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SEX PHEROMONE COMPONENTS OF CASUARINA MOTH, *Lymantria xylina*

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Abstract—*cis*-7,8-Epoxy-2-methyleicosane is a sex pheromone component of the Casuarina moth, *Lymantria xylina* Swinhoe. The compound was extracted from pheromone glands of female moths and was identified by coupled gas chromatographic–electroantennographic detection (GC-EAD) and GC–mass spectrometry. In field experiments in Taiwan, traps baited with either or both of (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyleicosane (>99% ee) [termed here (+)-xylinalure] and (7*S*,8*R*)-*cis*-7,8-epoxy-2-methyleicosane (>99% ee) [termed here (−)-xylinalure] captured male *L. xylina*. Addition of further candidate pheromone components to xylinalure did not enhance its attractiveness. Demonstration of whether or not female *L. xylina* produce both optical isomers of xylinalure, and determination of the ratio, will require pheromone extract analyses on a chiral, enantiomer-separating column (as yet unavailable) or derivatization of epoxides in accumulated gland extracts. Attraction of male *L. xylina* to either enantiomer of xylinalure contrasts with enantiospecific production of, and/or response to, epoxy pheromones in congeners. With no other nocturnal lymantriid moth known in Taiwan to utilize xylinalure for pheromonal communication, enantiospecific "fine tuning" of xylinalure, or evolution of a more complex pheromone blend, may not have been neces-

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sary for *L. xylina* to maintain specificity of sexual communication. Racemic xylinalure will be appropriate for pheromone-based detection surveys of *L. xylina* in North America.

Key Words—Lepidoptera, Lymantriidae, *Lymantria xylina*, *Lymantria dispar*, *Lymantria monacha*, *Lymantria fumida*, sex pheromone, reproductive isolation, (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyleicosane, (7*S*,8R)-*cis*-7,8-epoxy-2-methyleicosane, 2-methyl-*Z*7-eicosene, (7*R*,8*S*)-*cis*-7,8-epoxy-2-methylnonadecane, (7*S*,8*R*)-*cis*-7,8-epoxy-2-methylnonadecane, (7*R*,8*S*)-*cis*-7,8-epoxy-3-methylnonadecane, (7*S*,8*R*)-*cis*-7,8-epoxy-3-methylnonadecane, disparlure.

INTRODUCTION

The Casuarina moth, *Lymantria xylina* Swinhoe (Lepidoptera: Lymantriidae), formerly known as *Lymantria sakaguchii* Matsumura (Kishida, 1995), occurs in southern Japan (Kyushu, Okinawa, and islands between), Taiwan, and southeastern China (Inoue, 1957; Li et al., 1981; Xiao, 1992). Caterpillars feed on many hardwood trees, especially *Casuarina* spp. (Li et al., 1981; Chao et al., 1996). Outbreak populations have completely defoliated host trees, especially *Casuarina*, on both sides of the Taiwan Straits (Sonan, 1936; Li et al., 1981; Chang and Weng, 1985; Xiao, 1992; Schaefer and Gries, unpublished data).

Pheromone-based detection surveys in North America offer the best means of early detection and eradication of invading Eurasian forest defoliators, such as *L. xylina*. Yet, the sex pheromone of *L. xylina* has not been thoroughly investigated. Y.-S. Chou (1982, unpublished data) captured limited numbers of *L. xylina* males in traps baited with disparlure (*cis*-7,8-epoxy-2-methyloctadecane), the sex pheromone of the gypsy moth, *Lymantria dispar* L. (Bierl et al., 1970). Similarly, P. W. Schaefer succeeded in 1994, but not in 1996 and 1997, in capturing male *L. xylina* in milk-carton traps (Ecogen Inc., Langhorne, Pennsylvania) baited with (+)-disparlure [1994: laminate bait type (Hercon Environmental Company, Emigsville, Pennsylvania); 1996–1997: string bait type (Phero Tech Inc., Delta, British Columbia, Canada)]. We report identification and field testing of sex pheromone components of *L. xylina*.

