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SEX PHEROMONE COMPONENTS OF THE GEOMETRID MOTHS *Lobophora nivigerata* AND *Epirrhoe sperryi*[1]

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Abstract—3Z,6Z,9Z-Nonadecatriene (3Z,6Z,9Z-19:H; other abbreviations follow the same pattern) has been identified as a female sex pheromone component of the geometrid moth *Epirrhoe sperryi* (H.). 3Z,6Z,9Z-18:H and 6Z,9Z-19:H were also identified in pheromone gland extracts but had no apparent biological activity. 3Z,6Z,9Z-21:H was tentatively identified as a female sex pheromone component of a second geometrid species, *Lobophora nivigerata* (Wlk.). Attraction of male moths to this compound was strongly synergized by the addition of small amounts of 6Z,9Z-21:H to lures.

Key Words—Sex attractant, pheromone, 3Z,6Z,9Z-octadecatriene, 3Z,6Z,9Z-nonadecatriene, 3Z,6Z,9Z-heneicosatriene, 6Z,9Z-nonadecadiene.

INTRODUCTION

Unsaturated hydrocarbons with one to four *cis* double bonds separated by single methylene units have been identified as sex attractants and sex pheromone components for a variety of moths from several insect families. Compounds of this type are usually produced by the female but can also be produced by males (Heath et al., 1988; Descoins et al., 1990). These types of compounds have been found as single component pheromones in the winter moth and Bruce spanworm (Roelofs et al., 1982; Underhill et al., 1987), which both use

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1,3Z,6Z,9Z-nonadecatetraene as a single-component pheromone. More commonly, unsaturated hydrocarbons occur as blends, either with each other or with similar components such as unsaturated epoxides, aldehydes, or ketones (Millar et al., 1990a, and references therein). Hydrocarbon blends may occur as mixtures of homologues with the same number of double bonds [*Anticarsia gemmetalis* (Heath et al., 1983); *Mocis disseverans* (Landolt et al., 1986); *Caenurgina erechtea* (Underhill et al., 1983)] or as mixtures of compounds with the same or different carbon-chain lengths, with different numbers of double bonds [*Arctia villica* (Einhorn et al., 1984); *Utetheisa ornatrix* (Jain et al., 1983); *Alsophila pometaria* (Wong et al., 1984); *Paleacrita vernata* (Millar et al., 1990a)].

Wong et al. (1985) reported 3Z,6Z,9Z-nonadecatriene (3Z,6Z,9Z-19:H; other abbreviations follow the same pattern) as a sex attractant for male *Epirrhoe sperryi* (H.) moths. We report here (1) the identification of 3Z,6Z,9Z-18:H, 3Z,6Z,9Z-19:H, and 6Z,9Z-19:H in *E. sperryi* female pheromone glands, (2) the tentative identification of 3Z,6Z,9Z-21:H in *L. nivigerata* female pheromone glands, and (3) the results of field tests with blends of the identified components.

METHODS AND MATERIALS

Insects and Electroantennography. Male moths were captured in light traps, in sweep nets, or in traps baited with synthetic sex attractants. Female moths of unknown mating status were taken in light traps or in sweep nets. Male antennal responses to synthetic compounds were measured by EAG (Chisholm et al., 1975), using 1-μg amounts of compounds applied to filter-paper disks and by the GC-EAD method of Arn et al. (1975). Synthetic compounds tested in EAG and/or GC-EAD studies included C_{17-22} chain lengths of the following types of compounds: 6Z,9Z-, 3Z,6Z,9Z-, 1,3Z,6Z,9Z-, 3Z,6Z,9Z,11Z-, and 3Z,6Z,9Z,11*E*-unsaturated hydrocarbons; combined mixtures of the monoepoxide regioisomers from nonselective oxidation of the diene or triene hydrocarbons above (combined monoepoxides, CME); racemic and chiral forms of 3Z,6Z-*cis*-9,10-, 3Z,9Z-*cis*-6,7-, and 6Z,9Z-*cis*-3,4-epoxydienes; and racemic and highly enantiomerically enriched forms of 6Z-*cis*-9,10- and 9Z-*cis*-6,7-epoxymonoenes.

