Sigma-Aldrich Corp.) with $CrO_3 + H_2SO_4$, and β-phellandrene was from dipentene (Millennium Chemicals, Cockeysville, MD).

Experiment 2: olfactory response to compounds in synthetic mixtures. We used GC-EAD to test sensitivity of female *S. nigricornis* antennae to 23 commercially obtained compounds associated with healthy, stressed, decaying, and bark beetle–killed pines (Bookwalter et al. 2019, Flechtmann et al. 1999, Mirov 1961, Sullivan et al. 1997) and bark beetle pheromone components (Skillen et al. 1997): 4-allylanisole, (±)-borneol, (±)-*endo*-brevicomin, (±)-camphor, *p*-cymen-8-ol, *p*-cymene, *p*-α-dimethylstyrene, ethanol, (±)-fenchone, (±)-frontalin, (±)-ipsdienol, (±)-ipsenol, (±)-limonene, (–)-myrtenal, (–)-myrtenol, (±)-α-pinene, (±)-β-pinene, (–)-*trans*-pinocarveol, (±)-terpinen-4-ol, γ-terpinene, (±)-α-terpineol, and (±)-verbenone (Sigma-Aldrich Corp.; Fluka; Acros; Bedoukian Research Inc., Danbury, CT; Aaper Alcohol and Chemical Co., Shelbyville, KY; Albany International, Willoughby, OH; PheroTech Inc., Delta, BC, Canada). Isopinocamphone was synthesized as indicated above; enantiomeric composition was unknown. Purities of tested volatiles were at least 85% and generally greater than 95%. All insects were captured in funnel traps by using previously described methods in Grant Parish (31.594°N, 92.416°W) in November 2006 and maintained as described previously.

To minimize possible reduction in response amplitudes due to sensory adaptation by previously eluting olfactory stimulants, compounds were divided into two separate mixtures to allow at least 7 s of separation between antennal exposures (i.e., 12-s difference in retention times) to each compound. However, in electroantennogram studies with *S. noctilio*, Simpson (1976) observed no effects of adaptation with just 1-s recovery time between exposures to stimuli. Compounds were diluted approximately 1.5–2.5 mg/ml in hexane, and 9–11 females were tested

with each mixture. One microliter of each test mixture was injected in split mode (1/20) into the GC-EAD with the same column indicated previously. The GC oven temperature was 40°C for 1 min, 16°C/min to 80°C, 7°C/min to 230°C, and then held for a final 8 min.

EAD deflection amplitudes were corrected to account for variation in antennal responsiveness among insects and for any loss of antennal vigor over the duration of the run as follows. A bark beetle pheromone component and strong olfactory stimulant identified in preliminary studies, (±)-frontalin, was included in both mixtures at approximately the same concentration as the test compounds. In addition, test mixtures were diluted 1/50 by volume in mineral oil, and 10 μl was applied to a strip of filter paper in a Pasteur pipette. Purified air (30 ml/min for 2 s) was puffed through the pipette into the airstream over the antenna at both the beginning and end of each GC-EAD run, and the difference in response amplitudes was assumed to be the result of a linear decline in antennal responsiveness. Response voltages to all compounds in each run were normalized relative a line with the same x-intercept as the decline function and intersecting the point where X and Y equaled the retention time and response amplitude to (±)-frontalin, respectively. A compound was classed as an olfactory stimulant if four or more EAD traces indicated a deflection exceeding the 90th percentile of the amplitude of background noise (binomial probabilities test, $\alpha = 0.05$) at the compound's retention time. By considering split ratio, the FID/EAD split, the amount injected, and sample concentration, we estimate that antennae were exposed to 0.03–0.05 μg of each compound.

