*Journal of Chemical Ecology, Vol. 30, No. 5, May 2004 (© 2004)*

# VOLATILES ASSOCIATED WITH PREFERRED AND NONPREFERRED HOSTS OF THE NANTUCKET PINE TIP MOTH, *Rhyacionia frustrana*

C. ASARO,[1,*] B. T. SULLIVAN,[2] M. J. DALUSKY,[1] and C. W. BERISFORD[1]

[1]*Department of Entomology, University of Georgia, 413 Biological Sciences, Athens, Georgia 30602, USA*

[2]*Southern Research Station, USDA Forest Service, 2500 Shreveport Hwy, Pineville, Louisiana 71360, USA*

(Received August 26, 2003; accepted January 2, 2004)

**Abstract**—Ovipositing female Nantucket pine tip moth, *Rhyacionia frustrana*, prefer loblolly pine, *Pinus taeda* L., to slash pine, *Pinus elliottii* Engelm. except during the first spring following planting of seedlings. Host discrimination by *R. frustrana* increases as seedlings develop, suggesting that changes in the chemical composition of seedlings may mediate the moth's host preferences. Volatile compounds from slash and loblolly pine seedlings were collected using solid-phase microextraction (SPME) during the first year following planting. Four collection periods coincided with adult emergence and oviposition during each of four annual generations of *R. frustrana* in the Georgia Coastal Plain. Infestation of slash pine peaked during the second tip moth generation and was similar to the loblolly pine infestation level. By the fourth tip moth generation, slash pine infestation levels had declined and diverged considerably from those of loblolly pine. Significant differences in relative quantities of $\beta$-pinene, $\alpha$-phellandrene, limonene, $\beta$-phellandrene, bornyl acetate, $\beta$-caryophyllene, and an unidentified sesquiterpene occurred between slash and loblolly pine during the fourth generation. However, no strong correlation was observed between any individual compound and host damage that could readily explain the temporal changes in *R. frustrana* host preference. Gas chromatographic–electroantennographic detection (GC–EAD) analyses of standards identified 19 different seedling-associated compounds that elicited antennal responses from *R. frustrana* females, indicating that a blend of terpenoids may mediate host discrimination.

**Key Words**—Tortricidae, *Pinus taeda*, *Pinus elliottii*, terpenes, host selection, solid-phase microextraction, electroantennogram.

* To whom correspondence should be addressed. E-mail: casaro@uga.edu



0098-0331/04/0500-0977/0 © 2004 Plenum Publishing Corporation

## INTRODUCTION

The Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (Lepidoptera: Tortricidae), is an important pest of intensively managed loblolly pine (*Pinus taeda* L.) plantations throughout the southeastern United States. Female tip moths oviposit on needles, buds, and shoots. Larvae mine needles initially, and then bore into the bud or shoot, severing the vascular tissue and killing the apical meristem (Yates et al., 1981; Berisford, 1988; Asaro et al., 2003). Reduced growth and development of poor form may result (Cade and Hedden, 1987; Berisford et al., 1989; Nowak and Berisford, 2000; Asaro et al., 2003). *Rhyacionia frustrana* is multivoltine, with two to five generations per year in different parts of its range (Berisford, 1988; Fettig et al., 2000; Asaro et al., 2003).

Slash pine, *Pinus elliottii* Engelm., is generally resistant to *R. frustrana* attack (Yates, 1962). Hood et al. (1985) reported that *R. frustrana* oviposits almost exclusively on loblolly pine even when growing adjacent to slash pine. However, anecdotal observations suggest that slash pine seedlings are susceptible to tip moth oviposition and successful attack during the first growing season following planting with infestation rates decreasing to typical, low levels by the end of the first or second year (Yates, 1966; Hood et al., 1985; Berisford, 1988), although this has never been confirmed experimentally.

Monoterpenes are important host finding and oviposition cues for some Lepidoptera (Städler, 1974; Hanula et al., 1985; Leather, 1987; Åhman et al., 1988; Shu et al., 1997). Ross et al. (1995), using 5- to 29-month-old loblolly and slash pine seedlings, attempted to determine whether the oviposition preference of Nantucket pine tip moth was based on monoterpene emissions or cuticular lipids. They found a significantly greater amount of $\beta$-pinene and lower amounts of myrcene in slash pine compared to loblolly, whereas relative proportions among cuticular lipids varied between these two species. However, these differences have not yet been linked directly to oviposition preference.

Our primary objective was to identify olfactory cues for *R. frustrana* that could mediate this pest's ability to discriminate between slash and loblolly pine seedlings. We documented differences between the profiles of volatiles of these two host species during the first growing season following planting, and we attempted to correlate these differences with *R. frustrana* damage levels and, by association, oviposition preference during the same interval. We used headspace solid-phase microextraction (SPME) to obtain a more complete analysis of the total volatile profile of slash and loblolly pine than analyses reported previously (Ross et al., 1995). Evidence suggests that SPME is a more sensitive technique for detecting trace compounds than traditional methods for headspace sampling (Flechtmann et al., 1999; Thomsen, 1999). In addition, we used electroantennography to evaluate *R. frustrana*'s olfactory sensitivity to compounds identified in the profiles

of volatiles of the seedlings and to thereby distinguish possible semiochemicals utilized during host selection.

