*Journal of Chemical Ecology, Vol. 32, No. 3, March 2006 (©2006)*
DOI: 10.1007/s10886-005-9025-4

# FEMALE SEX PHEROMONE OF A CARPENTER MOTH, *Cossus insularis* (LEPIDOPTERA: COSSIDAE)

XIONG CHEN,[1,4] KIYOSHI NAKAMUTA,[1,*] TOMOAKI NAKANISHI,[2] TADAKAZU NAKASHIMA,[1] MASAHIKO TOKORO,[1] FUMIAKI MOCHIZUKI,[3] and TAKEHIKO FUKUMOTO[3]

[1]*Department of Forest Entomology, Forestry and Forest Products Research Institute, PO Box 16, Tsukuba Norin Kenkyu Danchi-nai, Tsukuba, Ibaraki 305-8687, Japan*

[2]*Fruit Tree Research Institute, Tokushima Prefectural Agriculture, Forestry and Fisheries Technology Center, Nue, Katuura, Tokushima 773-4301, Japan*

[3]*Shin-Etsu Chemical Co., Ltd., Specialty Chemicals Research Center, 28-1, Nishifukushima, Kubiki-ku, Joetsu, Niigata 942-8601, Japan*

(Received May 26, 2005; revised October 7, 2005; accepted October 8, 2005)

**Published Online April 26, 2006**

**Abstract**—This study describes the identification of a sex pheromone component of a cossid moth, *Cossus insularis*. Coupled gas chromatographic–electroantennographic detection (GC–EAD) analysis of solid-phase microextraction (SPME) collections of volatiles released by live female moths showed that two compounds elicited EAG responses from the antennae of male moths. These compounds were identified as (*E*)-3-tetradecenyl acetate (*E*3-14:Ac) and (*Z*)-3-tetradecenyl acetate (*Z*3-14:Ac) by mass spectral analysis and retention index comparisons with synthetic standards. The ratio of *E*3-14:Ac and *Z*3-14:Ac was 95:5 in the effluvia of a female. In field bioassays, sticky traps baited with blends of *E*3-14:Ac and *Z*3-14:Ac showed that *E*3-14:Ac is an essential component of the pheromone. However, the role of *Z*3-14:Ac is unclear, because *E*3-14:Ac as a single component was as attractive to male moths as blends of *E*3-14:Ac and *Z*3-14:Ac, including the 95:5 blend released by live female moths.

**Key Words**—Carpenterworm moth, *Cossus insularis*, (*E*)-3-tetradecenyl acetate, (*Z*)-3-tetradecenyl acetate, GC–EAD, SPME, sex pheromone.

* To whom correspondence should be addressed. E-mail: nakamuta@ffrpi.affrc.go.jp
[4] Present address: Texas Agricultural Experimental Station, Texas A & M University System, 6500 Amarillo Boulevard, Amarillo, TX 79106, USA.

## INTRODUCTION

The carpenterworm moth, *Cossus insularis* (Staudinger) (Lepidoptera: Cossidae) is found in Honshu, Kyushu, and Tsushima Islands of Japan (Tadauchi and Inoue, 2000). Although host plants of this species are not well known, Kitajima et al. (1998) reported an aggregation of 236 larvae from a 3.2-cm-diameter branch of a willow tree. Recently, Nakanishi (2005) reported the first incidence of *C. insularis* larvae in Japanese pear, *Purys pyrifolia* var. culta. On both host trees, aggregations of larvae were observed to bore into woody stems, causing significant damage and frequent mortality. Because burrows are not exposed, insecticides are not a practical measure for control. Isolation and identification of the sex pheromone of *C. insularis* would be useful to help monitor and possibly control populations of this species.

In the Family Cossidae, sex pheromones for *Cossus cossus* L. (Capizzi et al., 1983), *Holcocerus hippophaecolus* Hua (Fang et al., 2005), *Holcocerus insularis* (Zhang et al., 2001), *Prionoxystus robinae* Peck (Solomon et al., 1978), and *Zeuzera pyrina* L. (Tonini et al., 1986) have been identified, and sex attractants for three other species, *Acossus centerensis* Lintner (Doolittle et al., 1976), *Cossus mongolicus* Erschoff (Qi et al., 1985), and *Prionoxystus piger* Grote (Landolt et al., 1985) have been reported. Most of the attractive components for these cossid moths are mono- or diunsaturated 10-, 12- 14-, or 18-carbon alkenyl acetates. The aim of the present study was to isolate and identify the sex pheromone of *C. insularis*.