Extracts of sex pheromone glands from field-collected female moths were prepared by a 20-min pentane extraction of the terminal three or four abdominal segments. The extracts were concentrated under a stream of nitrogen, internal standards (*n*-heptadecane and *n*-tetracosane) were added, and the extract was submitted to coupled gas chromatography–electroantennogram detection (GC-EAD) as described previously (Arn et al., 1975). The signals from the flame ionization detector and the insect antenna were recorded simultaneously with a

matched pair of Hewlett-Packard 3392A integrating chart recorders (Hewlett-Packard, Avondale, PA).

Coupled gas chromatography–mass spectrometry (GC-MS) analyses were carried out in the selected ion monitoring (SIM) mode, using chemical ionization (methane), with a Finnigan 4000E instrument interfaced to an Incos 2300 data system. A DB-5 column (50 m × 0.32-mm ID; J&W Scientific, Folsom, CA) or an Ultra-2 column (50 m × 0.32-mm ID; Hewlett-Packard) were used for analyses.

GC-MS analysis of an *E. sperryi* female pheromone gland extract was conducted as follows. Full-scan CI mass spectra of synthetic standards were obtained to select characteristic ions. A pentane solution of the standards with octadecane and tetracosane internal standards (100 or 400 pg of each compound injected) was then run in the SIM mode, followed by a blank run. Finally, the concentrated pentane extract of the pheromone glands from field-collected female moths of unknown mating status was analyzed in SIM mode (pentane solution of octadecane and tetracosane internal standards added prior to injection).

Insect Trapping. Field experiments were carried out in a mixed forest area approximately 100 km northeast of Saskatoon. Phero Tech wing traps (Phero Tech Inc., Delta, B.C., Canada), similar in design to Pherocon 1 CP traps (Scentry Inc., Buckeye, AZ) were used throughout and were baited with red rubber septa containing the synthetic lure blends. These lure blends (usually 500 μg of test compound total weight) were loaded onto septa as hexane solutions. Baits were stabilized by the addition of several drops of a dilute acetone solution of butylated hydroxytoluene.

Traps were hung from branches 10–15 m apart, at a height of ca 1.5 m, and were set out in a randomized block design. Moth captures were recorded twice weekly throughout the flight season. Because some treatments had no captures, the raw data were subjected to a nonparametric Kruskal–Wallis test, followed by a Waller–Duncan *K*-ratio comparison of means test (SAS Institute, 1988).

Field survey traps in 1984 were baited with the following C_{18-22} compounds, alone or as binary mixtures at ratios of 10:1 and 1:10: 3*Z*,6*Z*,9*Z*-trienes, 6*Z*,9*Z*-dienes, mixtures of the combined monoepoxides derived from nonspecific monoepoxidation of those dienes and trienes (CME-X:H), racemic *cis*-3,4-, *cis*-6,7-, and *cis*-9,10-epoxydienes, and 3*Z*,6*Z*,9*Z*,11*E*-tetraenes.

Field survey traps in 1985 were baited with the following compounds, generally in chain lengths of C_{16-22}, alone or as binary mixtures at ratios of 10:1 and 1:10: 3*Z*,6*Z*,9*Z*,12*Z*-, 3*Z*,6*Z*,9*Z*,13*E*-, 3*Z*,6*Z*,9*Z*,13*Z*-, 3*Z*,6*Z*,9*Z*,14*Z*-, or 3*Z*,6*Z*,9*Z*,14*E*-tetraenes with 3*Z*,6*Z*,9*Z*-trienes; 3*Z*,6*Z*,9*Z*-trienes with enantiomers of *cis*-6,7- and *cis*-9,10-epoxydienes; and epoxide enantiomers with each other or with regioisomers. Blends were made up with compounds of the same chain length or differing by one carbon in chain length.

Field survey traps in 1986 were baited with the following compounds, generally in chain lengths of C_{16-21}, alone or as binary mixtures at ratios of 10:1 and 1:10: 3Z,6Z,9Z-trienes; 1,3Z,6Z,9Z-tetraenes; enantiomers of 6Z,9Z-*cis*-3,4-epoxydienes; 6Z,9Z-dienes; enantiomers of 6Z-*cis*-9,10- and 9Z-*cis*-6,7-epoxymonoenes; and 6Z,9Z-dien-3-ols and 6Z,9Z-dien-3-ones.