## METHODS AND MATERIALS

*Study Site.* The study was conducted on two plots in Effingham County, Georgia, approximately 2 km west of Rincon, in a portion of the Georgia Coastal Plain where *R. frustrana* has four generations per year (Fettig et al., 2000). On January 5, 2000, 400 bareroot seedlings were hand planted 20 rows wide by 20 trees long with alternating rows of slash and loblolly pine at 1.8 × 3.6 m spacing on each plot. At one plot, seedlings were planted on bedded soil (soil that is mounded up into low ridges or "beds," a common forestry practice on flat, poorly drained sites). The bedded plot was row-treated with Velpar® /Oust® (Hexazinone/Sulfometuron methyl) in the spring and received a broadcast treatment of Arsenal® (Imazapyr) during summer to control competing vegetation. The unbedded plot was mowed prior to planting, and the herbicide Accord® (Glyphosate) was applied during summer in a 0.5 m circle around each tree.

*Collection of Volatiles.* Tree odors were collected on four dates to coincide with the adult emergence/oviposition period of each generation of *R. frustrana*: March 2, May 15, July 6, and August 25, 2000. Collections were executed during a 3-hr interval at dusk (March 2, 5–8 P.M.; May 15, 6:45–9:45 P.M.; July 6 and August 25, 7–10 P.M.) to coincide with the mating flight and oviposition of *R. frustrana* (Webb and Berisford, 1978; Berisford, 1988). Monoterpene emissions from southern pines are high during this time interval and are relatively constant over a broad temperature range (Tingey et al., 1980). Volatiles were obtained from 12 seedlings of slash and loblolly pine from each plot using SPME fibers coated with 50 $\mu$m of cross-linked divinylbenzene (DVB), carboxen, and polydimethylsiloxane (PDMS) (Supelco, Bellefonte, PA). Prior to first use, each fiber was conditioned at 270°C for 4 hr. Fibers were thermally conditioned again for 10 min at 270°C prior to each subsequent use, and gas chromatography analysis confirmed that this adequately eliminated contaminants. For sampling, each fiber was extruded and attached to a top-whorl shoot using a clothespin. A 0.95 l (17.8 × 20.3 cm) plastic (LDPE) freezer bag was placed over the shoot and fiber and partially sealed at the base with binder clips to enclose a headspace. After 3 hr, fibers were retracted into the syringe needle, sealed at the tip with a Teflon plug, placed in screw-top culture tubes, and stored on ice for transport back to the laboratory. Fibers were subsequently stored at −80°C for up to 2 wk.

*Damage Estimates.* At the end of each *R. frustrana* generation, top-whorl damage estimates were obtained from 20 trees of each species at each plot, including those trees sampled for volatiles. Percent damage per tree was calculated by counting the total number of shoots and damaged shoots in the top whorl, which is well correlated with whole tree damage (Fettig and Berisford, 1999; Asaro et al.,

2003). Damaged shoots were identified by the presence of a pitch mass on or near the terminal bud accompanied by dry, brown needles and buds. Because of an overlap of *R. frustrana* with the northern extreme of the range of the subtropical pine tip moth, *R. subtropica* Miller, 25 pupae were collected on October 23, 2000, from each pine species to verify that *R. frustrana* was causing the observed damage. Pupae were identified according to Yates (1967).

*Chemical Analysis.* Analyses were performed by desorbing each fiber in the injector of an Hewlett-Packard (H-P) GCD G1800A coupled gas chromatograph–mass spectrometer (GC–MS) equipped with an SPME inlet liner (Supelco, Bellafonte, PA) and an HP-INNOWax column (60 m × 0.25 mm i.d.; 0.33 $\mu$m film thickness) (Hewlett-Packard Corp., Palo Alto, CA). Prior to injection, each fiber sample was exposed to the equilibrated headspace of >98% heptyl acetate (C7Ac) within a 100 ml bottle at room temperature for 5 sec to provide a semiquantitative internal standard. Specifically, the bottle cap was removed and the opening sealed with aluminum foil. After waiting 5 min for the headspace to equilibrate with any introduced air, an SPME fiber was attached to its specialized holder (Supelco), and the needle was placed through the foil without exposing the fiber. The needle was removed and immediately inserted into the GC, and the fiber was desorbed. The repeatability of this method was confirmed (mean variation 11.6% for 10 injections).

The carrier gas was helium at a flow rate of 0.9 ml/min with a 0.7 min splitless injection time. The GC inlet temperature was 220°C, and the temperature program was 40°C for 2 min, then 16°C/min to 130°C, then 6°C/min to 210°C, then 30°C/min to 240°C for 4 min. A subsample of three fibers per sampling date were reinjected immediately after a sample run to confirm that all compounds were completely desorbed onto the column. All compound identifications were based on mass spectral and retention time matches with known standards. For statistical comparisons, headspace compounds were quantified as C7Ac equivalents (i.e., the quotient of the raw integration areas of analyte peaks within the total ion chromatograms divided by the integrated area of the internal standard C7Ac).

