

Volatile Profile Differences and the Associated *Sirex noctilio* Activity in Two Host Tree Species in the Northeastern United States

Katalin Böröczky · Kelley E. Zylstra ·
Nathaniel B. McCartney · Victor C. Mastro ·
James H. Tumlinson

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Abstract *Sirex noctilio* females are known to be attracted to stem sections of stressed pine trees for oviposition. The volatile profiles and attractiveness of Eastern white pine (*Pinus strobus*) and two chemotypes of Scots pine (*P. sylvestris*) were compared after stem injection with herbicide. In general, trap captures on herbicide-treated trees were higher than on controls. The high-carene chemotype of Scots pine captured the highest numbers of females, followed by the low-carene chemotype, and finally the Eastern white pine. Herbicide-treated trees of both species emitted larger quantities of volatiles than the controls. The herbicide treatment induced higher volatile emission rates in the Scots pine chemotypes than in white pine, although there was no difference between the two chemotypes. However, qualitative

differences were found between the volatile profiles of the two species as well as between the two Scots pine chemotypes, which could account for the differential attractiveness of the species and chemotypes tested.

Keywords *Sirex noctilio* · Lure development · Stem injection · High-carene Scots pine · Host volatiles · Hymenoptera · Invasive insect

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K. Böröczky (✉) · N. B. McCartney · J. H. Tumlinson
Center for Chemical Ecology, Department of Entomology,
The Pennsylvania State University,
University Park, PA 16802, USA
e-mail: katalin.by@gmail.com

K. E. Zylstra
USDA, APHIS, PPQ, CPHST,
374 Northern Lights Drive,
North Syracuse, NY 13212, USA

V. C. Mastro
USDA, APHIS, PPQ, CPHST, Otis Laboratory,
Bldg. 1398, W. Truck Rd.,
Buzzards Bay, MA 02542, USA

Present Address:
K. Böröczky
Department of Entomology, North Carolina State University,
Raleigh, NC 27695, USA

Introduction

Plants produce a wide array of volatile compounds by which they lure pollinators and natural enemies of their herbivorous pests (Bruce and Pickett 2011). However, these compounds frequently serve as host finding cues for the herbivores themselves. Having co-evolved with their plant hosts, insects have developed an olfactory system that houses olfactory receptor neurons (ORNs) finely tuned for specific volatile compounds (Hansson et al. 1999). At any time, insects encounter blends of volatiles and receive numerous components simultaneously at the peripheral level, but the behavioral output, i.e., directed flight towards or away from a source and avoidance or acceptance, is formed in higher centers of the brain (Baker 2009).

Wood-feeding insects also rely on volatile compounds emitted by host and non-host tree species to make the right choice for landing, although it varies to which extent primary attraction plays a role in pre-landing behavior (Saint-Germain et al. 2007). While conifer monoterpenes are important cues in host finding behavior of bark beetles (Gijzen et al. 1993), in some species integration of visual and olfactory cues is necessary for successful assessment of the potential host (Campbell and Borden 2009).

In our study system, the woodwasp *Sirex noctilio* F. (Hymenoptera: Siricidae), females disperse soon after emergence (Morgan and Stewart 1966) and find their preferred host for oviposition, mostly *Pinus* species (Hurley et al. 2007). As many other wood-feeding insects, *S. noctilio* females are attracted to physiologically stressed trees: the stem of suppressed pines, stem sections conditioned by caged females (Madden 1968), or artificially stressed pine trees (Zylstra et al. 2010). Females possess a ventral ovipositor in a sheath modified for inserting eggs about a centimeter deep into the wood of their host (Schiff et al. 2006). With each drill, whether just probing the wood or ovipositing, females inject mucus and the arthrospores of the symbiotic fungus, *Amylostereum aerolatum* (Coutts and Dolezal 1969). The fungus is essential for the development of the larvae. In areas where *S. noctilio* populations are dense, successive attacks may result in the death of healthy trees (Morgan and Stewart 1966) due to the combined effects of the mucus and the fungus (Spradbery 1973).

Host finding behavior of a female *S. noctilio* could be viewed as a three-step process: 1) terminating the search flight and landing on a possible host, i.e., prealighting behavior (Singer 1986); 2) drilling into the wood to assess wood quality; and 3) laying eggs if the tree is accepted as a suitable host. We have been investigating the volatile compounds that may determine the behavior of the woodwasp in the first step.

According to Madden (1968), the physiological changes that occur in the host upon the introduction of natural and artificial stressors are similar. Interrupted translocation of soluble solids and increased respiratory activity of the phloem tissues lead to depletion of food reserves. After high mechanical girdling, for example, the stem section below the treatment becomes attractive to females. The diffusion rate of carbon dioxide as well as of water vapor and monoterpenes through that stem section becomes more rapid compared to healthy parts (Madden 1968, 1988). Attraction of female woodwasps to stressed trees has been attributed to enhanced emissions of monoterpenes (Madden 1988).