Synthetic Chemicals. The syntheses of the compounds used in the field surveys and EAG or GC-EAD studies described in this paper have been published elsewhere, as follows: 6Z,9Z-and 3Z,6Z,9Z-unsaturated hydrocarbons and the corresponding racemic *cis*-3,4-, *cis*-6,7-, and *cis*-9,10-monoepoxides (Underhill et al., 1983; Wong et al., 1985; Millar et al., 1987); enantiomers of *cis*-9,10-epoxydienes (Wong et al., 1985); enantiomers of *cis*-6,7-epoxydienes (Millar and Underhill, 1986); and enantiomers of *cis*-3,4-epoxydienes (Millar et al., 1990d).

With regard to the specific experiments described in this report, the trienes and dienes were >99% chemically and isomerically pure by capillary gas chromatography (DB-5 and DB-1701 columns).

RESULTS

Lobophora nivigerata *Wlk.* Male moths of this species were caught in low numbers for several years in traps baited with lures containing 3Z,6Z,9Z-21:H as a single or major component. For example, in 1984, 35 specimens were attracted to 17 different lures, 13 of which contained 3Z,6Z,9Z-21:H as the major component.

In EAG studies, strong antennal responses (approx 2 mV) were obtained by antennal stimulation with 3Z,6Z,9Z-21:H, 3Z,6Z,9Z-20:H, and 3Z,9Z-*cis*-6,7-epoxy-20:H and -21:H. Smaller responses (approx 1.5 mV) were seen to 6Z,9Z-21:H, 6Z,9Z-20:H, and homologues of the above compounds. In GC-EAD studies, a male antenna gave strong responses to C_{20-21} trienes and 3Z,9Z-*cis*-6,7-epoxy-21:H and lesser responses to 6Z,9Z-21:H and to homologues.

GC-EAD analysis of a female gland extract revealed a single strong response at the retention time of 3Z,6Z,9Z-21:H (Figure 1, bottom traces). In the same run, a flame ionization detector (FID) peak was obtained at the retention time of 3Z,6Z,9Z-21:H but not at that of 6Z,9Z-21:H.

A pheromone gland extract from a second female moth was analyzed by coupled gas chromatography–mass spectrometry, using chemical ionization (CI; methane) in the selected ion monitoring (SIM) mode, with a DB-5 column. An exact retention time match and a fair ion ratios match were obtained for 3Z,6Z,9Z-21:H versus a 100-pg standard [standard, *m/z* 291 (74, M + 1), 289 (100, M − 1), 275 (23); insect extract *m/z* 291 (83), 289 (100), 275 (11)]. 6Z,9Z-21:H was not detected in the extract.