To estimate the absolute proportions among different compounds in the headspace samples, quantities expressed as C7Ac equivalents were corrected using response factors calculated by exposing fibers to known quantities of commercially obtained standards. Compounds for which no standard was available had response factors assigned based on their structural similarity to compounds with known response factors. For compounds whose response factors were unknown but were present in very low amounts in our field samples (<0.5% of the total volatile profile), no response factors were applied to raw peak areas.

*Cold Storage Test.* Tests were performed to determine whether cold storage of SPME fibers for up to 2 wk led to any sample loss. Ten fibers were exposed

to an evaporated pentane solution (5 $\mu$l) containing 10 ng/$\mu$l each of $\alpha$-pinene, $\beta$-pinene, myrcene, $\alpha$-phellandrene, terpinolene, heptyl acetate, and terpinen-4-ol within a sealed 40 ml vial for 10 min. Five of these fibers were immediately injected into the GC–MS for analysis, while other five were stored at −80°C for 2 wk and subsequently analyzed.

*Electrophysiology.* Gas chromatographic–electroantennographic detection (GC–EAD) analyses were performed on 14 male and 14 female *R. frustrana* that had emerged up to 5 d previously from loblolly pine shoots clipped in Oconee Co., Georgia on February 10, 2003. Prior to analyses, moths were housed in foam-plugged plastic vials with pieces of moistened paper towel at 8°C and a 14:10 (L:D) hr light regime. Electrical contact was made by inserting a glass/pipette Ag/AgCl reference electrode into a moth's excised head and inserting the distal segments of one intact antenna into a second, glass/pipette Ag/AgCl recording electrode. Both pipettes were filled with Beadle–Ephrussi saline containing 0.5% polyvinylpyrrolidone (Bjostad, 1998) and 0.01% Triton X-100 (Union Carbide Midland, MI), a wetting agent which improved saline contact with the antennal tip. The antennal preparation was positioned at the opening of a stainless steel tube (8 mm diam.) that delivered a continuous stream (400 ml/min) of charcoal filtered, humidified air.

GC–EAD analyses were carried out with an H-P GC 5890 instrument fitted with a 60 m HP-INNOWax column. The temperature program was 40°C for 1 min, then 6°C/min to 230°C. for 10 min; the injector temperature was 200°C. Effluent from the column was split 1:1 and mixed with makeup gas in a union cross (Gerstel, Berlin, Germany). Deactivated, fused silica tubing (0.32 mm diam.) delivered half of the column effluent to a flame ionization detector and the other half through a heated transfer line (240°C.; Syntech, Hilversum, The Netherlands) that exited into the stimulus delivery tube (65 mm upwind from the antennal preparation). Samples consisted of a synthetic mixture of 22 compounds (Table 1) identified in seedling foliage headspace and diluted to ~45 ng/compound/$\mu$l of hexane (~90 ng/compound/$\mu$l for chiral compounds available as racemic mixtures). Samples (1 $\mu$l) were injected splitless into the GC.

Signals from the recording electrode were amplified by a high impedance guarded input AC/DC probe (Syntech) and then filtered and further amplified by an AutoSpike IDAC-2/3 signal connection interface (Syntech). Acquisition and analysis of antennal responses were performed with PeakSimple chromatography analysis software (Version 2.74) interfaced with a PeakSimple Chromatography Data System (SRI Instruments, Torrance, CA). For each run, the EAD trace was inverted, reprocessed with a moving average filter (1 sec wide), and assigned a baseline. Heights were calculated for all EAD peaks that occurred within a 23-min window that enclosed the retention times of the 22 test compounds (320–380 peaks per trial). A compound eluting from the GC was considered to have

TABLE 1. VOLATILE COMPOUNDS DESORBED FROM SPME FIBER SAMPLES FROM LOBLOLLY AND SLASH PINE

| Volatile compound | Tree species |
|---|---|
| 1. $\alpha$-Pinene | Loblolly and slash |
| 2. Camphene | Loblolly and slash |
| 3. $\beta$-Pinene | Loblolly and slash |
| 4. Sabinene[a] | Unbedded loblolly only |
| 5. Myrcene | Loblolly and slash |
| 6. $\alpha$-Phellandrene | Loblolly and slash |
| 7. $\alpha$-Terpinene[a] | Loblolly and slash |
| 8. Limonene | Loblolly and slash |
| 9. $\beta$-Phellandrene | Loblolly and slash |
| 10. $\gamma$-Terpinene[a] | Loblolly and slash |
| 11. *p*-Cymene[a] | Slash only |
| 12. Terpinolene | Loblolly and slash |
| 13. Linalool | Loblolly and slash |
| 14. Camphor[a] | Loblolly only |
| 15. Bornyl acetate | Loblolly and slash |
| 16. Terpinen-4-ol[a] | Loblolly and slash |
| 17. $\beta$-Caryophyllene | Loblolly and slash |
| 18. Myrtenal[a] | Slash only |
| 19. *trans*-Verbenol[a] | Loblolly and slash |
| 20. 4-Allylanisole[a] | Slash only |
| 21. $\alpha$-Terpineol[a] | Loblolly and slash |
| 22. Borneol[a] | Loblolly and slash |
| 23. $\alpha$-Humulene | Loblolly and slash |
| 24. Verbenone[a] | Loblolly and slash |
| 25. Unknown sesquiterpene | Loblolly and slash |

[a] Compounds found in trace amounts (<0.5% of total volatile profile).

produced a significant EAD response (i.e., one distinct from random noise), when the coinciding EAD spike fell in the 90% percentile for height more often than three times out of 14 runs ($P \leq 0.044$, table of cumulative binomial probabilities; Sokal and Rohlf, 1995).