Although monoterpene constituents of the resin of Monterey pine (*Pinus radiata*), such as α -pinene, β -pinene, δ -3-carene, β -myrcene, and β -phellandrene, elicit strong electrophysiological response in the antenna of females (Simpson 1976), chemical lures containing these compounds individually or in combination have not been as successful as herbicide-injected lure trees in capturing the insect in the Northeastern United States (unpublished data of the authors).

Sirex noctilio is an invasive pest in North America (Hoebeker et al. 2005; Hurley et al. 2007). Preferred host species in the current *S. noctilio* range on this continent are the native Jack pine (*P. banksiana*), red pine (*P. resinosa*), and Eastern white pine (*P. strobus*),

and the introduced Scots pine (*P. sylvestris*) (Dodds et al. 2010). In our trapping study conducted in the summer of 2008 over ten sites, Scots pine and white pine captured much higher numbers of *S. noctilio* females when conditioned by stem-injection with the herbicide Dicamba (3,6-dichlororo-2-methoxybenzoic acid) compared to untreated controls (unpublished data of the authors). In addition, there was a strong trend with herbicide-treated (HT) Scots pine capturing higher numbers of woodwasps than HT white pine. Volatile compounds were collected from trap trees at only one of the ten sites. Herbicide treatment caused increased volatile emissions in both species, however, more so in Scots pine (unpublished data of the authors). These results prompted us to perform a field trapping experiment combined with collection of volatiles emitted by trap trees of the two pine species.

Our goal in this study, conducted in 2009, was to investigate volatile production and simultaneously monitor trapping efficiency of two host species of *S. noctilio*, Scots pine (*P. sylvestris*) and white pine (*P. strobus*), during the flight season. The high-carene and low-carene producer chemotypes of Scots pine (Chalchat et al. 1985) were included as two different treatment groups. We developed a system to collect volatiles from stem sections of living trees causing minimal disturbance, which allows us to monitor the volatile production of the same trees throughout the trapping season. Based on our findings we suggest the testing of specific compounds with electrophysiological methods and in behavioral assays to identify chemicals that mediate host finding behavior in *S. noctilio* and possibly facilitate the development of a chemical lure.

Methods and Materials

Experimental Setup and Trapping Mixed sites with both Scots pine and white pine were selected in the Syracuse area of New York. Only trees with no signs of infestation were used in the experiments. An attempt was made to keep uniformity of height, stem diameter, and foliage. Trees of the same treatment were grouped in clumps of three within about a 5 m circle to enhance trapping efficiency. For a simple overview of the experimental setup see Table 1.

Our treatment groups reflected the fact that Scots pine occurs in two chemotypes. Scots pine chemotypes were determined by analyzing the resin composition before the start of the season. Resin was collected from Scots pine trees (153 trees altogether) at four potential sites in May by inserting a 1-ml screw cap vial into a 2–3 cm deep hole drilled in the stem at 45°, and the samples were analyzed for δ -3-carene (see Sample Preparation and Chemical Analysis). Three sites were found to have sufficient numbers of both chemotypes and were selected for simultaneous

Table 1 Experimental setup

Treatment groups	High-carene Scots pine: HT and control Low-carene Scots pine: HT and control White pine: HT and control
Nr. of sites	3
Nr. of replicates within site	3 trees per clump ^a 1 clump of each HT group per site 1 clump of each control group per site
Frequency of trap checking	Weekly
Type of traps used	Multiple-funnel traps
Arrangement of traps	1 trap per tree
Volatile collection time-points	Week 0, 3, 4, 5, 6, 7, 8, 9, 11

HT herbicide-treated, control: untreated

^a Except for low-carene Scots pine: one of the sites had only two trees in the HT and two in the control clump due to herbicide treatment of the wrong tree and misidentification of the chemotype, respectively

trapping and volatile collection. The treatment groups were: herbicide-treated (HT) and control high-carene Scots pine, HT and control low-carene Scots pine, and HT and control white pine.

In summary, we had three sites each of which had one clump for each of the six treatment groups, and each clump had three trees (except at one site the low-carene HT and control clump had only two trees, due to herbicide-injection of the wrong tree in the HT clump and misidentification of the chemotype in the control clump). Each tree in the study had a multiple-funnel trap attached (Lindgren 1983). Thus, the number of replicates was 9 for HT and control high-carene Scots pine and white pine, and 8 for HT and control low-carene Scots pine. Stem injection of Dicamba to stress the HT trees was performed in the week of June 8 (week 0) according to the methods described by Zylstra et al. (2010). Traps were checked weekly between the weeks of June 29 (week 3) and Sept 21 (week 15). Volatiles were collected from all HT and control trees first in week 0, then every week between week 3 and week 9, and finally in week 11. Volatiles were not collected on rainy days.

At the end of the trapping season, one tree from each treatment group was felled and bucked into equal-sized billets and brought back to the USDA laboratory in Syracuse, NY. The ends of all of the billets were coated with Waxlor (Willamette Valley Co., Eugene, OR, USA) to prevent drying. Billets from the same tree were placed into drums with screens and maintained at 26°C and <20% RH. Overhead fluorescent lights were turned on at 7:30 am and turned off at 4:30 pm. Once emergence commenced, siricids were collected, identified, and tallied daily until no more *Sirex* individuals were emerging from the material.

