*Journal of Chemical Ecology, Vol. 23, No. 4, 1997*

# SEX PHEROMONE COMPONENTS OF THE APPLE LEAFMINER, *Lyonetia prunifoliella*

REGINE GRIES,[1] GERHARD GRIES,[1,*] G. G. SKIP KING,[2] and CHRIS T. MAIER[3]

[1]*Centre for Pest Management, Department of Biological Sciences*
[2]*Department of Chemistry*
*Simon Fraser University*
*Burnaby, British Columbia V5A 1S6, Canada*
[3]*Department of Entomology*
*The Connecticut Agricultural Experiment Station*
*New Haven, Connecticut 06504*

(Received May 2, 1996; accepted December 1, 1996)

**Abstract**—Three methylated hydrocarbons, 10,14-dimethyloctadec-1-ene (10Me14Me-1-ene-18Hy = 5Me9Me-17-ene-18Hy), 5,9-dimethyloctadecane (5Me9Me-18Hy), and 5,9-dimethylheptadecane (5Me9Me-17Hy), are synergistic sex pheromone components of the leafminer *Lyonetia prunifoliella*. Compounds extracted from female pheromone glands were identified by coupled gas chromatographic-electroantennographic detection (GC-EAD), and one compound, 10Me14Me-1-ene-18Hy, also by coupled GC-mass spectrometry. In field trapping experiments, 10Me14Me-1-ene-18Hy, 5Me9Me-18Hy, and 5Me9Me-17Hy singly were unattractive to males but in ternary combination attracted numerous male moths. Attractiveness of the three-component blend significantly exceeded that of two-component blends. No attraction of males to pheromone lures without 10Me14Me-1-ene-18Hy indicates that this compound is essential for pheromone communication of *L. prunifoliella*. Common C-5 and C-9 methyl branches in lyonetiid pheromone hydrocarbons suggest a common biosynthetic pathway; the presence of 5Me9Me-17Hy and 5Me9Me-18Hy in pheromone blends of *L. prunifoliella* and *Leucoptera malifoliella* provides evidence for phylogeny of lyonetiid chemical communication. Determination of the stereoisomeric composition is required to completely describe the pheromone blend of *L. prunifoliella* and to support the hypothesis of phylogenetically related sex pheromones.

**Key Words**—Lepidoptera, Lyonetiidae, *Lyonetia prunifoliella*, *Perileucoptera coffeella*, *Lyonetia clerkella*, *Leucoptera malifoliella*, sex pheromone,

*To whom correspondence should be addressed.



0098-0331/97/0400-1119$12.50/0 © 1997 Plenum Publishing Corporation

synergism, 10,14-dimethyloctadec-1-ene, 5,9-dimethyloctadecane, 5,9-dimethylheptadecane.

## INTRODUCTION

*Lyonetia prunifoliella* (Hübner) [formerly *L. speculella* (Schmitt et al., 1996)] (Lepidoptera: Lyonetiidae) is a small polyphagous leafminer. Host plants include five rosaceous species in three genera, two *Betula* spp. (Betulaceae) and possibly two *Ceanothus* spp. (Rhamnaceae) (Maier, 1995). Females insert eggs singly into the lower surface of new leaves. After hatching, larvae form serpentine mines in the upper half of leaves. Second instars enlarge their mines and third instars occupy full-depth blotch mines (Maier, 1988). Within blotches, larvae consume all leaf tissue between the upper and lower epidermis (Maier, 1988). Pupation occurs in a silken cocoon suspended from the underside of a leaf in a silken hammocklike structure constructed by the larvae (Frost, 1924). There are several generations per year, each commencing with eggs deposited in young expanding leaves (Brown, 1989). In fruit orchards in Connecticut and West Virginia, *L. prunifoliella* injures apple, *Malus domestica* (Borkh.), and, to a lesser extent, other species of fruit trees (Brown, 1989; Maier, 1995). Heavily damaged leaves of apple may abscise prematurely (Maier, 1988; and C.T.M., unpublished data).

Three other lyonetiid species, *L. prunifoliella malinella* (Matsumura), *Leucoptera malifoliella* (Costa) (formerly *scitella* Zeller) and *Lyonetia clerkella* L. are pests of fruit trees in Europe and Asia (Kremer, 1963; Esmaili, 1971; Naruse, 1978; Sekita and Yamada, 1979; Seprõs, 1985). Because of the pestiferous reputations of these close relatives and increasing abundance and damage by *L. prunifoliella* in eastern West Virginia (Brown, 1989) and Connecticut (Maier, 1988), *L. prunifoliella* is considered a pest of apple in eastern North America (Brown, 1989). Abundant populations in 1995 provided the opportunity to study the pheromone blend of this leafminer. We report the identification and field testing of pheromone components of *L. prunifoliella.*

## METHODS AND MATERIALS

### *Experimental Insects and Pheromone Analysis*

Apple leaves with *L. prunifoliella* pupae were field-collected at Lewis Farms, Southington, Hartford County, Connecticut, sent to Simon Fraser University, and kept at 20°C, 70% relative humidity, and a 14L : 10D photoperiod. Emergent adults were placed individually into filter-paper-lined Petri dishes to avoid mating and subsequent decline of pheromone titers. Pheromone glands of

2-day-old, calling females were removed 0.5 hr into the photophase and extracted for 10 min in HPLC-grade hexane.