## METHODS AND MATERIALS

*Insects.* Late instar larvae and pupae of *C. insularis* were collected from heavily infested logs of willow trees at the Forestry and Forest Products Research Institute, Tsukuba, Japan, in March 2004. Field-collected larvae were reared individually on artificial diet (Insecta LF®, Nihon Nosan, Yokohama, Japan) under 14L:10D at 25 ± 1°C. Newly emerged female and male moths were used for solid-phase microextraction (SPME) collection of volatiles and coupled gas chromatographic–electroantennographic detection (GC–EAD) analyses, respectively.

*Collection of Volatiles from Female Moths.* We used SPME for collecting airborne volatiles emitted by the female moths. SPME fibers (100 μm polydimethylsiloxane, Supelco, Bellefonte, PA, USA) were preconditioned for 30 min at 250°C. Newly emerged females were isolated in a 100-ml glass bottle (Sibata, Tokyo, Japan) with a screw cap lined with an acrylic nitryl butadiene rubber/Teflon septum (45 mm diameter) with a hole (26 mm ID). Two SPME fibers were inserted into each bottle through a small hole in the septum. The

fibers were exposed to the moths for approximately 16 hr, from 1700 hr until 0900 hr next morning, at 25 ± 1°C. The loaded SPME fiber was then desorbed into the injection port of either a GC–EAD or a GC–MS system. The bottles for volatile collection were ultrasonicated in a detergent solution for 10 min, rinsed, and dried at 100°C before use.

To evaluate the SPME sampling method, we placed 1 mg each of (*E*)-3-tetradecenyl acetate (*E*3-14:Ac) and (*Z*)-3-tetradecenyl acetate (*Z*3-14:Ac) in 100 µl *n*-hexane solution on the bottom surface of a 100-ml glass bottle. An SPME fiber was inserted into the bottle after the *n*-hexane evaporated and kept for one night at 25 ± 1°C. Ten different SPME fibers were used to collect volatiles from the headspace of the bottle and were then desorbed in the injection port of a GC.

*Coupled Gas Chromatographic-Electroantennographic Detection (GC–EAD) Analysis.* Volatiles collected on the SPME fiber were analyzed by GC–EAD in splitless mode with a Hewlett-Packard 5890 series II GC equipped with a DB-23 column (30 m × 0.32 mm ID, 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA). Oven temperature was 45°C/1 min, 15°C/min to 150°C, 2°C/min to 180°C, and then 10°C/min to 230°C, where it was held for 20 min.

Although the GC effluent splitter followed the general design of Struble and Arn (1984), some parts were replaced with those made from inactivated material. To achieve proper elution of the sample to the flame ionization detector (FID) and the EAD, nitrogen makeup gas (30 ml/min) was introduced into the stainless T-union, in which the end of the analytical capillary column (0.32 mm ID) was inserted into a deactivated fused-silica capillary column (0.53 mm ID, Agilent Technologies, Wilmington, DE, USA). The effluent was split between the FID and the EAD with a press-fit Y splitter (Agilent Technologies). Deactivated fused-silica capillary columns (0.53 mm ID, Agilent Technologies) were also used as transfer lines from the Y splitter to each detector. The GC effluent for EAD emptied into a glass transfer tube (15 mm ID) mounted on the GC and was mixed with humidified air (300 ml/min, 20°C) passing through the tube.

An excised antenna was placed on the antenna holder of an EAG probe (Syntech, Hilversum, the Netherlands). The antenna was connected with the recording and indifferent electrode with electrode gel (Spectra® 360, Parker Lab. Inc., Orange, NJ, USA). EAG and FID signals were fed into a computer through an analog-to-digital conversion board (IDAC-232, Syntech). Signal reception and analysis was performed with GC–EAD software (Syntech).