METHODS AND MATERIALS

Insect Culture and Pheromone Extraction. On May 23, 1997, *L. xylina* pupae were collected in coastal plantings of *Casuarina equisetifolia* L. in Kuanyin, Taoyuan County, Taiwan, and shipped to the Biological Control Laboratory, Taiwan National University in Taipei. Female pupae (Xiao, 1992) were kept in individual plastic cups at a photoperiod of 14L : 10D, 24–26°C, and 60–80% relative humidity, whereas male pupae were kept at ~15°C to retard development. Abdominal tips with pheromone glands of calling, 1- to 3-day-old

virgin female moths were removed and placed in redistilled hexane. After 15–30 min of extraction, the supernatant was withdrawn, syringed into ampoules, and shipped (together with male pupae) to Simon Fraser University.

Laboratory Analyses and General Procedures. Aliquots of 1 female equivalent (FE) of pheromone gland extract were analyzed by coupled gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975), by using a Hewlett-Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25 or 0.32 mm ID) coated with either DB-210, DB-5, or DB 23 (J&W Scientific, Folsom, California). GC–mass spectrometry (MS) of synthetic or insect-produced compounds in full-scan electron ionization mode employed a Varian Saturn II Ion Trap GC-MS fitted with the DB-5 column referred to above. Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on a Bruker AMX-400 spectrometer at 400.13 MHz for ^{1}H NMR spectra. ^{1}H chemical shifts are reported as parts per million (ppm) relative to TMS (0.00 ppm). Column chromatography refers to flash chromatography that used silica gel 60 (230–400 mesh, E. Merck, Darmstadt) (Still et al., 1978).

Synthesis of 2-Methyl-Z7-eicosene (4) and cis*-7,8-Epoxy-2-methyleicosane (5) (Figure 1).* Coupling of 40 ml (166.7 mmol) of dodecylbromide with the dianion of 5-hexyne-1-ol (**1**; 10 g, 102 mmol) in the presence of HMPA, and subsequent hydrogenation (Ni-P2 catalyst) of 5-octadecyn-1-ol (**2**) afforded (*Z*)-5-octadecen-1-ol (**3**) (25.5 g, 95 mmol, 93% yield). Mesylation at 0°C of **3** with methanesulfonyl chloride (100 mmol) in the presence of triethylamine (150 mmol) and subsequent copper(I)-catalyzed (10 mmol of CuI) coupling of mesylate with 2-bromomagnesium propane (190 mmol) at −25°C produced 2-methyl-*Z*7-eicosene (**4**). Column chromatography of the crude product with hexanes and

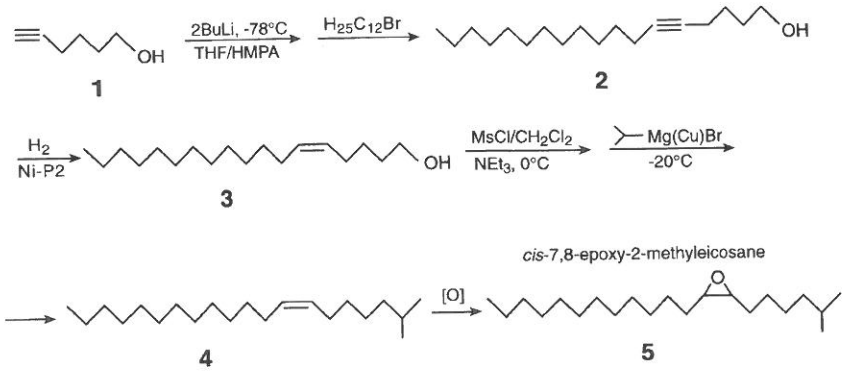

FIG. 1. Schemes for synthesis of 2-methyl-*Z*7-eicosene and *cis*-7,8-epoxy-2-methyleicosane.

then hexanes–ether (4 : 1) as eluents allowed purification of hydrocarbon **4** (8.48 g, 30% yield) and recovery of starting alcohol **3** (13 g, 51%), which was used for repeated mesylate–Grignard coupling. Combined yields of **4** were 19.28 g (69% yield based on alcohol **3**). Stirring **4** (10.8 g, 36.7 mmol) with 1.5 molar excess of *m*-chloroperoxybenzoic acid in dichloromethane at 0°C for 3 hr, and then overnight at room temperature, afforded *cis*-7,8-epoxy-2-methyleicosane (**5**) (10.82 g, 95% yield after purification by column chromatography that used 2% of ether in hexanes as the eluent). ^{1}H NMR of **5**: 2.90 (quint, 2H), 1.49 (m, 4H), 1.15–1.42 (m, 27H), 0.88 (t, 3H), 0.87 (d, 6H).