*Statistical Analyses.* Damage estimates among tree species and plots were compared within generations using ANOVA followed by Tukey's test for means separation or Kruskal–Wallis ANOVA followed by Dunn's test if normality or equal variance assumptions were violated (SigmaStat® 2.0, Jandel Corporation, San Rafael, CA). Nonparametric statistics were preferred over parametric statistics on transformed data because no single transformation function produced normality and equal variance in all cases. Relative quantities of headspace analytes were compared between loblolly and slash pine at each sampling time using a *t* test or Mann–Whitney Rank Sum test if assumptions were violated (SigmaStat® 2.0). Significance levels for all tests were set at $\alpha = 0.05$.

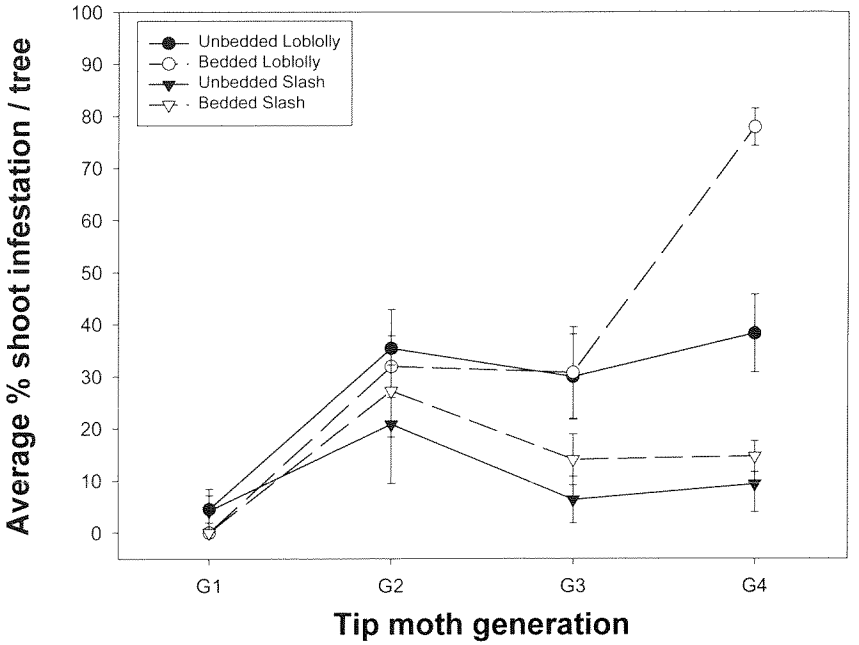


FIG. 1. Average (±SE) top-whorl percent shoot infestation of slash and loblolly pine by *Rhyacionia frustrana* on two separate plots near Rincon, GA, during four tip moth generations (G1-4).

## RESULTS AND DISCUSSION

All pupae collected from shoots were identified as *R. frustrana*, so the observed damage was attributed to this species. Damage estimates for slash and loblolly pine were not significantly different at either plot during generations 1 ($H = 6.07$; df = 3; $P = 0.11$) and 2 ($H = 4.12$; df = 3; $P = 0.25$), but damage to loblolly pine was higher in generations 3 ($H = 7.93$; df = 3; $P = 0.048$) and 4 ($H = 43.28$; df = 3; $P < 0.001$) (Figure 1). Damage to loblolly pine was three to four times greater than to slash pine during the fourth generation (Figure 1). Damage to slash pine peaked at 26% during the second generation (Figure 1). These results confirm previous observations that, although loblolly pine is more susceptible to tip moth damage overall, slash pine may be equally susceptible during early stages of seedling establishment. The results likewise suggest that host preferences of ovipositing *R. frustrana* shifted in favor of loblolly pine during the seedlings' first growing season, a phenomenon suggested but not documented previously.

No loss of adsorbed volatile compounds was detected ($P \geq 0.05$) on SPME fibers exposed to standards and stored up to 2 wk. Twenty-five compounds,

TABLE 2. ANTENNAL RESPONSES OF MALE AND FEMALE *R. frustrana* EXPOSED TO A SYNTHETIC MIXTURE OF HOST-ASSOCIATED COMPOUNDS USING COUPLED GAS CHROMATOGRAPHY–ELECTROANTENNOGRAPHIC DETECTION (GC–EAD)