Volatile Collection A portable two-pump volatile collection system with a teflon-sheet chamber (FEP100 fluoropolymer

film; Dupont, Wilmington, DE, USA) was developed to sample sections of a living tree without inflicting damage (Fig. S1 in Supplemental Information). The collection chamber is constructed from a wire frame wrapped with a FEP sheet and secured to the trunk with straps. Two grommet-reinforced holes in the FEP film located on opposite sides of the tree allowed us to introduce purified air on one side and install a filter filled with 30 mg of SuperQ sorbent (Alltech Associates, Deerfield, IL, USA) (SuperQ filter) on the opposite side to collect volatiles. One pump pushed air into the chamber through a charcoal filter, and the other pulled air out of the chamber through the SuperQ filter.

Volatiles were collected for 30 min at a flow rate of 0.6–0.7 l/min measured through the SuperQ filter. The ingoing flow rate of purified air was set to 1.0–1.2 l/min. Flow rates were checked with variable area flow meters (Key Instruments, Treviso, PA, USA) and adjusted if needed every morning. Before each collection 1 µl of a solution containing 100 mg/ml of 1,4-diisopropyl benzene in hexane was added to a small glass cartridge filled with glass-wool. The glass cartridge was inserted at the end of the ingoing air tube. This compound was an indicator of pump performance and air flow. Filters on which volatile compounds had been collected were sealed with Teflon[®] tape at both ends, wrapped in aluminum foil, and kept at room temperature until analysis (typically for 1–2 weeks). Filters were washed with a sequence of acetone, dichloromethane, and hexane, and were stored in aluminum foil packages between experiments.

Sample Preparation and Chemical Analyses All samples were taken to the Penn State laboratory for analysis. For the determination of the Scots pine chemotype, a small volume (5–10 µl) of resin was diluted in 500 µl of hexane (Mallinckrodt Baker, Phillipsburg, NJ, USA), and the sample was analyzed under the same conditions as the volatile collection samples (see below). Resin samples were only examined for the presence of δ -3-carene based on comparison of the retention time to that of the purchased reference. SuperQ filters were eluted with 150 µl of a solution containing 50 µg/ml of nonyl acetate (internal standard) dissolved in a 1:1 mixture of hexane and dichloromethane (Burdick & Jackson, Morristown, NJ, USA). Nitrogen flow was applied onto the filters to assist elution.

All samples were analyzed in an Agilent 6890 gas chromatograph-flame ionization detector system (GC-FID) equipped with an Equity-5 (30 m×0.2 mm×0.2 µm; Supelco, Bellefonte, PA, USA) column. Helium was used as carrier gas at an average linear velocity of 25 cm/s. Samples were injected in the splitless mode, and the injector was changed to split mode after 0.75 min. The initial oven temperature was held at 45°C for 1 min, then programmed

to 300°C at a rate of 10°C/min, and maintained at that temperature for 10 min. The injector and the detector temperatures were set to 280°C and 300°C, respectively. The enantiomeric ratio of α -pinene was determined by injecting samples on an Rt- β DEX (30 m \times 0.25 mm \times 0.25 μ m; Restek, Bellefonte, PA, USA) column installed in the above mentioned GC-FID. The oven program was the following: 45°C (1 min hold)-3°C/min to 140°C, then 10°C/min to 210°C (10 min hold). The injector and the detector were both kept at a temperature of 210°C. Other parameters were the same as above.

To identify blend components selected samples were analyzed in a GC-MS system consisting of an Agilent 6890 N gas chromatograph interfaced with an Agilent 5973 N mass selective detector. The capillary column was equivalent to that used in the GC-FID. Helium was used as carrier gas at an average linear velocity of 30 cm/s. Samples were injected in splitless mode with a split time of 0.75 min. Temperature settings were the same as in the GC-FID, except that here the transfer line was held at 300°C. The MS was used in electron impact (EI) ionization mode with the default temperature settings (ion source: 230°C, and quadrupole: 150°C). Compounds were identified by matching their spectra to spectra from the NIST 02 and Wiley mass spectral libraries. The identity of the following terpenes was confirmed by comparison of spectra with those of reference standard compounds purchased from Sigma-Aldrich: α -pinene, β -pinene, δ -3-carene, camphene, β -myrcene, limonene, γ -terpinene, terpinolene, eucalyptol, verbenone, α -cedrene, caryophyllene, and (*E*)- β -farnesene.

Quantification of compounds in SuperQ samples was based on GC-FID peak area compared to the peak area of nonyl acetate as the internal standard. Peak area values were corrected according to the relative response factors. For components with a purchased reference standard available (see above), the response factors relative to that of nonyl acetate were determined based on the slope value of the respective calibration curves. Terpenes identified based on their mass spectrum had the relative response factor of one of the reference compounds assigned depending on which compound class they belong to (aliphatic, monocyclic, or bicyclic monoterpene hydrocarbon, aliphatic or cyclic sesquiterpene hydrocarbon, monoterpene alcohol, or ketone). Since limonene and β -phellandrene were not separable on the Equity-5 column, they were quantified as one compound.