Results

Experiment 1: olfactory responses to compounds in steam–water distillates of stressed and dying pines. Olfactory responses by *S. nigricornis* antennae coincided with 29 identifiable FID peaks in the woodchip distillates or turpentine (Table 1; Fig. 1A). The sexes did not differ in compounds eliciting a response. In the woodchip distillates, 15 compounds (12 only in the spring samples and 3 in both spring and fall samples) that elicited antennal responses in *S. nigricornis* could not be identified, whereas 12 olfactory stimulants could not be identified in the turpentine (9 unique to turpentine). We were unable to identify these compounds due to poorly resolved mass spectra, absence of an adequate match in our mass spectral library, or the lack of a standard for making a confirmation. The parasitoid *I. leucospoides* responded to 23 identified volatiles in the woodchip distillates and/or turpentine that were likewise antennally active for *S. nigricornis* (Table 1; Fig. 1B) as well as 7 unidentified compounds (1 only in the woodchip distillates, 3 only in the turpentine, and 3 in both). The four woodchip distillates (i.e., from trees cut 1 or 2 mo posttreatment with wood fumigant in either spring or fall) did not differ in numbers of identifiable olfactory stimulants for *S. nigricornis* (Table 1). Turpentine had a less complex composition and contained fewer identifiable olfactory stimulants (15) than the woodchip distillates (28). One olfactory stimulant, *trans*-verbenol, was detected only in turpentine.

Experiment 2: olfactory response to synthetic compounds. Antennae of female *S. nigricornis* responded to 18 of the 23 of compounds in the synthetic

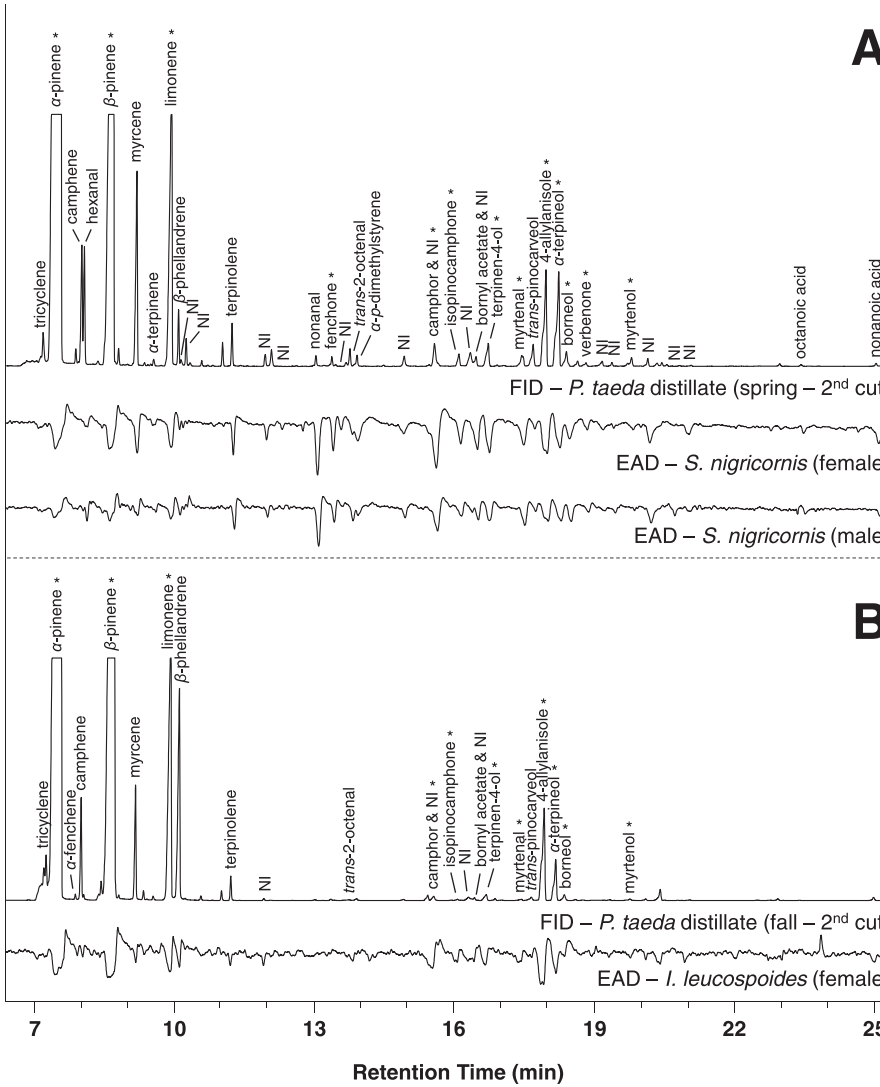


Fig. 1. Output from the FID and summed traces from the EAD in GC-EAD analyses. (A) Nine female and eight male *S. nigricornis* antennae responding to compounds in a distillate of chips from *P. taeda* cut in the spring following artificially induced, heavy colonization by subcortical insects. (B) Two female *I. leucospoides* responding to a distillate of chips of *P. taeda* in the same condition, but cut in the fall. Only FID peaks that coincided with a statistically verified olfactory response (see text) are labeled. Compounds whose antennal activity was confirmed in GC-EAD tests with a standard (Fig. 2) are marked with an asterisk (*). NI, unidentified compound.