Synthetic standards of *cis*-7,8-epoxy-2-methylnonadecane; *cis*-7,8-epoxy-3-methylnonadecane, (7*R*,8*S*)-*cis*-7,8-epoxy-3-methylnonadecane (*cis*-7*R*,8*S*-epoxy-3me-19Hy), and (7*S*,8*R*)-*cis*-7,8-epoxy-3-methylnonadecane (*cis*-7*S*,8*R*-epoxy-3me-19Hy), were available from previous work (Gries et al., 1996). Enantiospecific syntheses of (7*R*,8*S*)-*cis*-7,8-epoxy-2-methylnonadecane (*cis*-7*R*,8*S*-epoxy-2me-19Hy), (7*S*,8*R*)-*cis*-7,8-epoxy-2-methylnonadecane (*cis*-7*S*,8*R*-epoxy-2me-19Hy), (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyleicosane (*cis*-7*R*,8*S*-epoxy-2me-20Hy) and (7*S*,8*R*)-*cis*-7,8-epoxy-2-methyleicosane (*cis*-7*S*,8*R*-epoxy-2me-20Hy) followed methods previously developed by R. Hahn.

Field Experiments. Field experiments were conducted in several coastal plantings of *C. equisetifolia* in Kuanyin and Taoyuan, Taoyuan County, Taiwan. Self-made sticky 2-liter Delta milk carton traps (Gray et al., 1984) were suspended from trees ~2 m above ground in complete randomized blocks with trap spacings of 15–20 m. Traps were baited with gray sleeve stoppers (The West Company, Lionville, Pennsylvania) impregnated with test chemicals in HPLC grade hexane. Experiments 1–4 tested the major candidate pheromone components *cis*-7*R*,8*S*-epoxy-2me-20Hy and *cis*-7*S*,8*R*-epoxy-2me-20Hy singly and in combination, each at 50 μg (experiment 1), 5 μg (experiment 2), 0.5 μg (experiment 3), and 0.05 μg (experiment 4). Experiments 5–7 tested *cis*-7*R*,8*S*-epoxy-2me-20Hy (50 μg) singly and in combination with additional candidate pheromone components, such as either and both of: *cis*-7*R*,8*S*-epoxy-2me-19Hy (5 μg) and *cis*-7*S*,8*R*-epoxy-2me-19Hy (5 μg) (experiment 5); *cis*-7*R*,8*S*-epoxy-3me-19Hy (5 μg), and *cis*-7*S*,8*R*-epoxy-3me-19Hy (5 μg) (experiment 6); and with 2-methy-*Z*7–20Hy at 0.5, 5, and 50 μg. Experiment 8 tested *cis*-7*R*,8*S*-epoxy-2me-20Hy plus *cis*-7*S*,8*R*-epoxy-2me-20Hy at increasing doses (5, 50, and 500 μg each). Final experiment 9 tested either *cis*-7*R*,8*S*-epoxy-2me-20Hy (500 μg) plus *cis*-7*S*,8*R*-epoxy-2me-20Hy (500 μg) or *cis*-7*R*,8*S*-epoxy-2me-18Hy [(+)-disparlure; 500 μg)] alone or in combination.

RESULTS AND DISCUSSION

GC-EAD analyses of pheromone gland extract of female *L. xylina* revealed four compounds that consistently elicited responses from male moth antennae