Aliquots of one female equivalent (FE) of pheromone extracts were subjected to gas chromatographic analyses [Hewlett Packard (HP) 5890A] with both flame ionization and electroantennographic detection (GC-EAD) (Arn et al., 1975), employing three fused silica columns (DB-5, DB-210, and DB-23; 30 m × 0.25 or 0.32 mm ID; J&W Scientific, Folsom, California). Coupled GC–mass spectrometry (MS) in electron impact (70 eV) mode employed a HP 5985B fitted with the DB-5 column referred to above.

*General Procedures*

Nuclear magnetic resonance (NMR) spectroscopy was conducted on a Bruker AMX-400 spectrometer at 400 Mhz in deuteriochloroform solution. $^1H$ chemical shifts are reported in parts per million (ppm, $\delta$) and relative to TMS (0.00 ppm). Mass spectra were obtained on a Varian Saturn II Ion Trap GC-MS system fitted with a DB-5-coated fused silica column (30 m × 0.25 mm ID; J&W Scientific). GC analyses were carried out on a HP 5880 fitted with a DB-1-coated fused silica column (30 m × 0.25 mm ID). THF was freshly distilled from sodium–benzophenone ketyl. Recovered hexane–ether solvent mixtures were distilled from $P_2O_5$ via a Dufton fractionating column. Chemicals from commercial sources were used without further purification. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography using Silica Gel 60 (230–400 mesh E. Merck, Darmstadt) (Still et al., 1978).

*Syntheses*

*Ethyl 4-methyloctanoate (**1**; Figure 1).* This compound was synthesized as previously reported (Gries et al., 1994).

*4-Methyloctanol (**2a**; Figure 1).* Ester **1** was dissolved in ether, lithium aluminum hydride (0.21 g, 5.5 mmol) was added, and the mixture refluxed overnight. The reaction was quenched by dropwise addition of 5% NaOH (5 ml). A granular precipitate formed that was removed by filtration and washed three times with ether. Evaporation of solvent gave 0.55 g (3.8 mmol, 75% yield) of **2a**; $^1H$ NMR ($CDCl_3$); $\delta$ 3.61 (2H, t, $J$ = 7 Hz), 1.6–1.1 (12H, m), 0.85 (6 H, m).

*1-Bromo-4-methyloctane (**2b**; Figure 1).* Alcohol **2a** (0.55 g, 3.8 mmol) was dissolved in $CH_2Cl_2$. To this was added triphenylphosphine (1.4 g, 5.4 mmol), followed by dropwise addition of bromine (0.9 g, 5.6 mmol) until excess $Br_2$ was present. After stirring 30 min, the solution was quenched with methanol. The mixture was diluted with mixed solvent (100 ml), washed (water; 5% $Na_2S_2O_3$; sat. brine), and dried. After solvent evaporation, the residue was