Volatile Emission Calculations The hourly volatile emission rate of the whole trunk section of the trees through the bark was estimated using the calculated amount of volatiles collected in 30 min based on the GC data. The collection covered a surface area of about 0.365 m². Considering the trunk of an average pine tree to be a truncated cone with a

height of about 12 m, a base diameter of about 25 cm, and the top diameter of about 2 cm, the surface area of such a trunk is about 5.244 m². Thus, we used a factor of 14 for the surface area and a factor of 2 for the time period resulting in a combined factor of 28. This factor was multiplied by the measured emission rate to yield total emission rates per tree-trunk per hour.

Statistical Analyses Data were analyzed starting from week 4 since no *Sirex* was captured on the traps prior to that week. Outlier volatile emission rate values were not included for one of the two reasons: 1) The tree was substantially damaged during trap setup causing elevated volatile emission rates at the beginning of the season, or 2) there was resin contamination in the chamber (which usually happened towards the end of the season).

Total trap captures and the chemical composition data were analyzed for differences between treatment groups using a mixed model (Littell et al. 2006) with treatment as fixed effect, and site and site*treatment interaction as random factors. In the analysis of weekly trap captures and weekly emission rates, week and treatment*week interaction were also added as fixed effects with site*week and site*treatment*week interactions as random factors. Tree-to-tree variation was accounted for in the residual variances. Competing models (separate residual variances vs. common residual variances) were compared using Akaike's Information Criterion (AIC), and the model with the lowest AIC was selected. Separate residual variances were used for each treatment when necessary to attain normality and homogeneity of variances. All trapping and volatile emission data were square-root transformed, and the chemical composition data were square-root transformed when necessary to attain normality. For pairwise comparison of the Least Square Means (LS Means), Tukey multiple-range test was performed using the pdmix800 SAS macro (Saxton 1998). No statistical tests were performed on the emergence data due to extremely low sample sizes.

Results

Needles began to turn brown in both pine species as a symptom of stress by the 4th week after stem injection with the herbicide Dicamba. No such effect was observed with the control pine trees. The whole canopy of herbicide-treated (HT) Scots pines (*Pinus sylvestris*) turned brown by the 7th week whereas it happened to white pines (*P. strobus*) only by the 9th week.

Trapping Only female *Sirex noctilio* were captured in our study: a total number of 120 at all three sites combined. As

in 2008, the majority of females were caught by HT Scots pines (92), about two-thirds of which were caught by the high-carene chemotype (64). There was a significant difference in the total trap captures between treatment groups (4.93, 0.016, *F*, *P*). The numbers of *S. noctilio* females caught by HT trees were higher than the numbers caught by the respective controls, and the difference was significant for the high-carene chemotype (*P*=0.033) (Fig. 1). The difference between HT and control was not significant in low-carene Scots pine or white pine. Herbicide-treated trees of both Scots pine chemotypes captured more females than HT white pine, and the difference was significant between HT high-carene Scots pine and HT white pine (*P*=0.037).

The peak in trap captures was in the second half of July, 6 to 7 week after the herbicide treatment, about a week later than in the previous year. The statistics showed a weak overall week effect (Table 2), with week 7 being statistically different from week 4, and marginally statistically different from week 10 (*P*=0.036 and 0.081, respectively). The overall treatment effect was highly significant (Table 2), but there was no treatment*week interaction (1.30, 0.138, *F*, *P*). Weekly trap captures of herbicide-treated trap trees peaked 6 to 7 weeks after stem injection for the high-carene Scots pine and at week 5 to 6 for the low-carene chemotype (back-transformed LS Means are presented in Fig. 2a). White pine trap captures increased slightly in the middle of the season but stayed at a low level. The weekly trap captures differed significantly

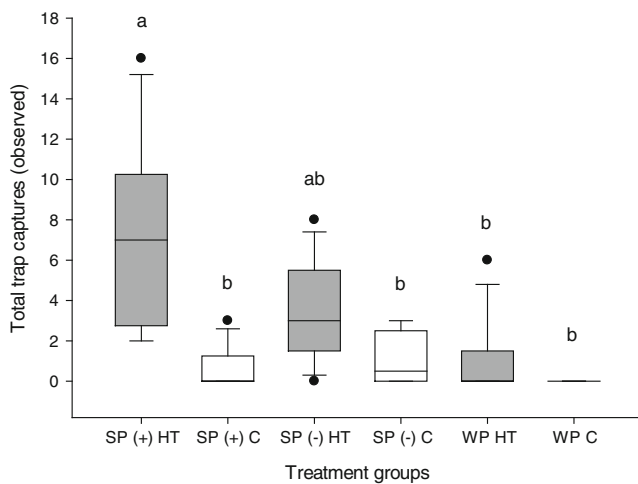


Fig. 1 Total number (observed data) of *Sirex noctilio* females captured by trap trees of the different treatment groups: SP (+) HT: herbicide-treated high-carene Scots pine, SP (+) C: control high-carene Scots pine, SP (-) HT: herbicide-treated low-carene Scots pine, SP (-) C: control low-carene Scots pine, WP HT: herbicide-treated white pine, and WP C: control white pine. Sample size for low-carene Scots pine is *N*=8, otherwise *N*=9. A mixed model was used to analyze the square-root transformed data (see Methods) and a significant treatment effect (*F*=4.93, *P*=0.016) was found. For pairwise comparison of back-transformed LS Means Tukey multiple-range test was used at α =0.05 significance level. Treatment groups not connected by the same letter are significantly different