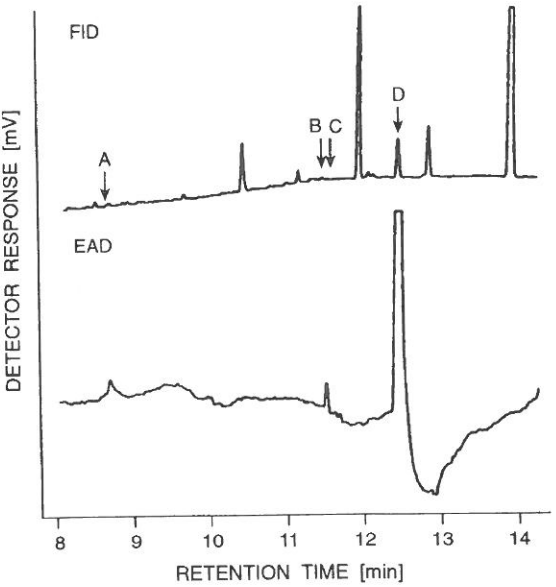

FIG. 2. Flame ionization detector (FID) and electroantennographic detector (EAD: male *L. xylina* antenna) responses to 1 FE of pheromone extract. Hewlett-Packard 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25 mm ID) coated with DB-23: temperature program: 100°C (1 min), 10°C/min to 200°C.

(Figure 2). Most abundant and most EAD-active compound **D** had retention indices (Dool and Kratz, 1963) ca. 200 units higher than those of disparlure on all three analytical columns (DB-5, DB-210, and DB-23), indicative of an epoxide homologous to disparlure. The mass spectrum of **D** with molecular ion *m/z* 311 (M+1) and fragmentation ion *m/z* 211 (Figure 3) suggested *cis*-7,8-epoxy-2me-20Hy as a potential molecular structure. Corresponding retention times and mass spectra as well as comparable antennal activity of insect-produced **D** and synthetic *cis*-7,8-epoxy-2me-20Hy support the structural assignment. Moreover, mass spectra of synthetic *cis*-6,7- or *cis*-8,9-epoxy-2me-20Hy were inconsistent with insect-produced **D**. EAD-active compound **A** with retention indices 200 units higher than those of the olefin analog of disparlure (2-methyl-*Z*7-octadecene) was hypothesized and, through comparative GC-EAD on all three columns, confirmed to be 2-methyl-*Z*7-eicosene. EAD-active compound **B** (not visible in Figure 2) cochromatographed on all employed analytical columns (DB-5, DB-210, and DB-23) with synthetic *cis*-7,8-epoxy-3-methylnonadecane (Gries et al., 1996). EAD-active compound **C** with retention indices on all three columns consistently ca. 100 units lower than that of **D** was hypoth-

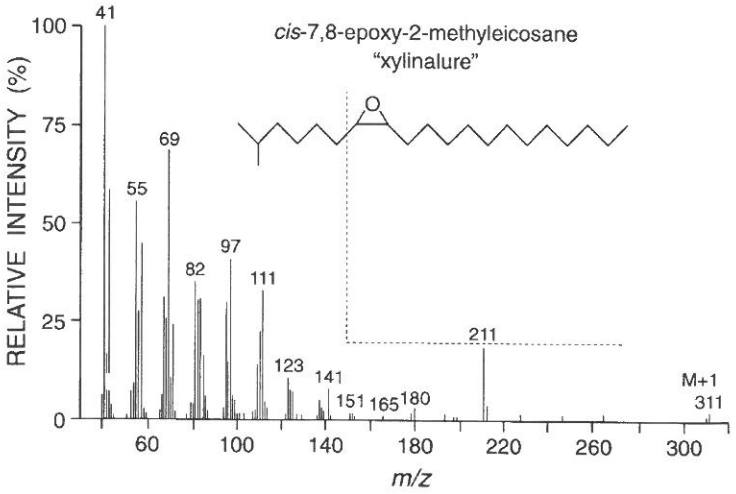

FIG. 3. Full-scan electron ionization mass spectrum of *cis*-7,8-epoxy-2-methyleicosane (xylinalure) present in pheromone gland extracts of female *L. xylina*; Varian Saturn II Ion Trap GC-MS.

esized and, through comparative GC-EAD with an authentic standard, confirmed to be *cis*-7,8-epoxy-2me-19Hy.