Table 1. Responses from antennae of *S. nigricornis* males and females and *I. leucospoides* females to compounds in steam–water distillates of chips from stressed, dying *P. taeda*, and commercial *P. taeda* turpentine.

Compound*	Antennal Response**	Distillate Source***
Tricyclene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
α-Pinene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
α-Fenchene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Camphene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Hexanal	mS, fS	Fa1, Fa2, Sp1, Sp2
β-Pinene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Myrcene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
α-Terpinene	mS, fS	Fa1, Fa2, Sp1, Sp2
Limonene	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
β-Phellandrene	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Terpinolene	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Nonanal	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Fenchone	mS, fS	Fa1, Fa2, Sp1, Sp2
trans-2-Octenal	mS, fS, I	Fa1, Fa2, Sp1, Sp2
p-α-Dimethylstyrene	mS, fS	Fa1, Fa2, Sp1, Sp2, T
Camphor and NI	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Isopinocamphone	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Bornyl acetate and NI	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Terpinen-4-ol	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Myrtenal	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
trans-Pinocarveol	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
4-Allylanisole	mS, fS, I	Fa1, Fa2, Sp1, Sp2
trans-Verbenol	mS, fS, I	T
α-Terpineol	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Borneol	mS, fS, I	Fa1, Fa2, Sp1, Sp2
Verbenone	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Myrtenol	mS, fS, I	Fa1, Fa2, Sp1, Sp2, T
Octanoic acid	mS, fS	Fa1, Fa2, Sp1, Sp2
Nonanoic acid	mS, fS	Fa1, Fa2, Sp1, Sp2

Table 1. Continued.

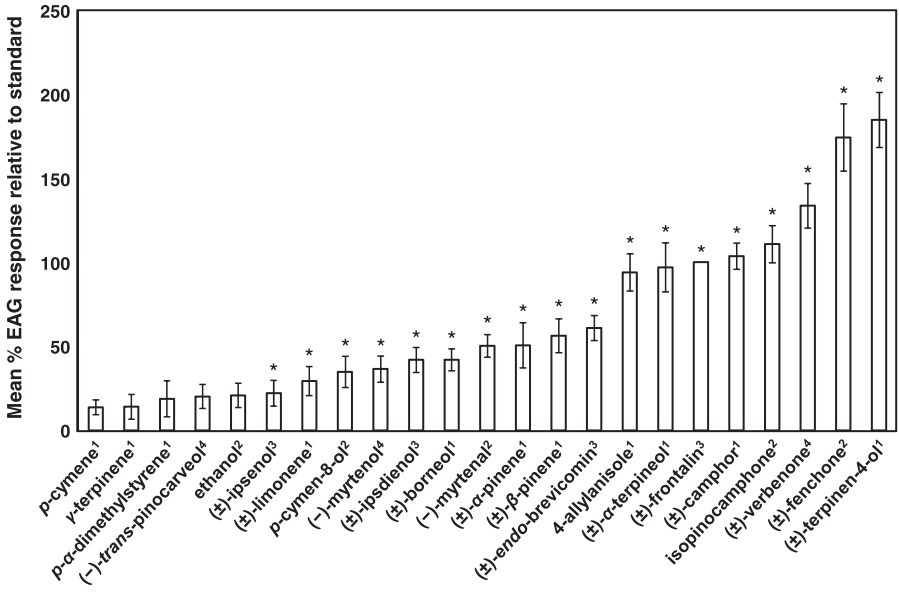
* Identities of the FID peaks that coincided with the EAD deflections and were subsequently confirmed using GC-MS with commercially obtained or synthesized standards. Compounds are presented in order of increasing retention time. Compounds in boldface font also produced a significant response in the GC-EAD tests of synthetic mixtures. NI, coeluting compound not identified.
** EAD trace deflections were detected in analyses of antenna of multiple individuals (see text). mS, male *S. nigricornis*; fS, female *S. nigricornis*; I, *I. leucospoides*.
*** T, commercial turpentine. Source of steam–water distillates: Fa1, artificially weakened tree cut in fall after first insect attacks observed; Fa2, artificially weakened tree cut in fall after bole was heavily infested and loose bark observed; Sp1,2, as stated for Fa1 and Fa2, but in spring.