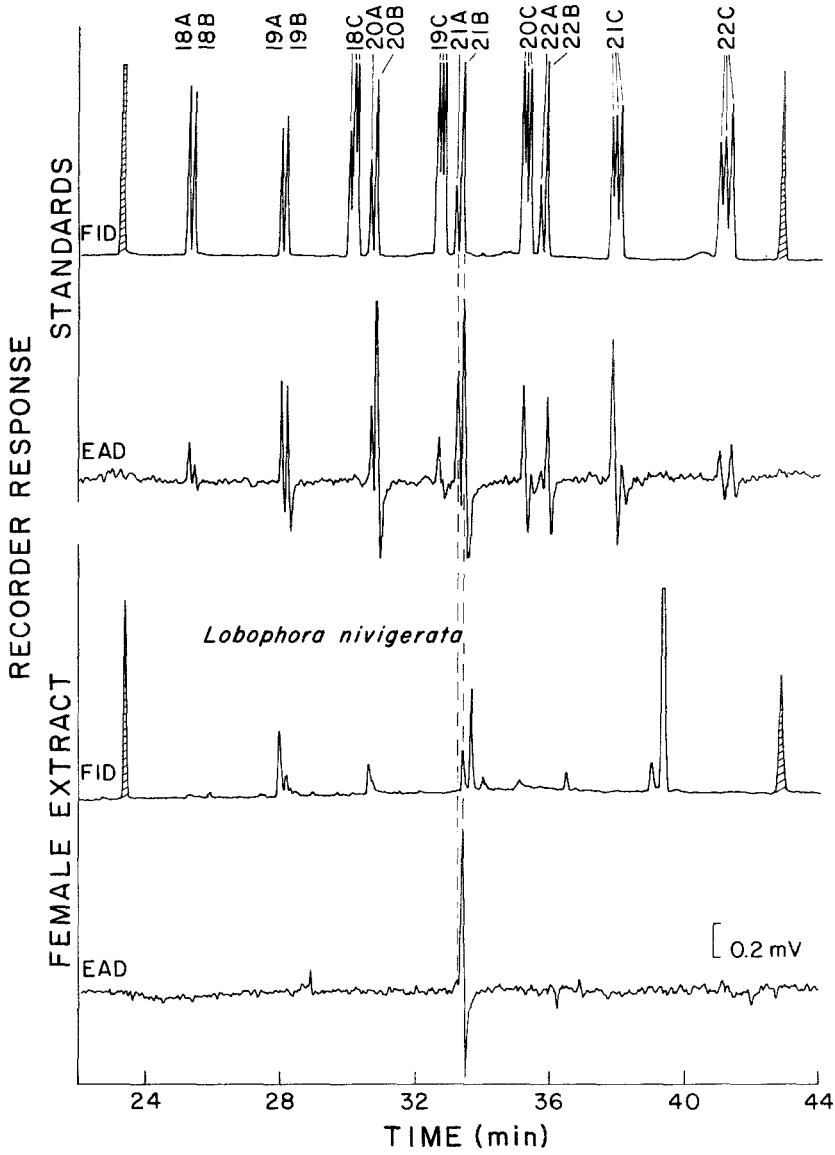

FIG. 1. Simultaneously recorded flame ionization detector (FID) and electroantennographic detector (EAD) traces using antennae of male *Lobophora nivigerata*. Upper pair of traces is in response to a mixture of C_{18-22} 6Z,9Z-diene hydrocarbons (designated by the letter A and preceded by the carbon-chain length), 3Z,6Z,9Z-triene hydrocarbons (designated by the letter B), and their monoepoxydiene analogues (designated by the letter C); the latter eluted from the DB-5 column in the order 6,7-, 3,4-, and 9,10-monoepoxydiene. Internal standards: 17:H and 24:H (shaded). Lower pair of traces is in response to a pheromone gland extract from a conspecific female. GC conditions: splitless, 40°C/0.5 min, 20°/min to 90°C, 4°/min to 230°C and hold.

In 1985 field tests, 3Z,6Z,9Z-21:H and 6Z,9Z-21:H as single components and a 1:1 blend were inactive, whereas mixtures of 19:1 and 4:1 (triene:diene) were attractive (Table 1). There was strong synergism between the two components, and the blend ratio was obviously important.

Epirrhoe sperryi *(H).* GC-EAD analysis of a female gland extract revealed EAD-active compounds at the retention times of 3Z,6Z,9Z-18:H, 3Z,6Z,9Z-19:H, and 6Z,9Z-19:H (Figure 2). The compounds were present at a ratio of 1:98:1, respectively.

A second female extract was submitted to GC-MS analysis in the SIM mode on an Ultra-2 column with methane CI. Exact retention time matches were obtained for all three compounds, and excellent ion ratio matches versus synthetic standards were obtained for 3Z,6Z,9Z-18:H [400-pg standard, *m/z* 249 (45, M + 1), 247 (47, M − 1), 109 (100); insect extract, *m/z* 249 (45), 247 (46), 109 (100)] and 6Z,9Z-19:H [400-pg standard, *m/z* 263 (100, M − 1), 249 (12), 109 (50); insect extract 263 (100), 249 (19), 109 (52)]. The SIM match between the 3Z,6Z,9Z-19:H standard [400 pg, *m/z* 263 (45, M + 1), 261 (48, *M* − 1), 247 (6), 206 (18), 109 (100)] and the compound in the insect extract [*m/z* 263 (100), 261 (100), 247 (18), 206 (46), 109 (100)] was poor because the amount of material in the extract was so large that the resulting signal saturated the detector.