In field experiments (Figure 4, experiments 1–4), traps baited with either or both *cis*-7*R*,8*S*-epoxy-2me-20Hy [termed here (+)-xylinalure] and *cis*-7*S*,8*R*-epoxy-2me-20Hy [termed here (−)-xylinalure] captured male *L. xylina*. Other candidate pheromone components, such as *cis*-7*R*,8*S*-epoxy-2me-19Hy and/or *cis*-7*S*,8*R*-epoxy-2me-19Hy (Figure 5, experiment 5), *cis*-7*R*,8*S*-epoxy-3me-19Hy and/or *cis*-7*S*,8*R*-epoxy-3me-19Hy (Figure 5, expriment 6), or 2-methyl-*Z*7-20Hy (Figure 5, experiment 7) neither enhanced nor reduced attractiveness of xylinalure, indicating that they are not part of the pheromonal blend.

Variation in relative attractiveness of (+)-, (−)-, and (+)- plus (−)-xylinalure in experiments 1–4 (Figure 4) may be explained, in part, by the heterogeneity of trapping sites, particularly those of experiments 2 and 3. Furthermore, low pheromone doses in experiments 2–4, attracting male moths mainly in the first night of trapping, possibly coupled with low emergence of new males in subsequent nights, may have prevented normalization of skewed trapping results. As demonstrated in the leaf-mining moth *Eriocrania semipurpurella* (Löfstedt et al., 1998), it is also possible that genetically related subsets of male *L. xylina* exhibited preference for (+)-, (−)-, or (+)- plus (−)- pheromone enantiomers in temporally and/or spatially separated experiments 1–4.

With the similar attractiveness of (+)- and (−)-xylinalure, even at lure load-

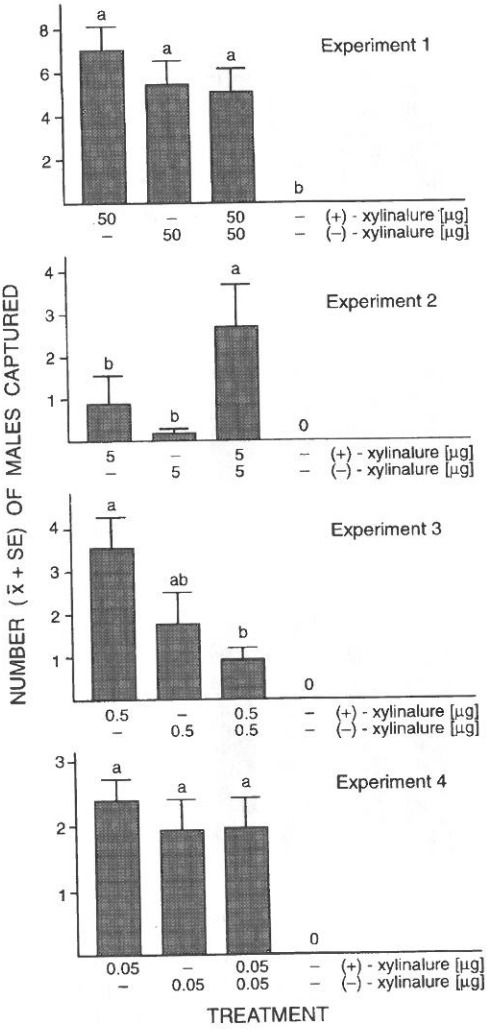

FIG. 4. Mean number (+SE) of male *L. xylina* captured in sticky 2-liter Delta milk carton traps baited with either or both (+)-xylinalure [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyleicosane] and (–)-xylinalure [(7*S*,8*R*)-*cis*-7,8-epoxy-2-methyleicosane], each at 50 μg (experiment 1), 5 μg (experiment 2), 0.5 μg (experiment 3), and 0.05 μg (experiment 4); 10 replicates per experiment; May 20–21, 1998 (experiment 1), May 22–24, 1998 (experiments 2 and 3), May 23–24, 1998 (experiment 4); near Kuanyin and Taoyuan, Taoyuan County, Taiwan. For each experiment, bars with the same letter are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni t test, $P < 0.05$] (SAS/STAT, 1988).