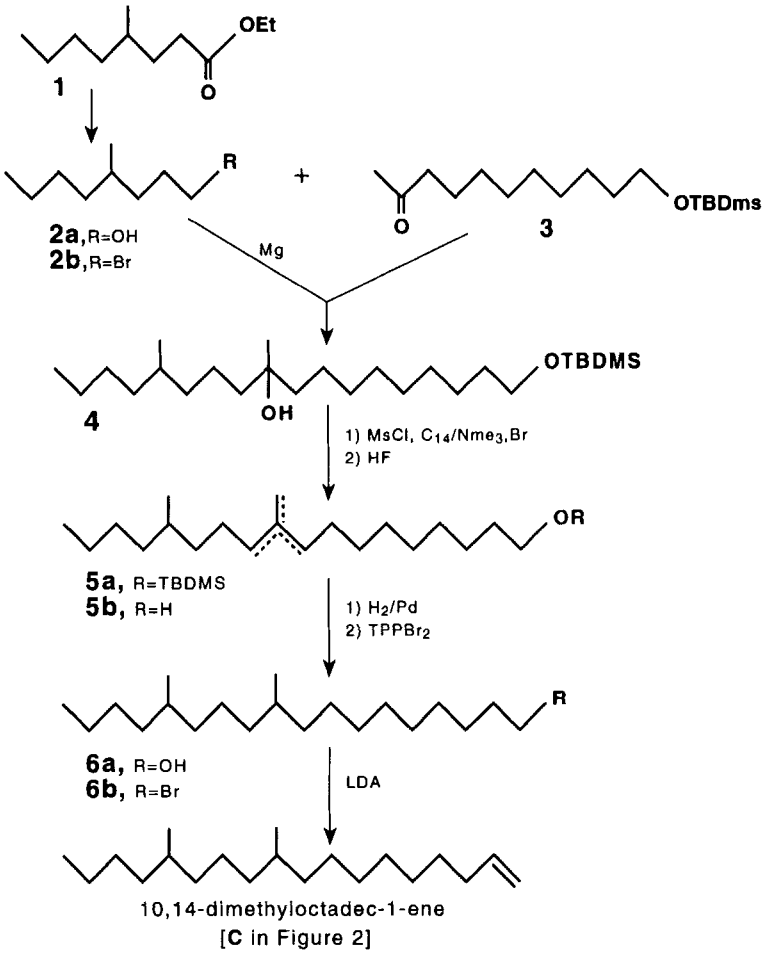


FIG. 1. Scheme for the synthesis of 10,14-dimethyloctadec-1-ene.

passed through silica gel with hexane as eluent to provide 0.8 g (3.8 mmol, 100% yield) of **2b**; $^1$H NMR ($CDCl_3$); $\delta$ 3.40 (2H, t, $J$ = 7 Hz), 1.8 (2H, m,), 1.4 (2H, m,), 1.25 (6H, m), 1.10 (1H, m), 0.88 (6H, m).

*1-tert-Butyldimethylsilyloxy-10-undecanone (3; Figure 1).* This compound was synthesized according to Nonaka et al. (1984).

*1-tert-Butyldimethylsilyloxy-10,14-dimethyl-10-octadecanol (4; Figure 1).* A Grignard reagent was prepared from **2b** (0.8 g, 3.8 mmol) and Mg (0.24 g, 10 mmol) in refluxing ether. After 1 hr, the mixture was cooled and **3** (1.2 g, 4 mmol) added. After stirring for several hours, the mixture was quenched with

aqueous $NH_4Cl$, diluted with mixed solvent, and the aqueous layer removed. The organic layer was washed (brine) and dried. Evaporation and chromatography gave 1 g (2.3 mmol, 60% yield) of **4**. $^1H$ NMR ($CDCl_3$); $\delta$ 3.60 (2H, t, $J$ = 7 Hz), 2.35 (1H, s), 1.5–1.3 (29H, m), 1.15 (3H, s), 0.90 (12H, bs), 0.87 (3H, d, $J$ = 7 Hz), 0.04 (6H, s).

*10,14-Dimethyloctadecanol (**6a**; Figure 1).* Compound **4** was dissolved in THF (15 ml) containing triethylamine (3 ml). To this was added MsCl (0.8 g), and 1 hr later trimethyltetradecylammonium bromide (1 g) (Corey and Snider, 1972). The mixture was stirred overnight, diluted with mixed solvent, and filtered. Chromatography provided 0.8 g of **5a** as a mixture of isomers.

This isomeric mixture was dissolved in $CH_3CN$ (40 ml) containing 2 ml of 40% HF (Newton et al., 1979). After 2 hr, starting material was not detectable by TLC. Water was added, the mixture neutralized with 5% NaOH, and extracted. Evaporation provided 0.4 g of **5b**. GC-MS showed identical spectra for all three isomeric compounds [*m/z* (relative intensity)]: 296 (3), 277 (2), 239 (4), 169 (15), 151 (20), 123 (21), 109 (41), 95 (83), 81 (80), 69 (84), 55 (100).

The isomeric mixture of unsaturated **5b** was dissolved in hexane, 5% catalytic Pd/C (200 mg) was added, and the mixture hydrogenated overnight at 1 atmosphere. Following catalyst removal by filtration and solvent evaporation, the crude product was chromatographed to give 0.3 g (1.0 mmol, 44% yield from **4**) of pure **6a.** $^1H$ NMR ($CDCl_3$); $\delta$ 3.60 (2H, t, $J$ = 7 Hz), 1.90 (2H, quintet, $J$ = 7 Hz), 1.3 (25H, m), 1.1 (4H, m), 0.85 (9H, m); MS [*m/z* (relative intensity)]: 152 (4), 151 (4), 125 (16), 111 (34), 97 (79), 83 (58), 69 (75), 55 (100).