| Peak number | Compound | Quantity into GC (ng)[a] | Purity (%) | Supplier[b] | Female EAD response ($\mu$V)[c] | Male EAD response ($\mu$V) |
|---|---|---|---|---|---|---|
| 1 | (±)-α-Pinene | 85 | 97 | Acros | 82 ± 28 | 73 ± 14 |
| 2 | (±)-Camphene | 63 | 82 | Aldrich | 38 ± 10 | ns[d] |
| 3 | (±)β-Pinene | 86 | 98 | Aldrich | 64 ± 13 | 88 ± 13 |
| 4 | (+)-Sabinene | 42 | 98 | Aldrich | 28 ± 4 | ns |
| 5 | Myrcene | 40 | 85 | Aldrich | 69 ± 13 | 86 ± 17 |
| 6 | (–) α-Phellandrene | 85 | 55 | Aldrich | 40 ± 8 | 48 ± 6 |
| 7 | α-Terpinene | 42 | 86 | Aldrich | ns | ns |
| 8 | (±)-Limonene | 84 | 99 | Aldrich | 75 ± 29 | 70 ± 13 |
| 9 | γ-Terpinene | 42 | 98 | Aldrich | 44 ± 11 | 61 ± 9 |
| 10 | *p*-Cymene | 43 | 98 | Aldrich | 40 ± 9v | ns |
| 11 | Terpinolene | 43 | 96 | Aldrich | ns | 30 ± 5 |
| 12 | (±)-Linalool | 87 | 97 | Aldrich | 120 ± 28 | 129 ± 16 |
| 13 | (±)-Camphor | 98 | 95 | Fluka | 87 ± 23 | 111 ± 18 |
| 14 | (–)-Bornyl acetate | 37 | 91 | Aldrich | ns | ns |
| 15 | (±)-Terpinen-4-ol | 93 | 96 | Aldrich | 104 ± 26 | 129 ± 15 |
| 16 | (–)-β-Caryophyllene | 90 | 91 | Aldrich | 50 ± 10 | 48 ± 12 |
| 17 | (–)-Myrtenal | 49 | 96 | Aldrich | 59 ± 17 | 75 ± 13 |
| 18 | 4-Allylanisole | 48 | 99 | Aldrich | 73 ± 18 | 78 ± 13 |
| 19 | α-Humulene | 44 | 99 | Fluka | 31 ± 4 | 42 ± 9 |
| 20 | (±)-α-Terpineol | 93 | 99 | Aldrich | 99 ± 25 | 103 ± 13 |
| 21 | (±)-Borneol | 97 | 96 | Fluka | 77 ± 26 | 104 ± 17 |
| 22 | (±)-Verbenone | 98 | 89 | Borregaard | 68 ±17 | 115 ± 13 |

[a] Amount injected into the gas chromatograph of the GC–EAD apparatus. Column effluent was split 1:1, hence antennae were exposed to approximately half this quantity.
[b] Acros Organics, Pittsburgh, PA (Acros); Aldrich Chemical Co., Milwaukee, WI (Aldrich); Fluka Chemical Corp., Milwaukee, WI (Fluka); Borregaard Chemical Co., Sarpsborg, Norway (Borregaard).
[c] Mean ± standard error.
[d] Antennae did not respond to the compound.

primarily monoterpenes and sesquiterpenes, were identified from SPME runs of both slash and loblolly pine, with 12 of these compounds present in only trace amounts (i.e., they did not appear in most runs or never averaged more than 0.5% of the total volatile profile throughout the study) (Table 1). Previous tests confirmed that none of the compounds identified as host volatiles were present as impurities from the fibers or polyethylene bags (data not shown). The relatively large number of compounds detected compared with previous research using Porapak® Q (Ross et al., 1995) suggests that SPME is a more sensitive method for sampling volatiles.

Significant differences were found between the total volatile profiles of slash and loblolly pine for all generations. For simplicity we have shown volatile profiles

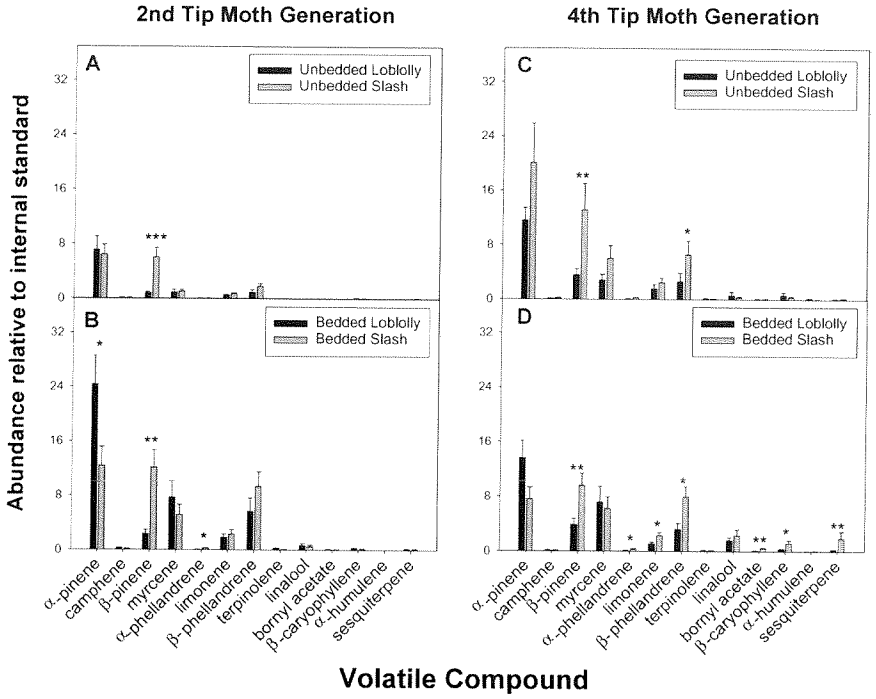


FIG. 2. Relative differences (average ± SE) in headspace composition between slash and loblolly pine during the second (A and B) and fourth (C and D) *Rhyacionia frustrana* generation on unbedded (A and C) and bedded (B and D) plots near Rincon, GA. Compounds showing a significant difference between slash and loblolly pine are indicated by a single ($P < 0.05$), double ($P < 0.01$), or triple ($P < 0.001$) asterisk.