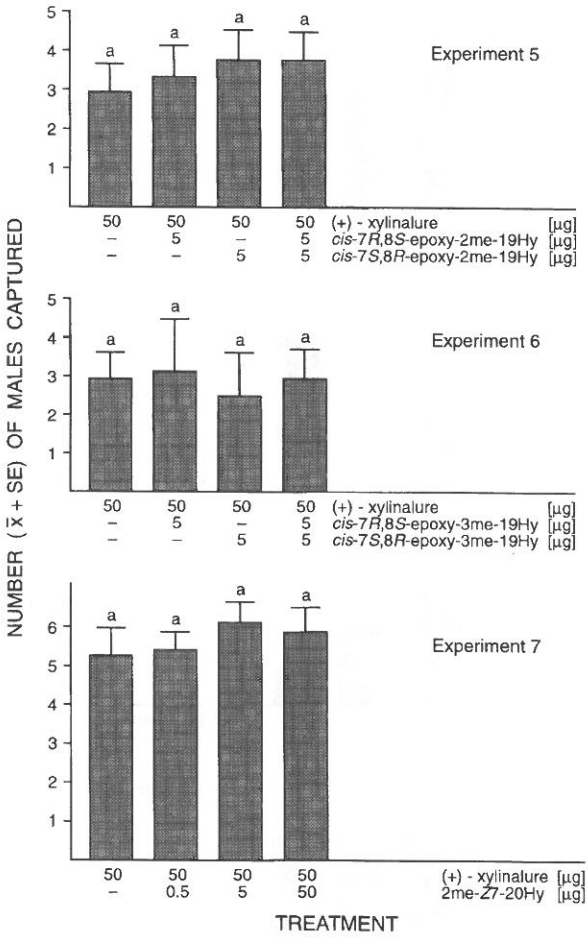

FIG. 5. Mean number (+SE) of male *L. xylina* captured in sticky 2-liter Delta milk carton traps baited with (+)-xylinalure [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyleicosane] singly and in combination with additional candidate pheromone components, such as either or both (7*R*,8*S*)-*cis*-7,8-epoxy-2-methylnonadecane (*cis*-7*R*,8*S*-epoxy-2me-19Hy) and (7*S*,8*R*)-*cis*-7,8-epoxy-2-methylnonadecane (*cis*-7*S*,8*R*-epoxy-2me-19Hy) (experiment 5), either or both (7*R*,8*S*)-*cis*-7,8-epoxy-3-methylnonadecane (*cis*-7*R*,8*S*-epoxy-3me-19Hy) and (7*S*,8*R*)-*cis*-7,8-epoxy-3-methylnonadecane (*cis*-7*S*,8*R*-epoxy-3me-19Hy) (experiment 6), and with 2-methyl-*Z*7-eicosene (2me-*Z*7-20Hy); 10 replicates per experiment; May 21–22, 1998; near Kuanyin and Taoyuan, Taoyuan Company, Taiwan. For each experiment, there were no significant differences between treatments; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni *t* test, $P > 0.05$] (SAS/STAT, 1988).

ings as low as 0.05 μg (Figure 4, experiment 4), and enantiomeric purities of test chemicals exceeding 99% ee (Hahn et al., unpublished), enantiomeric contamination could not have been responsible for the behavioral activity of either enantiomer. Demonstration whether or not female *L. xylina* produce both optical isomers of xylinalure, and determination of the ratio, will require GC-EAD and GC-MS analyses of pheromone extract on a chiral, enantiomer-separating column (as yet unavailable) or derivatization of epoxides (Oliver and Waters, 1995) in accumulated gland extracts.

Attractiveness of either enantiomer of xylinalure in *L. xylina* (Figure 4, experiments 1–4) contrasts with enantiospecific attractiveness of (+)-disparlure in the closely related gypsy moth, *L. dispar.* (+)-Disparlure attracts male *L. dispar*, whereas the presence of (−)-disparlure reduces attractiveness of the bait (Cardé et al., 1977a,b; Miller et al., 1977; Plimmer et al., 1977). The presence of 2-methyl-*Z*7-octadecene (the olefin analog of disparlure) also reduces attractiveness of disparlure to male *L. dispar* (Cardé et al., 1973; Miller et al., 1977), whereas analogous 2-methyl-*Z*7-eicosene in *L. xylina* has no behavioral effect.