*1-Bromo-10,14-dimethyloctadecane (**6b**; Figure 1).* Bromination reagent was prepared from triphenylphosphine (0.55 g, 2 mmol) and bromine (0.34 g, 2.1 mmol) in $CH_2Cl_2$. Alcohol **6a** (1 mmol) was added and the mixture stirred 0.5 hr. The reaction was diluted with hexane, washed (water; 2 × 50% aqueous MeOH; brine) and dried. Evaporation and chromatography gave 0.35 g (0.97 mmol, 96% yield) of **6b**. $^1H$ NMR ($CDCl_3$); $\delta$ 3.40 (2H, t, $J$ = 7 Hz), 1.85 (2H, quintet, $J$ = 7 Hz), 1.25 (24H, m), 1.05 (4H, m), 0.85 (9H, m).

*10,14-Dimethyloctadec-1-ene (Figure 1).* LDA (3.8 mmol) was prepared in THF (5 ml) from diisopropylamine (0.42 g) and 2.4 M of *n*-butyl lithium (1.6 ml). After 5 min, **6b** (0.35 g) was added in THF (5 ml). The solution was stirred overnight, then diluted with hexane, washed (10% $H_2SO_4$; brine), dried, and evaporated. After chromatography, 0.2 g (0.72 mmol, 71% yield) of the target compound was obtained. $^1H$ NMR ($CDCl_3$); $\delta$ 5.80 (1H, m), 4.99 (1H, d, $J$ = 18 Hz), 4.91 (1H, d, $J$ = 12 Hz), 1.9 (2H, m), 1.4–1.2 (22H, m), 1.10 (4H, m), 0.85 (9H, m).

5,9-Dimethylheptadecane and 5,9-dimethyloctadecane, previously synthesized by Francke et al. (1988) and Riba et al. (1990), were prepared by methods

analogous to the synthesis of 3,13-dimethylheptadecane (Gries et al., 1993). After purification by HPLC [Waters LC 625 high performance liquid chromatograph equipped with a Waters 486 variable wavelength UV-visible detector, a HP 3396 Series II integrator and a Nova-Pak $C_{18}$ (3.9 × 300 mm) column], compounds were >97% chemically pure.

*Field Experiments*

Field experiments (August 30–October 16, 1995) in apple orchards in Guilford, New Haven County, Connecticut, were set up in randomized complete blocks with traps and blocks at 20-m intervals. Pherocon 1 CP traps (Trécé Inc., Salinas, California) were suspended (north–south-oriented) from trees 2 m above ground and 1–2 m away from the main trunk. The lures were grey sleeve stoppers (The West Company, Lionville, Pennsylvania) that were impregnated with candidate pheromone components in HPLC-grade hexane and suspended on colored pins from trap tops. The first experiment tested 10Me14Me-1-ene-18Hy (25 μg), 5Me9Me-18Hy (25 μg), and 5Me9Me-17Hy (25 μg) singly and in ternary combination. The second and third experiments tested 10Me14Me-1-ene-18Hy, 5Me9Me-18Hy, and 5Me9Me-17Hy in all binary and ternary combinations at 25 μg (experiment 2) or 35 μg (experiment 3) per compound.

Despite transformation, data from male moths captured in all three field experiments were not normally distributed and were therefore subjected to nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means (Scheffé test, $P < 0.05$) (Zar, 1984; SAS Institute Inc., Cary North Carolina, Propietary Software Release 6.10).

## RESULTS AND DISCUSSION

GC-EAD analysis of female *L. prunifoliella* pheromone extract on three GC columns consistently revealed three antennal responses (Figure 2). Compounds A and B, with <10 pg/FE, were not FID detectable, whereas compound C occurred at >50 pg/FE and was the most EAD active. Retention indices (Van den Dool and Kratz, 1963) of all EAD-active compounds on DB-5, DB-23, and DB-210 columns were indicative of methylated hydrocarbons. Based on these indices and previously identified methylated hydrocarbon pheromone components in the Lyonetiidae (Figure 3), compounds A and B were hypothesized to be 5Me9Me-17Hy and homologous 5Me9Me-18Hy. Identical retention and comparable EAD characteristics of synthetic 5Me9Me-17Hy and female-produced A, and 5Me9Me-18Hy and female-produced B on all three columns referred to above confirmed these structural assignments.