collected during the second and fourth tip moth generations (Figure 2), because these two generations appeared to best represent the change in *R. frustrana* host preference that occurred during the first growing season. Specifically, the second generation caused moderate and similar damage levels in both pine species, whereas damage levels, and presumably *R. frustrana* oviposition preference, were higher for loblolly seedlings by the fourth generation (Figure 1).

Greater differences in volatiles between slash and loblolly pine emerged by the fourth generation (Figure 2a–d). Among the 25 compounds collected (Table 1), 12 (sabinene, camphor, *p*-cymene, myrtenal, 4-allylanisole, $\alpha$-terpinene, $\gamma$-terpinene, terpinen-4-ol, *trans*-verbenol, $\alpha$-terpineol, borneol, verbenone) were found in trace amounts and did not differ between slash and loblolly pine within either plot ($P > 0.1$) from one generation to the next (data not shown). Barring the possibility of temporal variation in the enantiomeric composition of chiral

members of this group (which our study did not examine), our data suggest that these 12 compounds likely do not play a role in host discrimination by gravid female *R. frustrana*.

At each plot, a number of volatile compounds, which were not present or detected in trace amounts during the second generation, became apparent or increased significantly during the fourth generation (Figure 2). Because damage diverged on the two host species after the second generation, we would also expect compounds acting as host selection cues to diverge quantitatively during this time. Six compounds were detected that quantitatively distinguished slash and loblolly pine during the fourth but not the second generation in the bedded plots ($\alpha$-phellandrene, limonene, $\beta$-phellandrene, bornyl acetate, $\beta$-caryophyllene, and an unidentified sesquiterpene) (Figure 2b and d). However, only one of these compounds ($\beta$-phellandrene) distinguished the two tree species in both the bedded and unbedded plots (Figure 2a–d).

Although no interspecific differences in relative amounts of myrcene were discovered, as in Ross et al. (1995), these data do support earlier studies showing that $\beta$-pinene is present in greater amounts in slash pine compared to loblolly pine. However, $\beta$-pinene did not fluctuate significantly in either host during the study. Because the chirality of $\beta$-pinene produced by each tree species was not determined, the possible role of $\beta$-pinene in host discrimination remains unclear.

The intensity of site preparation and corresponding growth rate of tree seedlings may have had an important effect on volatile emissions, because volatiles from trees on the bedded plot yielded a greater variety of compounds or showed greater differences between slash and loblolly pine. Weeds overtopped the pines on the unbedded plot but never became well established in the bedded area. By October 23, the trees on the unbedded plot averaged only 38.7 and 34.2 cm in height for loblolly and slash pine, respectively, whereas on the bedded site, they averaged 98.7 and 64.4 cm in height, respectively. However, the experiment was not originally designed to test differences in site preparation and, without replication, any conclusions regarding the effects of site preparation on production of volatiles are speculative.

Nineteen different compounds associated with host seedlings elicited responses from antennae of female *R. frustrana* (Figure 3, Table 2). Antennae of males generally exhibited a similar pattern of olfactory sensitivities as females, responding to 17 of 22 tested compounds. In addition, a significant antennal response was detected at the retention time of $\beta$-phellandrene, which was present as a contaminant in the synthetic test mixture. Our GC–EAD data indicate that *R. frustrana* are capable of sensing the majority of compounds in the blends of volatiles associated with host seedlings. The extent to which *R. frustrana* can distinguish these compounds or their enantiomers from one another is unknown; nonetheless our data suggest that a large number of compounds could potentially mediate host selection by female *R. frustrana*.