In 1985, 530 male moths were attracted to 56 lure blends, the majority of which contained 3Z,6Z,9Z-19:H as the major component. There was no clear indication of synergistic or antagonistic effects due to the minor components, which included dienes, tetraenes, and monoepoxydienes. In 1986, traps containing 37 different lure blends caught 619 specimens, of which 610 were caught in traps baited with 3Z,6Z,9Z-19:H as a significant (> 10%) or major component.

In a second 1986 field test (Table 2), 3Z,6Z,9Z-19:H alone was signifi-

TABLE 1. ATTRACTION OF *Lobophora nivigerata* MOTHS TO LURES CONTAINING 3Z,6Z,9Z-21:H AND 6Z,9Z-21:H AS SINGLE COMPONENTS OR BLENDS

Lure (μg)	Male moths captured[a]
3Z,6Z,9Z-21:H (500)	4 b
6Z,9Z-21:H (500)	1 b
3Z,6Z,9Z-21:H (475) + 6Z,9Z-21:H (25)	54 a
3Z,6Z,9Z-21:H (400) + 6Z,9Z-21:H (100)	47 a
3Z,6Z,9Z-21:H (250) + 6Z,9Z-21:H (250)	0 b

[a]Traps were replicated three times and set out June 18–July 10, 1985. Trap captures followed by the same letter are not significantly different ($P < 0.05$, Waller–Duncan *K*-ratio multiple-comparisons test).

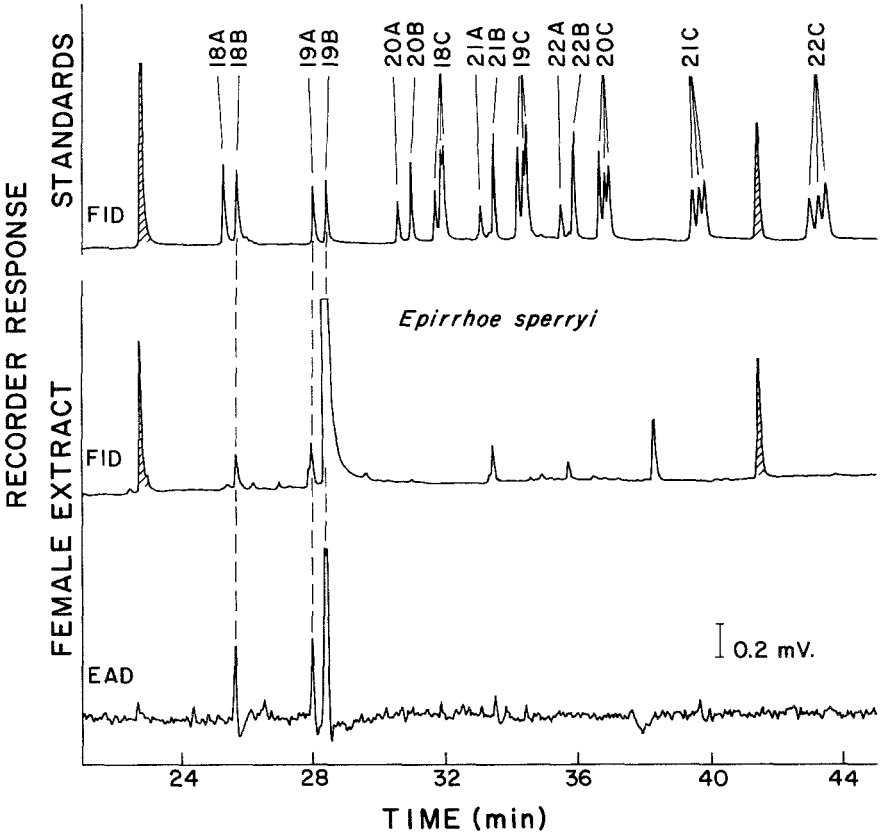

FIG. 2. FID and EAD traces (antennae of male *Epirrhoe sperryi*); upper trace is an FID trace from standards blend. Lower pair of traces is in response to a pheromone gland extract of a conspecific female. Number/letter designations and GC conditions the same as in the legend to Fig. 1.

cantly attractive, whereas 3Z,6Z,9Z-18:H and 6Z,9Z-19:H were not. When added in amounts equivalent to 1 or 10% of the total lure blend, 3Z,6Z,9Z-18:H had no significant effect on trap captures. 6Z,9Z-19:H had no significant effect at the 1% level but completely suppressed trap captures when present as 10% of the blend.