Most EAD-active C eluted later on DB-23 and DB-210 and earlier on DB-5 than did 5Me9Me-18Hy, indicative of at least one unsaturation in the

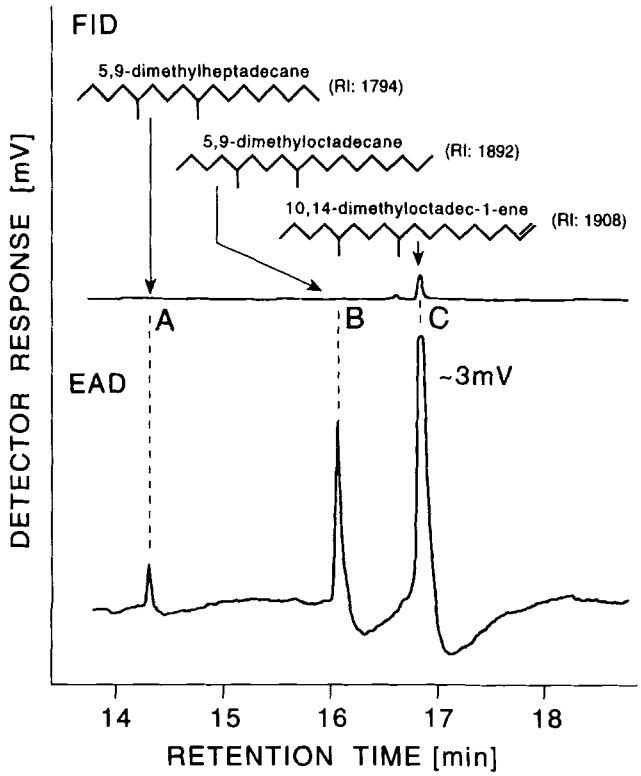


FIG. 2. Flame ionization detector (FID) and electroantennographic detector (EAD: male *L. prunifoliella* antenna) responses to one female equivalent of pheromone gland extract. Chromatography: splitless injection, injector and FID detector: 240°C; DB-23 column: temperature program: 50°C for 1 min, then 20°C/min to 70°C, then 5°C/min to 200°C; RI = retention index.

molecule. The mass spectrum of C also indicated unsaturation (Figure 4), and fragmentation ions *m/z* 85 and *m/z* 153 suggested methyl branches at C-5 and C-9. Because a methylated octadecene with terminal unsaturation [(14*S*)-methyloctadec-1-ene] is a pheromone component in *L. clerkella* (L.) (Sugie et al., 1984; Sato et al., 1985), and C-5 and C-9 are common methyl branches in lyonetiid hydrocarbon pheromones (Figure 3), C was hypothesized to be 10Me14Me-1-ene-18Hy (5Me9Me-17-ene-18Hy). Consistent mass spectra, identical retention characteristics on three fused silica columns (DB-23, DB-5, and DB-210), and comparable EAD activity of female-produced C and synthetic 10Me14Me-1-ene-18Hy confirmed the identification.

In field experiments, the ternary blend of 10Me14Me-1-ene-18Hy,

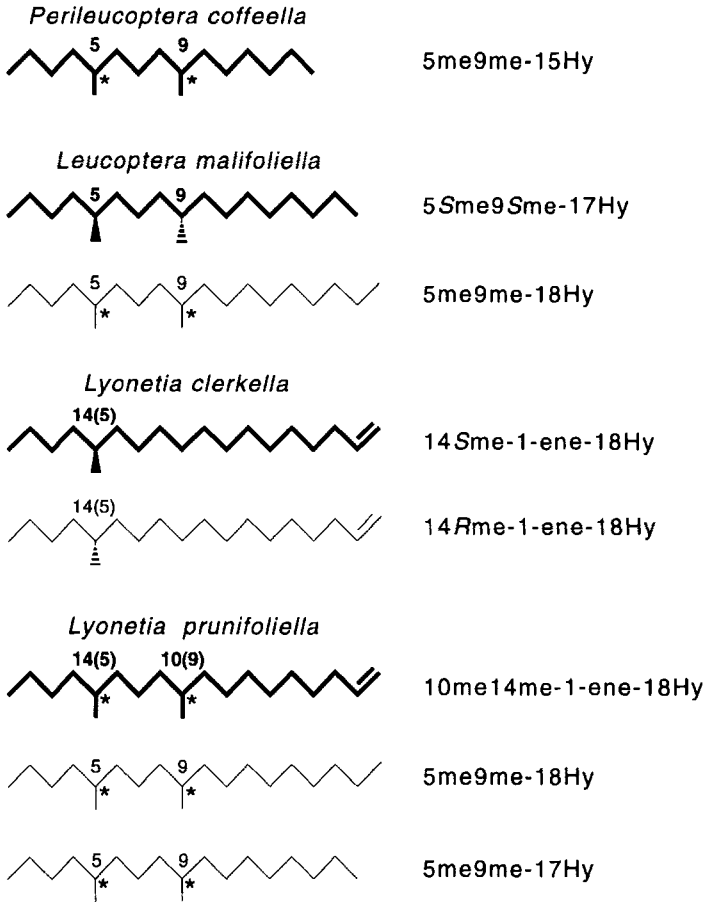


FIG. 3. Methylated hydrocarbon pheromone components of lyonetiids *Perileucoptera coffeela* (Francke et al., 1988), *Leucoptera malifoliella* (formerly scitella) (Francke et al., 1987; Tóth et al., 1989; Riba et al., 1990), *Lyonetia clerkella* (Sugie and Kumakura, 1984; Sato et al., 1985), and *Lyonetia prunifoliella* (this study). *Stereoisomers attractive to insects not yet determined.