Table 2 Tests of fixed effects in the mixed model for the trap capture and volatile emission rate data

Effect	Trap captures		Volatile emission rates	
	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Treatment	9.65	<0.001	14.09	<0.001
Week	2.34	0.033	4.46	0.004
Treatment*week	1.30	0.138	1.19	0.289

between treatment groups at week 6 and 7 (6.56, <0.001 and 6.40, <0.001, *F*, *P*, respectively). The trend among herbicide-treated groups in that period was such that high-carene Scots pine caught more wasps than low-carene Scots pine, which caught more wasps than white pine. However, significant difference was found only between HT high-carene Scots pine and HT white pine and only at week 6 (*P*=0.021).

Sites were different in the total number of *S. noctilio* females captured: 77, 28, and 15, and the site with the lowest number had a later peak in trap captures, at week 9, compared to the other sites (at week 6 to 7). These findings were reflected in the non-zero values of the covariance parameter estimates for site and site*week interaction in the statistical model.

Analysis of Volatiles The overall peak in emission rates was at week 5 to 6 after which the rates slowly declined with week 5 being significantly different from week 9 and 11 (Table 3). The back-transformed LS Means of the weekly volatile emission rates for each treatment group are presented in Fig. 2b. A strong treatment effect was observed, as in the trapping data, and also a strong week effect, but the treatment*week interaction was not significant (Table 2). While herbicide-treated Scots pines emitted significantly higher amounts of volatiles per hour than the respective control trees, stem injection did not cause a significant increase in the volatile emission rates of white pines (Table 3). When comparing the different herbicide-injected groups, Scots pines emitted significantly higher amounts per hour than white pine, but there was no significant difference in the emission rates of the two chemotypes of Scots pine (Table 3). As opposed to the trapping data, there were no site differences in the volatile emission rates, indicated by an estimated zero covariance for site as a random effect in the mixed model. However, the tree-to-tree variation was high, much higher than any other random effects (site*treatment, site*week, or site*treatment*week interactions).

In summary, when comparing quantities of volatiles emitted by high-carene Scots pine and white pine, higher trap captures paralleled higher emission rates. Nevertheless, while having comparable volatile emission rates to that of HT high-carene Scots pines, the low-carene Scots pines captured fewer *S. noctilio* females in comparison.