The three-component blend of 3Z,6Z,9Z-19:H, 3Z,6Z,9Z-18:H, and 6Z,9Z-19:H (100:1:1, the approximate ratio found in the pheromone gland extract) was not significantly more attractive than 3Z,6Z,9Z-19:H alone. However, the same three components at a ratio of 9:1:1 were significantly less attractive, supporting our finding that increased amounts of 3Z,6Z,9Z-18:H decreased trap captures.

TABLE 2. ATTRACTION OF *Epirrhoe sperryi* MOTHS TO 3Z,6Z,9Z-19:H, 3Z,6Z,9Z-18:H, AND 6Z,9Z-19:H AS SINGLE COMPONENTS OR BLENDS

Lure (μg)	Male moths captured[a]
3Z,6Z,9Z-18:H (500)	1 e
6Z,9Z-19:H (500)	0 e
3Z,6Z,9Z-19:H (500)	38 bc
3Z,6Z,9Z-19:H (500) + 3Z,6Z,9Z-18:H (5)	15 de
3Z,6Z,9Z-19:H (450) + 3Z,6Z,9Z-18:H (50)	51 ab
3Z,6Z,9Z-19:H (500) + 6Z,9Z-19:H (5)	26 cd
3Z,6Z,9Z-19:H (450) + 6Z,9Z-18:H (50)	0 e
3Z,6Z,9Z-19:H (500) + 6Z,9Z-19:H (5) + 3Z,6Z,9Z-18:H (5)	56 a
3Z,6Z,9Z-19:H (450) + 6Z,9Z-19:H (50)+ 3Z,6Z,9Z-18:H (50)	14 de
Blank	0 e

[a]Traps were replicated three times and set out May 28–June 26, 1986. Values followed by the same letter are not significantly different ($P < 0.05$, Waller–Duncan K-ratio multiple-comparisons test).

DISCUSSION

Male *L. nivigerata* moths were attracted to the synergistic blend of 3Z,6Z,9Z-21:H and 6Z,9Z-21:H, with the presence and ratio of both compounds being important. No other potential pheromone components were detected, either by GC-EAD or by GC-MS examinations of pheromone gland extracts. Blends of dienes and trienes are not uncommon as lepidopteran pheromones. For example, the sex pheromone of the noctuid species *Mocis latipes* consists of a blend of the same two components (Descoins et al., 1986), and the arctiid species *Creatonotos transiens, C. gangis*, and *Utetheisa ornatrix* have both these compounds admixed with several other components in their pheromones (Jain et al., 1983; Bell and Meinwald, 1986; Wünderer et al., 1986). There have also been several reports of homologous diene and triene blends as lepidopteran sex pheromones [*Paleacrita vernata* (Millar et al., 1990a)] or sex attractants [*Alsophila quadripunctata* (Szöcs et al., 1984); *Boarmia repandata* (Bogenschütz et al., 1985)].

Preliminary data on attraction of male moths of *E. sperryi* to 3Z,6Z,9Z-19:H have been published (Wong et al., 1985), along with EAG data showing strong responses of male moth antennae when stimulated by C_{18-20} 3Z,6Z,9Z-trienes and the combined monoepoxides derived therefrom. *E. sperryi* antennae are highly receptive to three components from the female pheromone gland, but only one of these, 3Z,6Z,9Z-19:H, is significantly attractive alone to males. The other two components have variable biological activity, depending on the

blend ratio at which they are presented. At low doses, the combination of the two minor components appears to synergize the response to the major component, while at higher doses, at least one of the minor components (6Z,9Z-19:H) appears to be inhibitory. It is possible that a wider ratio of 3Z,6Z,9Z-19:H to the other two components (e.g., 1000:1:1) may have enhanced trap catches.