*Preparative Gas Chromatography.* An HP 5890 Series II gas chromatograph modified for GC–EAD as described above was converted to a preparative GC by exchanging a press-fit Y splitter with a press-fit column connector (Agilent Technologies). The GC–EAD active compounds were trapped in a

glass capillary (5.5 mm ID, 107 mm long) placed on the column outlet after removing the collector nut, an igniter castle, a collector, and collector insulators from the FID port of the GC. The glass capillary was eluted with *n*-hexane, and the eluate was stored at −5°C until analysis. The same column and temperature program as the GC–EAD system was used to trap the compounds of interest except that nitrogen makeup gas flow was adjusted to 15 ml/min.

*Chemical Analysis.* Samples collected by SPME were analyzed with an Agilent 6890N GC interfaced to an Agilent 5973 mass-selective detector run in electron impact ionization mode at 70 eV. The GC–MS was equipped with a DB-23 column (30 m × 0.25 mm ID, 0.25 μm film thickness, J & W Scientific), using a temperature program of 120°C/1 min, 5°C/min to 200°C, and held for 10 min. Helium was used as carrier gas (1 ml/min).

Double bond positions and geometries in unsaturated compounds were determined from dimethyldisulfide (DMDS) adducts of the insect-produced compounds (Buser et al., 1983). Fifty microliters of DMDS and 5 μl of iodine solution (60 mg $I_2$ in 1 ml diethyl ether) were added to 50 μl of *n*-hexane solution containing EAD-active compounds from female volatiles collected by preparative GC. The mixture was stored at 60°C for 24 hr, and then quenched with 200 μl of 5% $Na_2S_2O_3$ solution in distilled water, and the mixture was extracted with 100 μl *n*-hexane. EI-MS spectra of the derivatives were obtained with the same GC–MS system described above equipped with an HP-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness, J & W Scientific), using a temperature program of 100°C/5 min, 5°C/min to 280°C.

Geometry of the double bond in each monounsaturated compound was determined by comparing the GC retention time (Rt) of DMDS adducts of a compound eliciting an antennal response with those of synthetic standards using the same HP-5MS column described above.

*Chemicals.* Samples of *E*3-14:Ac (purity > 95%) and *Z*3-14:Ac (purity > 95%) were kindly provided by Prof. Chul-Sa Kim of Kochi University, Japan, and Dr. Huang Yongping of Shanghai Institute of Plant Physiology and Ecology, China, respectively.

For field trapping we synthesized Δ3-14:Ac from Δ3-tetradecen-1-ol (Δ3-14:OH). Thus, the Grignard reagent from 1-dodecyne (Tokyo Kasei Kogyo Co., Ltd., Tokyo) was reacted with ethylene oxide in dry tetrahydrofuran. The product (3-tetradecyn-1-ol) was reduced to Δ3-14:OH by hydrogenation over Lindlar's catalyst. The *E*/*Z* ratio of synthesized Δ3-14:OH was 2:98. To enrich the *E* isomer, isolated Δ3-14:OH was isomerized by stirring for 3 hr at 80–90°C after mixing with 2% (w/w) of nitric acid as a catalyst (Terauchi et al., 2002), which was then acetylated with acetic anhydride by standard techniques. Crude *E*3-14:Ac of 76% isomeric purity was purified by a urea adduct method (Fukumoto et al., 1999). Thus, the crude *E*3-14:Ac was poured into a saturated methyl alcohol solution of urea at 50–60°C and stirred for 5 min. To precipitate

the urea adduct, the solution was cooled to room temperature over 2 hr and further to 10°C by immersion in an ice-water bath. The free acetate was removed by extraction of the cooled methyl alcohol solution with *n*-hexane. After removal of the *n*-hexane layer, precipitated *E* isomer-rich urea adduct was dissolved by warming the methyl alcohol to 50–60°C. Two cycles of *Z* isomer-rich free acetate removal from the urea adduct solution gave *E*3-14:Ac of > 99.9% isomeric purity.

To obtain *Z*3-14:Ac, Δ3-14:OH synthesized as above was acetylated and purified by the urea adduct method (Fukumoto et al., 1999), resulting in *Z*3-14:Ac of > 99.9% isomeric purity.