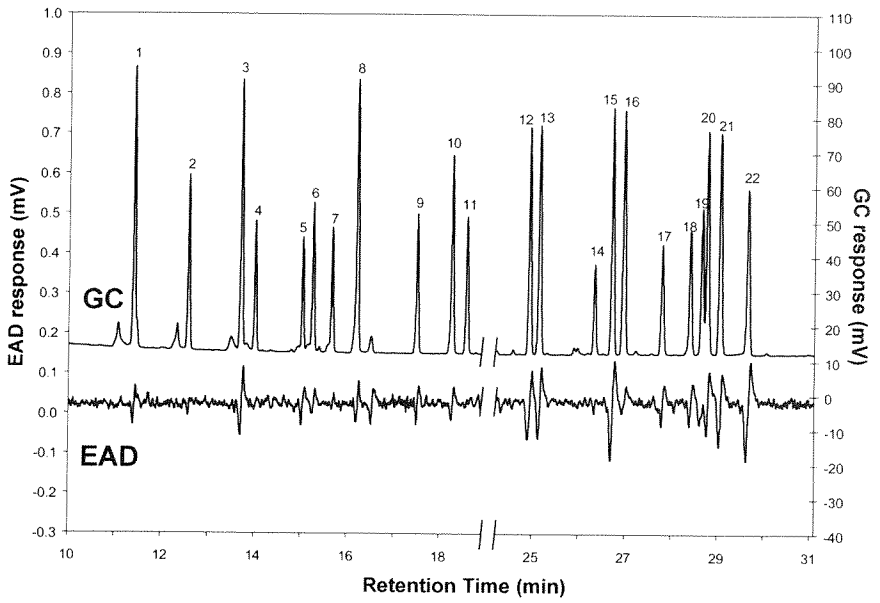


FIG. 3. Simultaneously recorded gas chromatographic (GC) and electroantennographic detection (EAD) traces from a single female *R. frustrana* antenna in response to a synthetic mixture of compounds found associated with foliage of potential host trees, *P. taeda* and *P. elliottii*. Identities of individual GC peaks are listed in Table 2. GC trace represents output from a flame ionization detector.

Location of host plants by phytophagous insects may be mediated by plant volatiles (Städler, 1974; Miller and Strickler, 1984; Hanula et al., 1985; Metcalf, 1987), whereas compounds on the plant surface may mediate oviposition preferences (Städler, 1986; Woodhead and Chapman, 1986; Ross et al., 1995). However, Honda (1995) cites accumulating evidence that plant volatiles may also mediate oviposition by female moths. Previous electrophysiological studies have demonstrated the ability of tortricid moths to detect and distinguish a large number of host-associated odors (Den Otter et al., 1978; Van der Pers, 1981; Rotundo and Tremblay, 1993). In addition, behavioral studies have shown that moth species from a diversity of insect families respond to a broad variety of host-associated compounds, including terpenes (Pivnick et al., 1994; Suckling et al., 1996; Shu et al., 1997; Raguso and Light, 1998; Burguiere et al., 2001). Suckling et al. (1996) stated that EAD was a poor predictor of oviposition-related attraction or inhibition in the light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae), a highly polyphagous species. However, they suggested that this approach might work better on monophagous species.

Whereas $\beta$-phellandrene was the only compound that met basic criteria for being a host selection cue, its modest correlation with damage levels suggests that this compound is not the only cue mediating host discrimination. The large number of volatile compounds detectable by *R. frustrana* suggests that tip moth oviposition preferences may be mediated by the perception of blends of compounds in specific proportions and not the absolute concentrations of individual semiochemicals. Furthermore, there is a strong possibility that *R. frustrana* can distinguish between the enantiomers of chiral compounds. Therefore, differences in the enantiomeric composition of phytochemicals between these two hosts may influence oviposition preference. Future studies should include enantiomeric analysis of host volatiles and EAD responses of *R. frustrana* to these enantiomers. In addition, the potential importance of visual cues in host selection and oviposition by *R. frustrana* and the possible interactions of visual with chemical cues should be addressed.

*Acknowledgments*—We thank International Paper for permission to conduct research on their properties. A special thanks to J. Seckinger, International Paper, for his assistance with site establishment, tree planting, and damage estimates. Thanks are also extended to C. Fettig, K. McCravy, and K. Seltmann for assistance with field collection of volatiles. This work was funded in part by the Pine Tip Moth Research Consortium and the Georgia Agricultural Experiment Station.

## REFERENCES

ÅHMAN, I., WIERSMA, N., and LINDSTRÖM, M. 1988. Electroantennogram responses in *Cydia strobilella* (L.) (Lep. Tortricidae) to flower and twig odors of its host plant *Picea abies* (L.) Karst. *J. Appl. Entomol.* 105:314–316.

ASARO, C., FETTIG, C. J., MCCRAVY, K. W., NOWAK, J. T., and BERISFORD, C. W. 2003. The Nantucket pine tip moth (Lepidoptera: Tortricidae): A literature review with management implications. *J. Entomol. Sci.* 38:1–40.

BERISFORD, C. W. 1988. The Nantucket pine tip moth, pp. 141–161, *in* A. A. Berryman (ed.). Dynamics of Forest Insect Populations: Patterns, Causes, Implications. Plenum, New York.

BERISFORD, C. W., GODBEE, J. F., JR., and ROSS, D. W. 1989. Impact of pine tip moth control, weed control, and fertilizer on growth and form of loblolly pine, pp. 130–136, *in* R. I. Alfaro and S. G. Glover (eds.). Insects Affecting Reforestation: Biology and Damage. Forestry Canada, Pacific Forestry Centre, Victoria, BC.

BJOSTAD, L. B. 1998. Electrophysiological methods, pp. 339–375, *in* J. G. Millar and K. F. Haynes (eds.). Methods in Chemical Ecology, Volume 1: Chemical Methods. Kluwer Academic, Boston.

BURGUIERE, L., MARION-POLL, F., and CORK, A. 2000. Electrophysiological responses of female *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to synthetic host odours. *J. Insect Phys.* 47:509–514.

CADE, S. C. and HEDDEN, R. L. 1987. Growth impact of pine tip moth on loblolly pine plantations in the Ouachita Mountains of Arkansas. *South J. Appl. For.* 11:128–133.