With at least three sympatric species of lymantriid moths that utilize disparlure as a pheromone component, including *L. dispar* (Bierl et al., 1970); the nun moth, *L. monacha* L. (Bierl et al., 1975); and *L. fumida* (Schaefer et al., unpublished), enantiospecificity in the production of (Hansen, 1984) and/or response to disparlure may have evolved to contribute to species-specific sexual communication. 2-Methyl-*Z*7-octadecene, serving as a pheromone component in both *L. monacha* (Grant et al., 1996; Gries et al., 1996) and *L. fumida* (Schaefer et al., 1999), but being repellent to male *L. dispar*, seems to enhance specificity of communication channels. Xylinalure, in contrast, is reported here for the first time as a pheromone component in lymantriid moths. With no other nocturnal lymantriids in Taiwan known to utilize xylinalure for pheromonal communication, enantiospecific fine tuning of xylinalure, or evolution of a more complex pheromone blend, may not have been necessary for *L. xylina* to maintain reproductive isolation.

The (almost) indiscriminate response by male *L. xylina* to (+)-xylinalure with or without (+)-disparlure (Figure 6, experiment 9) may be attributed to the absence of *L. dispar* in Taiwan. Whether sympatry and coseasonality of *L. xylina* and *L. dispar* on other islands, such as Okinawa, alter the response to heterospecific pheromone and/or diel periodicity of pheromonal communication is currently being investigated. This research has implications for pheromone-based detection surveys for Asian lymantriids in North America, as it will facilitate decisions whether pheromones of two or more lymantriids, such as *L. xylina* and *L. dispar*, can be economically combined in one lure without compromising optimal attraction of all target species.

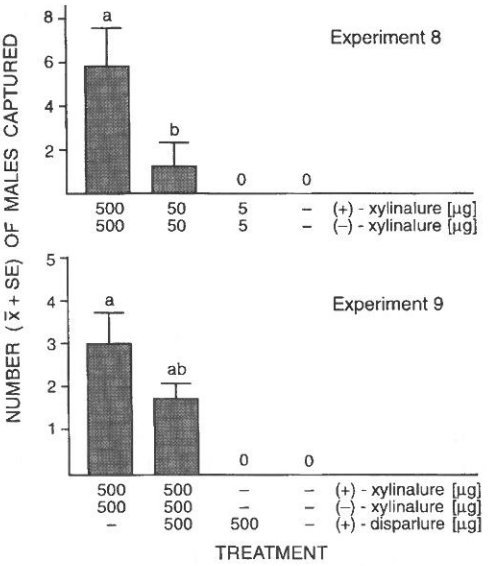

FIG. 6. Mean number (+SE) of male *L. xylina* captured in sticky 2-liter Delta milk carton traps baited with various doses of (+)- and (−)-xylinalure [(7*R*,8*S*)- and (7*S*,8*R*)-*cis*-7,8-epoxy-2-methyleicosane] (experiment 8), and (+)- plus (−)-xylinalure or (+)-disparlure [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane) singly and in combination (experiment 9); 10 replicates for each experiment; May 25–26, 1998; near Kuanyin and Taoyuan, Taoyuan Company, Taiwan. For each experiment, bars with the same letter are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni *t* test, $P > 0.05$] (SAS/STAT, 1988).

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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.

BIERL, B. A., BEROZA, M., and COLLIER, C. W. 1970. Potent sex attractant of the gypsy moth: Its isolation, identification and synthesis. *Science* 170:87–89.

BIERL, B. A., BEROZA, M., ADLER, V. E., KASANG, G., SCHRÖTER, H., and SCHNEIDER, D. 1975. The presence of disparlure, the sex pheromone of the gypsy moth, in the female nun moth. *Z. Naturforsch.* 30c:672–675.

CARDÉ, R. T., ROELOFS, W. L., and DOANE, C. C. 1973. Natural inhibitor of the gypsy moth sex attractant. *Nature* 241:474–475.

CARDÉ, R. T., DOANE, C. C., GRANETT, J., HILL, A. S., KOCHANSK, Y. J., and ROELOFS, W. L. 1977a. Attractancy of racemic disparlure and certain analogues to male gypsy moths and the effect of trap placement. *Environ. Entomol.* 6:765–767.

CARDÉ, R. T., DOANE, C. C., BAKER, T. C., IWAKI, S., and MARUMO, S. 1977b. Attractancy of optically active pheromone for male gypsy moth. *Environ. Entomol.* 6:768–772.