5Me9Me-18Hy, and 5Me9Me-17Hy was strongly attractive, whereas none of the three components singly attracted a significant number of *L. prunifoliella* males (Figure 5, experiment 1). Attractiveness of the ternary 1 : 1 : 1 blend exceeded that of binary 1 : 1 blends (Figure 6, experiments 2 and 3), indicating that all three compounds are part of the pheromone blend. Greatest abundance and antennal activity of 10Me14Me-1-ene-18Hy in GC-EAD recordings (Figure 2), and no field attraction of males to lures without 10Me14Me-1-ene-18Hy

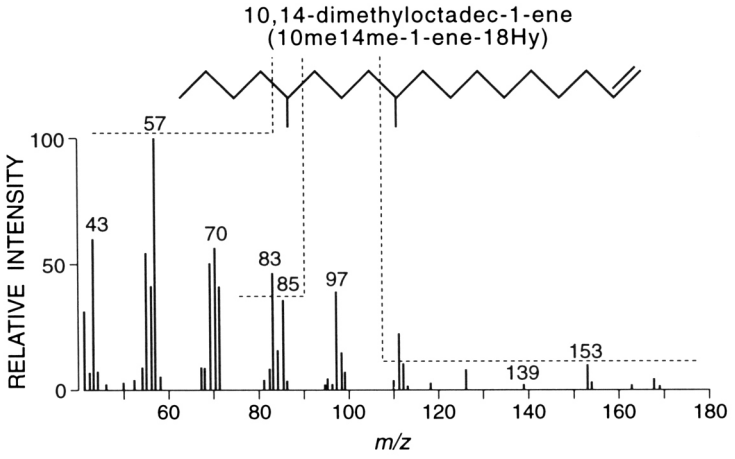


FIG. 4. Electron impact (70 eV) mass spectrum of synthetic 10,14-dimethyloctadec-1-ene. Fragmentation ions *m/z* 85 and *m/z* 153 are diagnostic of methyl branch positions C-14 (5) and C-10 (9).

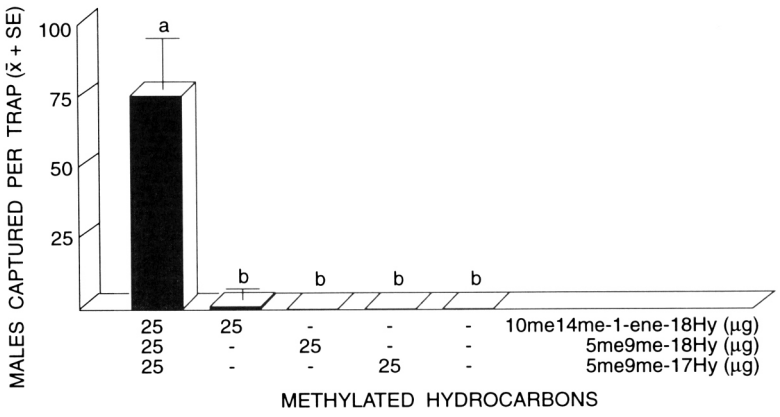


FIG. 5. Captures of *L. prunifoliella* males (experiment 1) in Pherocon-1 CP traps baited with 10,14-dimethyloctadec-1-ene (10Me14Me-1-ene-18Hy), 5,9-dimethyloctadecane (5Me9Me-18Hy), and 5,9-dimethylheptadecane (5Me9Me-17Hy) singly and in ternary combination at 25 µg each); Guilford, New Haven County, Connecticut, August 30–September 6, 1995; 10 replicates. Bars with the same superscript are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means (Scheffé test), $P < 0.05$.