*Field Trapping.* Delta traps with removable sticky bottoms (SE trap®, Sankei Chemical Co. Ltd., Tokyo, Japan) were baited with synthetic *E*3-14:Ac and *Z*3-14:Ac (1 mg in total) mixed in a series of different ratios (Table 1). The pheromone components were impregnated in gray halobutyl isoprene blend elastomer septa (West Co., Singapore). Three replicates of the trap treatments were placed in a randomized block design in a pear orchard in Tokushima, Japan, from June 7 to August 10, 2004. Traps were hung from tree branches at a height of about 2 m. Traps were inspected at 3- to 6-d intervals and shifted at each inspection to reduce positional effects. Sticky panels within the traps were replaced when they became saturated with captured moths.

*Statistics.* Data from field tests were analyzed using JMP (SAS Institute, Cary, NC, USA). The analyzed variable was the trap catch after $\sqrt{(X + 0.5)}$ transformation followed by ANOVA. Significantly different means were separated by Tukey–Kramer's HSD test.

TABLE 1. MEAN AND TOTAL CATCHES OF *C. insularis* IN TRAPS BAITED WITH MIXTURES OF (*E*)-3-TETRADECENYL ACETATE AND (*Z*)-3-TETRADECENYL ACETATE

| Blend tested % (*E*3: *Z*3) | Average catch/trap (mean ± SE) | Total number of moths captured |
|---|---|---|
| 100:0 | 173.0 ± 21.4a | 519 |
| 95:5 | 113.7 ± 28.3a | 335 |
| 80:20 | 124.3 ± 20.7a | 370 |
| 60:40 | 145.7 ± 23.1a | 429 |
| 20:80 | 74.3 ± 6.8b | 223 |
| 0:100 | 10.3 ± 4.5c | 31 |
| Control | 0 | 0 |

Means followed by the same letter are not significantly different ($P = 0.05$, Tukey–Kramer's HSD test). Control was omitted from ANOVA to avoid heterogeneity among variances (Levene test).

## RESULTS

Injections of SPME-collected volatiles released by an unmated female of *C. insularis* into the DB-23 column gave two consistent EAD responses with retention times of 13.72 (peak I) and 13.89 (peak II) min, respectively (Figure 1a). Although some EAD responses different from those to peak I and II were observed (Figure 1a), they were not consistent, suggesting that they were artifacts and, therefore, not subjected to further analysis.

Although it did not show a molecular ion, the EI-MS spectrum for GC peak I (Figure 2) showed *m*/*z* 61 ($CH_3COOH_2^+$), a diagnostic fragment ion for an acetate ester, and a characteristic fragment ion at *m*/*z* 194 ($M^+ - 60$). This suggested that the compound was a 14-carbon monounsaturated acetate.

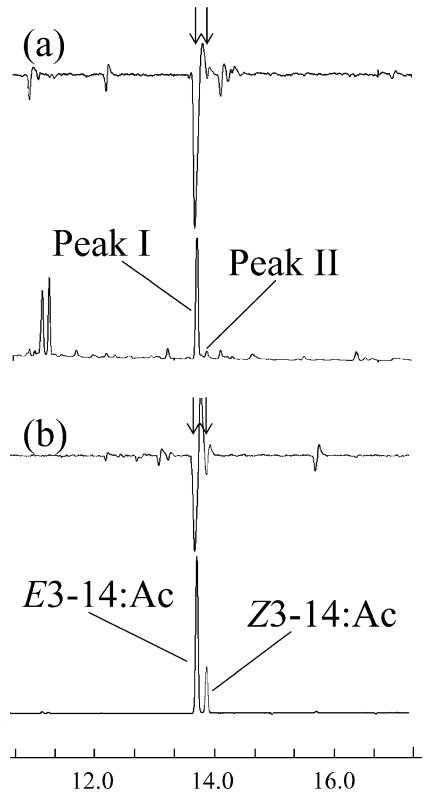


FIG. 1. Coupled GC–EAD responses from an antenna of a male *C. insularis* to female volatiles collected by SPME from an unmated female moth (a) and to synthetic *E*3-14:Ac and *Z*3-14:Ac (b).