mixtures (Fig. 2). The mean response amplitudes varied widely among compounds; however, there was a general tendency for oxygenated compounds to produce stronger olfactory responses than hydrocarbons, and the former were predominantly oxygenated monoterpenes. The largest response amplitudes were to (±)-verbenone, (±)-fenchone, and (±)-terpinen-4-ol (Fig. 2). Only *p*-cymene, *p*-α-dimethylstyrene, ethanol, (−)-*trans*-pinocarveol, and γ-terpinene failed to produce responses at the tested concentration.

Discussion

Our data indicate that *S. nigricornis* can sense an abundance of compounds associated with suitable host trees, implying that host selection and location could be mediated by a complex semiochemical mixture. *Sirex noctilio* antennae display a similar breadth of sensitivities and respond to >20 compounds in pine volatiles (Crook et al. 2012, Simpson 1976, Simpson and McQuilken 1976). Consequently, olfaction studies may provide limited guidance in targeting host-associated compounds for behavioral studies with *Sirex* (Crook et al. 2012). In addition, a very large number of olfactory stimulants in our host tree distillates could not be identified and thus would not be subject to behavioral evaluation without further chemical analysis. However, the apparent abundance of olfactory stimulants in our studies could be an artifact of the concentration of exposure we used in the GC-EAD analyses and thus the responses may have been occurring at levels of exposure that might not be experienced by woodwasps seeking or selecting a host tree. Dose–response studies may prove informative. It is noteworthy that male *Sirex* are not attracted to trap trees or lures attractive to females (Barnes et al. 2014, Hartshorn et al. 2016, Hartshorn 2021), despite having an essentially identical olfactory response profile.

The major pine monoterpenes α-pinene and β-pinene were olfactory stimulants for *S. nigricornis*. This was reported previously for *S. noctilio* (Simpson 1976, Simpson and McQuilken 1976) and is consistent with the attraction of *S. nigricornis* to a 70:30 mixture of α-pinene and β-pinene that is operationally used as a lure for *S. noctilio* (Bashford and Madden 2012, Coyle et al. 2012, Johnson et al. 2013). However, another reported semiochemical for *Sirex*, ethanol, did not produce an olfactory response at the concentration tested. Ethanol is released from stressed and dying trees (Kelsey 1994, Kelsey and Joseph 2003, Miller and Rabaglia 2009) and has been shown to be attractive to native siricids, including *S. nigricornis*, when



[1]Host resin constituent
[2]Compund associated with hosts in early states of decay
[3]Bark beetle pheromone component
[4]Both (2) and (3)

Fig. 2. Normalized antennal response amplitudes (±SE) for female *S. nigricornis* antennae exposed to commercially obtained or synthesized host volatiles and bark beetle pheromones. Compounds were tested on 9–11 antennae [(±)-frontalin tested on 20]. An asterisk (*) indicates that the mean EAD spike voltage at the retention time of the compound was significantly higher than the mean level of background noise.

combined with (−)-α-pinene (Erbilgin et al. 2017). However, ethanol did not significantly increase *S. nigricornis* catches when added to the operational α- and β-pinene *Sirex* lure (Barnes et al. 2014, Johnson et al. 2013).

The great majority of compounds that elicited olfactory responses in *S. nigricornis* have either failed to show behavioral activity with *Sirex* species, or no investigations have been reported for them. The bark beetle pheromone components frontalin, *endo*-brevicomin, ipsenol, and ipsdienol were all olfactory stimulants for *S. nigricornis*. These semiochemicals are released by *Dendroctonus* or *Ips* bark beetles that commonly are the first subcortical insects to arrive on weakened pines (Flamm et al. 1993), and our results are aligned with the hypothesis that they have behavioral activity. They could signal host availability and be indirect host location cues (kairomones) for *S. nigricornis*. Conversely, bark beetle–colonized trees are not attacked by *S. noctilio* (Gitau et al. 2013), which suggests these pheromones could function additionally or alternatively in signaling host unsuitability. However, pheromone components of sympatric *Ips* species

(ipsenol and ipsdienol) did not alter captures of native siricids by a host monoterpene lure (Johnson et al. 2013).