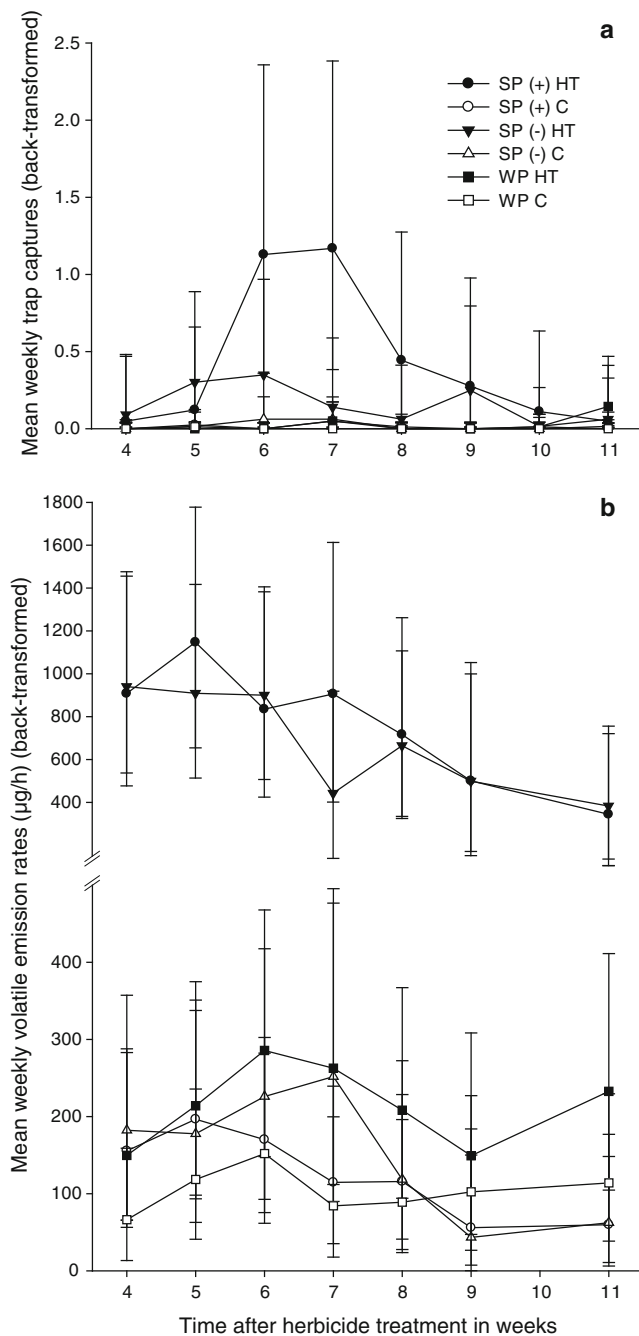


Figure 2 Mean weekly trap captures (back-transformed LS Means) of *Sirex noctilio* females by treatment groups ($N=8-9$), **a**, and the corresponding mean weekly volatile emission rates (back-transformed LS Means) ($N=4-9$), **b** Error bars represent the lower and upper 95% confidence intervals. A mixed model was used to analyze the square-root transformed data; for detailed results see Table 2 and 3

Qualitative differences were found in the volatile profiles of the stem injected treatment groups due to differential emission of some of the terpenes (Table 4). Percent composition of selected monoterpenes was compared at week 6 when there was a peak in trap captures with a significant treatment effect (6.56, <0.001 , F, P). The two monoterpenes α - and β -pinene

were major components in the volatile blend of all herbicide-treated groups. In high-carene Scots pine and white pine samples, δ -3-carene was a major component, too, although it was significantly less abundant in HT white pine compared to HT high-carene Scots pine ($P<0.001$). The sum of these three monoterpenes accounted for about 90% of the volatiles emitted in all herbicide-treated groups. Herbicide injection induced relatively higher emission rates of α -pinene in low-carene Scots pine only ($P=0.006$) (Table 4). The relative emission rates of β -pinene and δ -3-carene did not change significantly upon herbicide treatment for either species or chemotype. Some of the minor components, such as α -thujene, γ -terpinene, and terpinolene were present in significantly higher amounts in HT high-carene Scots pine compared to the other HT groups, and thus were positively linked to emission levels of δ -3-carene (Table 4). In addition, sabinene was significantly more abundant in high-carene Scots pine than in the low-carene chemotype and somewhat more abundant than in white pine. The relative emission rates of these minor components increased in high-carene Scots pine upon herbicide treatment. Other components, however, such as tricyclene, camphene, and limonene together with β -phellandrene, were emitted at a lower rate by HT Scots pines compared to HT white pine (trend only). The enantiomeric ratio (mean \pm SE) of α -pinene, (R)-/(S)-, in HT high-carene Scots pine tended to be higher (4.4 ± 1.2) than in the other two HT groups (1.7 ± 0.3 and 1.4 ± 0.2 for low-carene Scots pine and white pine, respectively), however, the difference was not significant. As a trend, herbicide treatment resulted in higher (R)-/(S)- ratios in HT high-carene compared to the control. Oxygenated monoterpenes occurred in traces in most of the samples, therefore, only the total amounts are listed in Table 4. The relative emission rate of these compounds was lower in HT Scots pines than in HT white pines, however not significantly. Sesquiterpenes could not be quantified due to the extremely small amounts detected in the samples. Thus, this compound group is not discussed in the paper.

Sirex noctilio Emergence Control trees of low-carene Scots pine and white pine had zero *S. noctilio* emergence from the sampled material, whereas the high-carene controls provided a few emerging woodwasps (5.67 ± 5.67). In HT trees, more *S. noctilio* emerged from the low-carene Scots pines (13.00 ± 5.15) than from the high-carene Scots pines (1.67 ± 5.15) and white pines (4.33 ± 5.15).

Discussion

Little is known about the mechanism by which the woodwasp *Sirex noctilio* finds a suitable pine host for oviposition. Regardless of the possible mode of action (attractant or

Table 3 Separation of treatment groups and weeks by volatile emission rates ($\mu\text{g/h}$)

High-carene Scots pine		Low-carene Scots pine		White pine		
HT	Control	HT	Control	HT	Control	
742 (510, 1017) ^a	119 (49, 217) ^b	658 (446, 910) ^a	141 (60, 255) ^b	212 (113, 341) ^b	102 (39, 195) ^b	
Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 11
319 (231, 420) ^{abc}	380 (283, 490) ^a	375 (279, 485) ^{ab}	295 (196, 415) ^{abc}	267 (186, 363) ^{abc}	183 (106, 280) ^{bc}	176 (112, 255) ^c

HT herbicide-treated, Control: untreated

Back-transformed LS Means are presented with the lower and upper 95% confidence intervals in parentheses. Treatment groups or weeks not connected by the same letter are significantly different (Tukey multiple-range test, $\alpha=0.05$)

arrestant) we have been looking for compounds emitted by stressed pines that may govern the host selection of the wasp. We analyzed volatiles from Scots pine (*P. sylvestris*) and white pine (*P. strobus*). Our behavioral assay was a field trapping study conducted in NY State in 2009. We demonstrated that high-carene Scots pines captured more females than the low-carene Scots pine chemotype or than white pines at the three mixed sites where trees had been stressed by stem injection with the herbicide Dicamba 4 week before the anticipated peak flight. Moreover, Zylstra et al. (2010) reported higher numbers of female woodwasps attracted to Scots pine than to red pine (*P. resinosa*), in a similar experimental setup. Thus, Scots pine, and especially the high-carene chemotype, is worthy of study as a potential source of kairomones that might be effective lure components.