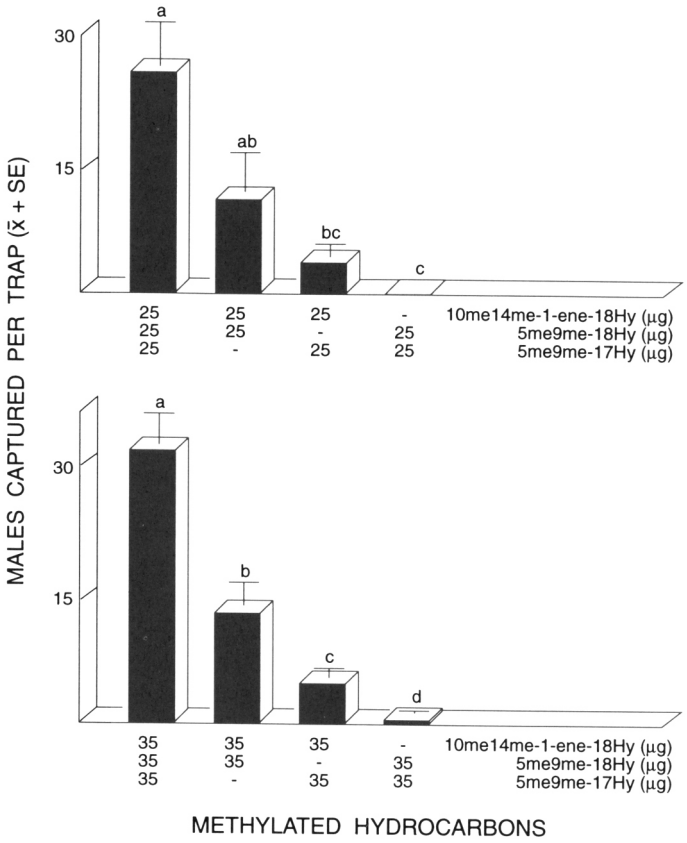


FIG. 6. Captures of *L. prunifoliella* males in Pherocon-1 CP traps baited with 10,14-dimethyloctadec-1-ene (10Me14Me-1-ene-18Hy), 5,9-dimethyloctadecane (5Me9Me-18Hy), and 5,9-dimethylheptadecane (5Me9Me-17Hy) in all binary and ternary combinations at 25 µg (experiment 2) and 35 µg (experiment 3) per compound; Guilford, New Haven County, Connecticut; experiment 2: September 18–25, 1995; 10 replicates; experiment 3: October 6–16, 1995; 8 replicates. For each experiment, bars with the same superscript are not significantly different; nonparametric analyses of variance by ranks (Friedman's test), followed by comparison of means (Scheffé test), $P < 0.05$.

(Figure 6), strongly suggest that this compound is essential for *L. prunifoliella* pheromone communication. It is the first unsaturated, dimethylated hydrocarbon identified as a pheromone component in the Lepidoptera.

Sex pheromone components of other lyonetiid moths also comprise methylated hydrocarbons (Figure 3). Structural similarities between pheromone components of congeners support taxonomic classifications. For instance, congeneric

*Lyonetia clerkella* and *L. prunifoliella* both produce a methylated octadecene, which is not part of the pheromone blend in heterogeneric *Perileucoptera coffeella* (Guérin-Meneville) or *Leucoptera malifoliella*. Occurrence of methyl-branches at C-5 and C-9 in all identified lyonetiid pheromone components (Figure 3) implies phylogeny of lyonetiid chemical communication and a common biosynthetic pathway, possibly from propionate (methylmalonate) and acetate units (Francke et al., 1987). Determination of all pheromone stereoisomers, such as (5*S*,9*S*)-5,9-dimethylheptadecane in *L. malifoliella* (Figure 3) (Tóth et al., 1989), is required to completely describe the pheromone blend of *L. prunifoliella* and to support the hypothesis of phylogenetically related sex pheromones.

*Acknowledgments*—We thank Greg Owen for mass spectrometry, Prof. Dr. Wittko Francke and one anonymous colleague for constructive review of the manuscript, and Jonathan Bishop and M. Stewart Ramsay for allowing us to conduct pheromone research in their orchards. The research was supported in part by a research grant of the Natural Sciences and Engineering Research Council of Canada to G.G.

## REFERENCES

ARN, H., STÄDLER, E., and RAUSCHER, S. 1975. The electroantennographic detector—a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30c:722–725.

BROWN, M. W. 1989. Spatial dynamics and sampling of *Lyonetia speculella* (Lepidoptera: Lyonetiidae), a new leafminer of apple. *Environ. Entomol.* 18:875–880.

COREY, E. J., and SNIDER, B. B. 1972. A synthesis of (±)-fumagillin. *J. Am. Chem. Soc.* 1972:2549.

ESMAILI, M. 1971. Four species of leaf miners attacking deciduous fruit trees in Iran's central province. *Z. Angew. Entomol.* 69:407–415.

FRANCKE, W., FRANKE, S., TÓTH, M., SZÖCS, G., GUERIN, P., and ARN, H. 1987. Identification of 5,9-dimethylheptadecane as a sex pheromone of the moth *Leucoptera scitella*. *Naturwissenschaften* 74:143–144.

FRANCKE, W., TÓTH, M., SZOCS, G., KRIEG, W., ERNST, H., and BUSCHMANN, E. 1988. Identifizierung und Synthese von Dimethylalkanen als Sexuallockstoff weiblicher Miniermotten (Lyonetiidae). *Z. Naturforsch.* 43c:787–789.