Host-associated olfactory stimulants for *S. nigricornis* reflected those reported for *S. noctilio*. Both species displayed strongest olfactory responses to oxygenated monoterpenes present in host-associated odors, and responses to oxygenated monoterpenes were generally stronger than to the hydrocarbon monoterpenes that quantitatively dominate host odors and have demonstrated attractiveness for *S. noctilio* (Crook et al. 2012, Simpson 1976). In experiment 2, the strongest response amplitudes were elicited from *S. nigricornis* by the oxygenated monoterpenes (±)-terpinen-4-ol, (±)-fenchone, (±)-verbenone, isopinocamphone, and (±)-camphor, and these responses were substantially greater than those elicited by the monoterpene hydrocarbons (±)-α-pinene, (±)-β-pinene, and (±)-limonene. These oxygenated monoterpenes are also among those eliciting the strongest electroantennogram responses from *S. noctilio* (Crook et al. 2012, Simpson 1976). Oxygenated monoterpenes increase in relative concentration in the volatile profile of pines and other conifers following infestation by subcortical insects and felling (Flechtmann et al. 1999, Simpson and McQuilkin 1976). *Sirex* attraction to trees likewise increases in the first days or weeks following felling or treatment with herbicide (Johnson et al. 2013, Simpson and McQuilkin 1976, Zylstra et al. 2010), suggesting a correlation between timing of *Sirex* attraction and increasing oxygenated monoterpene concentrations. However, oxygenated monoterpenes fenchone and verbenone, although exceptionally strong olfactory stimulants for *S. noctilio* and *S. nigricornis*, did not significantly alter captures of *S. noctilio* when included with an α- and β-pinene lure (Crook et al. 2012). We note that the ordering of response amplitudes among compounds may have been influenced by variation among exposure concentrations to the compounds (1.5–2.5 mg/ml was injected into the GC-EAD). However, variation in exposure concentration typically is associated with disproportionately smaller variation in antennal response amplitude (Byers 2013). In addition, the enantiomeric compositions of the tested compounds may not have reflected those produced by host trees or bark beetles in nature, and insects can have differing olfactory sensitivities to enantiomers.

The parasitoid *I. leucospoides* had a similar profile of olfactory responses to odors in distilled woodchips as did its host, displaying responses to most of the same hydrocarbon and oxygenated monoterpenes. Robertson (2014) found numerous olfactory stimulants, including many of the same hydrocarbon and oxygenated monoterpenes reported herein, in volatiles collected from *S. nigricornis* oviposition sites. This suggests the parasitoid may use similar cues in locating their *Sirex* hosts as *Sirex* use in locating host trees. In the laboratory, *I. leucospoides* are attracted to a combination of volatiles from the *S. noctilio* fungal associate, *A. areolatum*, as well as to (−)-α-pinene and ethanol (Faal et al. 2021). However, there is no published evidence that *I. leucospoides* is attracted to an artificial lure in the field.

Our results indicate numerous candidate compounds that might be investigated as attractants (or repellants) for both *S. nigricornis* and *I. leucospoides*. However, exploring their individual and combined potential as lure adjuvants would be very challenging and possibly fruitless given the sheer number of compounds and combinations that could be presented in lures. Pilot tests with *S. noctilio* that formulated lures from the strongest olfactory stimulants associated with hosts

indicated no significant improvement in attraction, suggesting there might be limited value in using electroantennogram techniques in targeting attractants (Crook et al. 2012).

Acknowledgments

The authors thank James Meeker, Billy Bruce, Saul Petty, and Chris Steiner, USDA Forest Service, for providing guidance, tree treatment or cutting, and live *S. nigricornis* for this project, and Zach Oliver, USDA Forest Service, for assisting with the distillations. Funding was provided by USDA Forest Service, Southern Research Station, RWU-4552.

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