CHANG, Y.-C., and WENG, Y.-C. 1985. Morphology, life habit, outbreak and control of casuarina tussock moth (*Lymantria xylina* Swinhoe). *Q. J. Chin. For.* 18:29–36.

CHAO, J.-T., SCHAEFER, P. W., FAN, Y.-B., and LU, S.-S. 1996. Host plants and infestation of casuarina moth, *Lymantria xylina* (Lepidoptera: Lymantriidae) in Taiwan. *Taiwan J. For. Sci.* 11:23–28.

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

GRANT, G. G., LANGEVIN, D., LÍSKA, J., KAPITOLA, P., and CHONG, J. M. 1996. Olefin inhibitor of gypsy moth, *Lymantria dispar*, is a synergistic pheromone component of nun moth, *L. monacha*. *Naturwissenschaften* 83:328–330.

GRAY, T. G., SLESSOR, K. N., SHEPHERD, R. F., GRANT, G. G., and MANVILLE, J. F. 1984. European pine shoot moth, *Rhyacionia buoliana* (Lepidoptera: Tortricidae): Identification of additional pheromone components resulting in an improved lure. *Can. Entomol.* 116:1525–1532.

GRIES, G., GRIES, R., KHASKIN, G., SLESSOR, K. N., GRANT, G. G., LÍSKA, J., and KAPITOLA, P. 1996. Specificity of nun and gypsy moth sexual communication through multiple-component pheromone blends. *Naturwissenschaften* 83:382–385.

HANSEN, K. 1984. Discrimination and production of disparlure enantiomers by the gypsy moth and the nun moth. *Physiol. Entomol.* 9:9–18.

INOUE, H. 1957. A revision of the Japanese Lymantriidae (II). *Jpn. J. Med. Sci. Biol.* 10:187–219.

KISHIDA, Y. 1995. Taxonomic notes on *Lymantria xylina* Swinhoe and *L. sakaguchii* Matsumura (Lymantriidae). *Yugato* 141:115–116.

LI, Y., CHEN, S. L., XIE, Q. M., CAI, Q. J., WU, J., LI, Y. W., ZHENG, X. Q., ZHU, Z. W., XHOU, B. T., and ZHENG, H. Q. 1981. Studies on the lymantriid moth, *Lymantria xylina* Swinhoe. *Acta Entomol. Sin.* 24:174–183 (in Chinese).

LÖFSTEDT, C., METCALFE, P., SVENSSON, G., KOZLOV, M., and FRANCKE, W. 1998. Sex pheromones and speciation among pheromone morphs and cryptic species in the leaf-miner *Erocrania semipurpurella*. Second International Symposium on Insect Pheromones. WICC-International Agricultural Centre, Wageningen, The Netherlands.

MILLER, J. R., MORI, K., and ROELOFS, W. L. 1977. Gypsy moth field trapping and electroantennogram studies with pheromone enantiomers. *J. Insect Physiol.* 23:1447–1453.

OLIVER, J. E., and WATERS, R. M. 1995. Determining enantiomeric composition of disparlure. *J. Chem. Ecol.* 21:199–211.

PLIMMER, J. R., SCHWALBE, C. P., PASZEK, E. C., BIERL, B. A., WEBB, R. E., MARUMO, S., and IWAKIM, S. 1977. Contrasting effects of (+)- and (−)-enantiomers of disparlure for trapping native populations of the gypsy moth in Massachusetts. *Environ. Entomol.* 6:518–522.

SAT/STAT. 1988. User's guide, release 6.03 edition. SAS Institute Inc., Cary, North Carolina 27513.

SCHAEFER, P. W., GRIES, G., GRIES, R., and HOLDEN, D. 1999. Pheromone components and diel peridodicity of pheromonal communication in *Lymantria fumida*. *J. Chem. Ecol.* 25:2305–2312.

SONAN, J. 1936. On damage by *Lymantria xylina* (Lymantriidae) to *Acacia* and *Casuarina*. Taiwan Agric. Rept. No. 350, pp. 51–57 (in Japanese).

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

XIAO, G. 1992. Forest Insects of China, 2nd ed. China Forestry Publ. House, Beijing, 1362 pp.