We note that trap captures provide no information on differences in egg density or larval performance, i.e., the suitability of the tree for the development of the insect (Morgan and Stewart 1972). Therefore, experiments that test host preference by keeping females in a confined space with wood material might deliver a different ranking of pine species. In fact, we did not find a big difference in the emergence between the two host species, despite lower trap captures in white pine. This suggests that while volatiles draw woodwasps to the host, other factors, such as resin pressure and moisture content of the trees, affect the choice to oviposit and the success of development therein.

The total number of *S. noctilio* captured at each site varied in a wide range (15–77). The sites are relatively small stands on private properties in the Syracuse, NY area (separated by

Table 4 Mean percent compositions of selected monoterpenes (observed data) in samples collected 6 weeks after stem injection

	High-carene Scots pine		Low-carene Scots pine		White pine		Treatment effect ^a
	HT ^b	Control	HT	Control	HT	Control	F/P
α -Pinene	52.9 \pm 5.8 ^b	54.0 \pm 2.7 ^b	80.9 \pm 3.8 ^a	64.4 \pm 3.0 ^b	56.4 \pm 2.7 ^b	55.0 \pm 2.3 ^b	7.67/<0.001
(R)-/(S)-	4.4 \pm 1.2 ^a	2.3 \pm 0.04 ^a	1.7 \pm 0.3 ^a	1.7 \pm 0.2 ^a	1.4 \pm 0.2 ^a	1.6 \pm 0.2 ^a	2.79/0.050
β -Pinene	13.6 \pm 4.3 ^a	15.3 \pm 2.3 ^a	12.6 \pm 3.8 ^a	20.7 \pm 2.4 ^a	26.4 \pm 3.5 ^a	25.4 \pm 2.7 ^a	3.27/0.030
δ -3-Carene	25.8 \pm 3.4 ^a	17.8 \pm 2.7 ^a	0.5 \pm 0.2 ^c	5.0 \pm 2.0 ^{bc}	5.1 \pm 1.6 ^b	6.7 \pm 1.8 ^b	20.12/<0.001
Tricyclene	0.17 \pm 0.02 ^b	0.29 \pm 0.05 ^{ab}	0.21 \pm 0.02 ^b	0.34 \pm 0.05 ^{ab}	0.42 \pm 0.06 ^a	0.42 \pm 0.06 ^a	7.61/<0.001
Thujene	0.08 \pm 0.01 ^a	0.02 \pm 0.01 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.01 \pm 0.01 ^b	0.01 \pm 0.01 ^b	9.04/<0.001
Camphene	0.7 \pm 0.1 ^b	1.1 \pm 0.1 ^a	1.2 \pm 0.1 ^a	1.4 \pm 0.1 ^a	2.8 \pm 0.6 ^a	2.3 \pm 0.4 ^a	7.83/0.001
Sabinene	0.62 \pm 0.11 ^a	0.10 \pm 0.06 ^b	0.02 \pm 0.01 ^b	0.06 \pm 0.06 ^b	0.37 \pm 0.17 ^{ab}	0.40 \pm 0.27 ^{ab}	7.37/0.002
β -Myrcene	1.2 \pm 0.1 ^a	0.8 \pm 0.1 ^a	0.9 \pm 0.1 ^a	0.6 \pm 0.1 ^a	1.2 \pm 0.3 ^a	1.0 \pm 0.2 ^a	1.61/0.348
Lim/ β -phell ^c	2.0 \pm 0.2 ^b	3.1 \pm 0.3 ^a	2.4 \pm 0.2 ^{ab}	3.3 \pm 0.3 ^a	3.2 \pm 0.2 ^a	3.3 \pm 0.3 ^a	4.12/0.004
γ -Terpinene	0.18 \pm 0.02 ^a	0.09 \pm 0.04 ^{ab}	0.01 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.02 \pm 0.01 ^b	0.01 \pm 0.01 ^b	24.89/<0.001
Terpinolene	1.27 \pm 0.18 ^a	0.58 \pm 0.20 ^{ab}	0.17 \pm 0.03 ^b	0.14 \pm 0.06 ^b	0.27 \pm 0.05 ^b	0.21 \pm 0.08 ^b	6.99/0.003
OMT ^d	0.3 \pm 0.1 ^a	1.0 \pm 0.6 ^a	0.8 \pm 0.5 ^a	0.3 \pm 0.2 ^a	0.9 \pm 0.4 ^a	0.8 \pm 0.4 ^a	0.93/0.500

^a A mixed model was used to analyze the data (see Methods). For pairwise comparison of LS Means Tukey multiple-range test was used at $\alpha=0.05$ significance level. Levels for a given compound not connected by the same letter are significantly different. Data analysis was performed on square-root transformed data for β -pinene, sabinene, γ -terpinene, and oxygenated monoterpenes (OMT)

^b HT herbicide-treated, Control: untreated

^c Lim/ β -phell limonene and β -phellandrene were quantified together as their GC peaks were not resolved on the Equity-5 column

^d OMT sum of oxygenated monoterpenes

up to 50 km), and the population density of *S. noctilio* varies significantly among them. Even though they were selected based on typical signs of attack by *S. noctilio*, such as resin beads, brown crowns, and emergence holes, the density of the wasp population in a particular pine stand is hard to predict before flight. An added challenge was to find enough Scots pines of both chemotypes at sites that also had white pines.