DEN OTTER, C. J., SCHUIL, H. A., and SANDER-VAN OOSTEN, A. 1978. Reception of host-plant odours and female sex pheromone in *Adoxophyes orana* (Lepidoptera: Tortricidae): Electrophysiology and morphology. *Entomol. Exp. Appl.* 24:370–378.

FETTIG, C. J. and BERISFORD, C. W. 1999. A comparison of three common indices used for estimating Nantucket pine tip moth damage in the field. *J. Entomol. Sci.* 34:203–209.

FETTIG, C. J., DALUSKY, M. J., and BERISFORD, C. W. 2000. Nantucket pine tip moth phenology and timing of insecticide spray applications in seven southeastern states. USDA For. Serv. Res. Paper SRS-18.

FLECHTMANN, C. A. H., DALUSKY, M. J., and BERISFORD, C. W. 1999. Bark and ambrosia beetle (Coleoptera: Scolytidae) responses to volatiles from aging loblolly pine billets. *Environ. Entomol.* 28:638–648.

HANULA, J. L., BERISFORD, C. W., and DEBARR, G. L. 1985. Monoterpene oviposition stimulants of *Dioryctria amatella* in volatiles from fusiform rust galls and second-year loblolly pine cones. *J. Chem. Ecol.* 11:943–952.

HONDA, K. 1995. Chemical basis of differential oviposition by lepidopterous insects. *Arch. Insect Biochem. Phys.* 30:1–23.

HOOD, M. W., BERISFORD, C. W., and HEDDEN, R. L. 1985. Oviposition preferences of the Nantucket pine tip moth (Lepidoptera: Tortricidae) on loblolly and slash pine. *J. Entomol. Sci.* 20:204–206.

LEATHER, S. R. 1987. Pine monoterpenes stimulate oviposition in the pine beauty moth, *Panoliss flammea*. *Entomol. Exp. Appl.* 43:295–303.

METCALF, R. L. 1987. Plant volatiles as insect attractants. *CRC Crit. Rev. Plant Sci.* 5:251–301.

MILLER, J. R. and STRICKLER, K. L. 1984. Finding and accepting host plants, pp. 127–157, *in* W. J. Bell and R. T. Cardé (eds.). Chemical Ecology of Insects. Sinauer Associates, Inc., Sunderland, MA.

NOWAK, J. T. and BERISFORD, C. W. 2000. Effects of intensive forest management practices on insect infestation levels and loblolly pine growth. *J. Econ. Entomol.* 93:336–341.

PIVNICK, K. A., JARVIS, B. J., and SLATER, G. P. 1994. Identification of olfactory cues used in host-plant finding by diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Chem. Ecol.* 20:1407–1427.

RAGUSO, R. A. and LIGHT, D. M. 1998. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and 'green leaf volatiles.' *Entomol. Exp. Appl.* 86:287–293.

ROSS, D. W., BIRGERSSON, G., ESPELIE, K. E., and BERISFORD, C. W. 1995. Monoterpene emissions and cuticular lipids of loblolly and slash pines: Potential bases for oviposition preferences of the Nantucket pine tip moth. *Can. J. Bot.* 73:21–25.

ROTUNDO, G. and TREMBLAY, E. 1993. Electroantennographic responses of chestnut moths (Lepidoptera: Tortricidae) and their parasitoid *Ascogaster quadridentatus* Wesmael (Hymenoptera: Braconidae) to volatiles from chestnut (*Castanea sativa* Miller) leaves. *Redia* 76:361–373.

SHU, S., GRANT, G. G., LANGEVIN, D., LOMBARDO, D. A., and MACDONALD, L. 1997. Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) elicited by monoterpenes and enantiomers from eastern white pine. *J. Chem. Ecol.* 23:35–50.

SOKAL, R. R. and ROHLF, F. J. 1995. Biometry, 3rd edn. Freeman, New York.

STÄDLER, E. 1974. Host plant stimuli affecting oviposition behavior of the eastern spruce budworm. *Entomol. Exp. Appl.* 17:176–188.

STÄDLER, E. 1986. Oviposition and feeding stimuli in leaf surface waxes, pp. 105–121, *in* B. E. Juniper and R. Southwood (eds.). Insects and the Plant Surface. Edward Arnold, London.

SUCKLING, D. M., KARG, G., GIBB, A. R., and BRADLEY, S. J. 1996. Electroantennogram and oviposition responses of *Epiphyas postvittana* (Lepidoptera: Tortricidae) to plant volatiles. *N. Z. J. Crop Hort. Sci.* 24:323–333.

THOMSEN, J. C. 1999. Comparative Evaluation of Different Isolation Methods for Volatile Analysis. PhD Thesis, Göteborg University, Göteborg, Sweden.

TINGEY, D. T., MANNING, M., GROTHUAS, L. C., and BURNS, W. F. 1980. Influence of light and temperature on monoterpene emission rates from slash pine. *Plant Physiol.* 65:797–801.

VAN DER PERS, J. N. C. 1981. Comparison of electroantennogram response spectra to plant volatiles in seven species of *Yponemeuta* and in the tortricid *Adoxophyes orana*. *Entomol. Exp. Appl.* 30:181–192.