FROST, S. W. 1924. Two little known leaf-miners of apple (Lepid.: Tineidae; Col.: Curculionidae). *Entomol. News* 35:132–134.

GRIES, G., KING, G. G. S., GRIES, R., WIMALARATNE, P. D. C., GRAY, T. G., SHEPHERD, R. F., LI, J., SLESSOR, K. N., and KHASKIN, G. 1993. 3,13-Dimethylheptadecane: Major sex pheromone component of the western false hemlock looper, *Nepytia freemani* Munroe (Lepidoptera: Geometridae). *J. Chem. Ecol.* 19:1501–1510.

GRIES, G., GRIES, R., PEREZ, A. L., OEHLSCHLAGER, A. C., GONZALES, L. M., PIERCE, H. D., JR., ZEBEYOU, M., and KOUAME, B. 1994. Aggregation pheromone of the African rhinoceros beetle, *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae). *Z. Naturforsch.* 49c:363–366.

KREMER, W. F. 1963. Major leaf miner species occuring in the South Tirolean fruit-farming region, and their control. *Pflanzenschutz-Nachr.* 16:1–6.

MAIER, C. T. 1988. A new leafminer, *Lyonetia speculella*, in Connecticut apple orchards. *Rep. Conn. Pomol. Soc.* 1987:6–12.

MAIER, C. T. 1995. Hosts and density of *Lyonetia* spp. (Lepidoptera: Lyonetiidae) mining the foliage of woody ericaceous and rosaceous plants. *Ann. Entomol. Soc. Am.* 88:739–747.

NARUSE, H. 1978. Defoliation of peach tree caused by the injury of the peach leaf-miner, *Lyonetia clerkella* L. I. Influence of larval density. *Jpn. J. Appl. Entomol. Zool.* 22:1–6.

NEWTON, R. F., REYNOLDS, D. P., FINCH, M. A. W., KELLY, D. R., and ROBERTS, S. M. 1979. An excellent reagent for the removal of the *t*-butyldimethylsilyl protecting group. *Tetrahedron Lett.* 41:3981–3982.

NONAKA, T., KANEMOTO, S. OSHIMA, K., NOZAKI, H. 1984. Pyridinium flurochromate or benzyltrimethylammonium chlorochromate for selective oxidation of alcohols. *Bull. Chem. Soc. Jpn.* 57:2019–2020.

RIBA, M., ROSELL, J. A., EIZAGUIRRE, M., CANELA, R., and GUERRERO, A. 1990. Identification of a minor component of the sex pheromone of *Leucoptera malifoliella* (Lepidoptera, Lyonetiidae). *J. Chem. Ecol.* 16:1471–1483.

SATO, R., ABE, N., SONNET, P., SUGIE, H., and TAMAKI, Y. 1985. Biological activity of (*R*)- and *S*)-14-methyl-1-octadecene, as the chiral component of the sex pheromone of the peach leafminer moth, *Lyonetia clerkella* Linné. *Appl. Entomol. Zool.* 20:411–415.

SEKITA, N., and YAMADA, M. 1979. Life history of *Lyonetia prunifoliella* Hübner subspecies *malinella* (Matsumura) (Lepidoptera: Lyonetiidae). *Appl. Entomol. Zool.* 14:285–292.

SCHMITT, J. J., BROWN, M. W., and DAVIS, D. R. 1996. Taxonomy, morphology, and biology of *Lyonetia prunifoliella* (Lepidoptera: Lyonetiidae), a leafminer of apple. *Ann. Entomol. Soc. Am.* 89:334–345.

SEPRŐS, I. 1985. Fontosabb Fitofáag Mikrolepidopterák. Aknázómolyok. Mém. Növényvédelmi és Agrokémiai Központja. Budapest.

STILL, W. C., KAHN, M., and MITRA, A., 1978. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* 43:2923–2925.

SUGIE, R., and KUMAKURA, M. 1984. Sex pheromone of the peach leafminer moth, *Lyonetia clerkella* Linné: Isolation and identification. *Appl. Entomol. Zool.* 19:323–330.

TÓTH, M., HELMCHEN, G., LEIKAUF, U., SZIRAKI, GY., and SZÖCS, G. 1989. Behavioural activity of optical isomers of 5,9-dimethylheptadecane, the sex pheromone of *Leucoptera scitella* L. (Lepidoptera: Lyonetiidae). *J. Chem. Ecol.* 15:1534–1543.

VAN DEN DOOL, H., and KRATZ, P. 1963. A generalization of the retention index system including linear temperature programmed gas liquid partion chromatography. *J. Chromatogr.* 2:463–471.

ZAR, J. H. 1984. Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, New Jersey, 718 pp.