E. sperryi is also unusual in that its behavioral responses to the attractive 3Z,6Z,9Z-19:H were not affected by a wide variety of homologues and other similar compounds, including 6Z,9Z-dienes, 3Z,6Z,9Z,X-tetraenes (*X* = 12*E* or *Z*, 13*E* or *Z*, 14*E* or *Z*), chiral or racemic *cis*-3,4-, 6,7-, or 9,10-epoxydienes, and 6Z,9Z-dien-3-ones or 6Z,9Z-dien-3-ols. Of all the compounds tested in combinations with 3Z,6Z,9Z-19:H, the only compound which significantly altered trap captures, either positively or negatively, was 3Z,6Z,9Z-20:H, which strongly suppressed trap captures, as had been previously reported (Wong et al., 1985). None of the epoxides, either of the same chain length or homologues, had any apparent effect, despite the strong responses to these compounds by male antennae in EAG studies (Wong et al., 1985).

In our region of Saskatchewan, *E. sperryi* fly from mid-May to early July (e.g., 1984, first moth caught May 21; last moth caught July 10). During this period, there is a wide variety of geometrid and noctuid moths flying, and 3Z,6Z,9Z-19:H is a common component of sex attractants and sex pheromones for some of these species (Wong et al., 1985; Millar et al., 1990b,c,d, 1991a). *E. sperryi* males were attracted to a wide variety of lures containing 3Z,6Z,9Z-19:H as a component (e.g., in 1986, 619 moths caught with 37 different lure blends), suggesting that other physiological or behavioral mechanisms (e.g., closely synchronized female calling and male response) are operative in minimizing male attraction to pheromone blends containing 3Z,6Z,9Z-19:H produced by female moths of sympatric species.

Obtaining hard analytical data to support conclusive identifications of the pheromone components has been difficult, as we have obtained only a few hand-collected female moths of unknown mating status for analysis of pheromone gland chemistry. The evidence that has been presented in support of the identification of 3Z,6Z,9Z-21:H as a pheromone component for *L. nivigerata* consists of retention time matches on two capillary GC columns and a reasonable CI SIM ion ratios match versus a synthetic standard, plus the strong electroantennogram activity and biological activity of the synthetic compound as an attractant. Furthermore, previous studies in our laboratory have shown that under the GC-EAD conditions used, only one (an *EEZ* isomer) of the eight possible geometric isomers of 3Z,6Z,9Z-21:H is chromatographically indistinguishable from 3Z,6Z,9Z-21:H (Millar et al., 1991b). However, until hard analytical evidence can be obtained to determine unambiguously the double-bond geometries and positions, our identification remains tentative.

The evidence for the identification of 6Z,9Z-21:H as a pheromone com-

ponent is tenuous, consisting of antennal responses to standards by male moth antennae in GC-EAD studies and antennal responses in EAG studies. This identification is strongly supported by the obvious synergism between this compound and 3*Z*,6*Z*,9*Z*-21:H in field trials (Table 1).

Much stronger evidence was obtained for the identifications of the components isolated from *E. sperryi* pheromone glands. The major biologically active compound, 3*Z*,6*Z*,9*Z*-19:H, had retention times identical to those of a synthetic standard on two capillary GC columns. The SIM mass spectral match was poor, due to the saturation of the detector by the large amount of 3*Z*,6*Z*,9*Z*-19:H in the gland extract. However, the major ions at *m/z* 263 ($M + 1$) and 261 ($M - 1$) provided support for the molecular weight and formula, and the major ion at *m/z* 109 $(C_8H_{12} + H)^+$ was diagnostic for the 3,6,9-triene structure (Heath et al., 1983). Furthermore, we have previously demonstrated that under the GC-EAD conditions used, only the *EEE* isomer and one of the *EEZ* isomers had retention times which were potentially indistinguishable from that of 3*Z*,6*Z*,9*Z*-19:H. Given the strong field attraction (Table 2) and EAD responses (Fig. 2) to this compound, the chance of the pheromone component being one of the other two isomers seems remote.

Evidence to support the identity of 3*Z*,6*Z*,9*Z*-18:H in pheromone gland extracts consisted of exact retention time and CI SIM matches versus a synthetic standard, including the presence of a strong *m/z* 109 ion confirming the 3,6,9 placement of the double bonds. The same types of data confirmed the gross structure of 6*Z*,9*Z*-19:H in the extract, and in addition, all four geometric isomers of this compound are readily distinguishable by capillary GC (Millar et al., 1990a).

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