We examined the relationship between trap captures and the volatile emission rates of herbicide-treated trees. While herbicide-treated high-carene Scots pines captured significantly more female woodwasps and emitted volatiles at a significantly higher rate than herbicide-treated white pines, there was no significant difference in the volatile emission rates between the two chemotypes of Scots pine, yet, the high-carene chemotype captured more woodwasps. Moreover, in a study conducted in 2006, we found that while herbicide-treated red pines emitted similar amounts of volatiles as Scots pines the trap captures were lower (unpublished data of the authors). Thus, quantitative differences in the volatile emission rates only partly explain the differences seen in trap captures.

We found qualitative differences among the volatile blends of the two chemotypes of Scots pine and white pine. It is not only δ -3-carene that distinguishes the volatile blends of high- and low-carene chemotypes (Thoss et al. 2007), but some minor monoterpenes, as well, namely thujene, sabinene, γ -terpinene, and terpinolene. As these components had elevated levels in high-carene Scots pine compared to the other groups, they are good target compounds for a behavioral assay. Although they did not elicit high electrophysiological responses when tested individually (Simpson 1976), these compounds still might be important components of an attractive blend.

The ratios of the (*R*)-(+)- and (*S*)-(–)-enantiomers of α -pinene were higher in the high-carene samples compared to the low-carene and white pine samples collected 6 week after stem injection, although not significantly. According to Sjödin et al. (1993) there was no significant treatment effect on the enantiomeric ratios of chiral components when Scots pine trees were mechanically wounded. Neither did sawfly (*Diprion pini*) oviposition on needles of *P. sylvestris* affect the enantiomeric ratios of needle volatiles (Mumm et al. 2003). Nevertheless, elevated (*R*)-(+)- α -pinene emission was found in artificially wounded *P. massoniana* trees that attracted the pine sawyer beetle *Monochamus alternatus* (Fan et al. 2007). The relevance of changes in enantiomeric ratios needs to be tested in bioassays.

It is not clear whether the compounds/blends emitted by ‘attractive’ trees act, in fact, as attractants from a long distance or rather, as arrestants after an “appropriate landing” (Finch and Collier 2000). Madden (1988) reported on the arrestment activity of α - and β -pinene in flight mill olfactometers, however, no data were presented. Visual cues also need to be considered when developing a trapping

method (Reddy et al. 2005 and the references therein). In the case of the mountain pine beetle, *Dendroctonus ponderosae*, Campbell and Borden (2006) found that olfactory and visual cues are integrated at close-range to the host.

When trying to explain why a certain host species is preferred, we often neglect the lack of non-host characteristics that might contribute to the ‘attractiveness’ of the host. In a comparative study of preferred (*P. sylvestris* and *P. nigra*) and non-preferred (*P. pinea*) host species of the pine sawfly, *Neodiprion sertifer*, the monoterpene limonene induced avoidance when present in higher relative amounts (Martini et al. 2010). In *S. noctilio*, the oxygenated monoterpene components of *P. radiata* resin are thought to be repellents, or compounds that mask the attractiveness of the monoterpene hydrocarbons (Taylor 1981), although never tested in a bioassay. One of the oxygenated monoterpene components of *P. radiata* resin, camphor, was detected in our samples as well. We found relatively low amounts of oxygenated monoterpenes in herbicide-treated Scots pines compared to the other herbicide-treated groups, although the very low amounts made it impossible to individually quantify these components.

The volatile collection method used in our study, i.e., collecting airborne compounds on a sorbent, is especially suitable for field collections when multiple emitters have to be sampled, and when there are no opportunities to analyze samples immediately after collection. Eluting the sorbent with a solvent allows the researcher to run multiple tests (chemical and behavioral) with the sample and to perform quantification of the compounds. Nevertheless, the detection of low-molecular weight compounds that elute with the solvent from the GC column is not possible using this method. It is likely that we missed volatile components relevant to the host-seeking behavior of *S. noctilio*. Although solvent-free methods such as thermodesorption (Helsper et al. 2006) or collection on solid-phase microextraction (SPME) fibers (Martini et al. 2010) provide only one-time analysis, they are useful techniques in the exploration of volatile chemical cues. Testing compounds more abundant in the volatile profile of attractive pine species, such as δ -3-carene, thujene, sabinene, γ -terpinene, and terpinolene in high-carene Scots pine, with electrophysiological methods and in behavioral assays will be a focal point of our investigations in the future. Such research is valuable because it could lead to the identification of a successful artificial lure that will improve detection efficacy of insect pests for forest managers working to conserve and protect ecologically significant habitats and economic commodities.

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