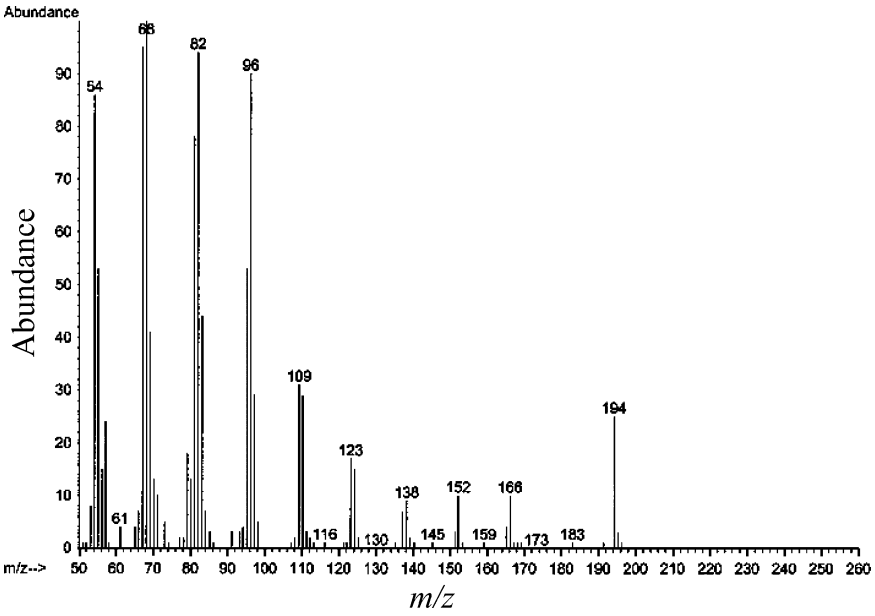


FIG. 2. Mass spectrum of peak I in Figure 1a.

The mass spectrum of the DMDS adduct of GC peak I showed diagnostic fragments at *m/z* 87 ($CH_3COOC_2H_4^+$, 31%), 147 ($CH_3COOC_2H_4CH{=}SCH_3^+$, 3%), 201 ($H_3CS^+{=}CH{-}C_{10}H_{21}$, 100%), and 348 ($M^+$, 11%), demonstrating that the peak I was a Δ3-14:Ac isomer.

Retention times of the DMDS adducts of GC peak I and peak II on the HP-5MS column were 34.39 and 34.29 min, respectively, which were the same as those of the DMDS adducts of the synthetic *E*3-14:Ac (34.38 min) and *Z*3-14:Ac (34.29 min). The GC retention times of the synthetic *E*3-14:Ac and *Z*3-14:Ac on a DB-23 column matched those of peak I and II compounds from female moths. Each of the synthetic compounds elicited as strong an EAG response as elicited by the natural compounds (Figure 1b).

The mixture ratio of the *E* and *Z* isomers of Δ3-14:Ac was estimated from the area of each peak obtained from the GC analysis of SPME-collected samples. The *E*:*Z* ratio was 95 ± 0.9% to 5 ± 0.9% ($N = 12$), indicating that individual variation in pheromone ratio among females was low. GC analysis of the samples collected by SPME from a 1:1 mixture of synthetic *E*3-14:Ac and *Z*3-14:Ac gave an *E*/*Z* ratio of 48.5 ± 2.0% *E* and 51.5 ± 2.4% *Z* isomer, which was not different from 1:1 ($P > 0.05$, Binomial test, $N = 10$). This demonstrated that the ratio of Δ3-14:Ac isomers collected by SPME reflected the actual ratio released by the female moths.

Traps baited with lures of *E*3-14:Ac and *Z*3-14:Ac mixed in different ratios captured 1907 male moths from June 7 to August 10. All the lures tested attracted *C. insularis* (Table 1). Numbers of males attracted to the 100:0 to 60:40 blends of *E*3-14:Ac and *Z*3-14:Ac were equivalent (Table 1), whereas the 20:80 and 0:100 blends of *E*3-14:Ac and *Z*3-14:Ac attracted fewer males. No moths were captured in control traps.

## DISCUSSION

Our data indicate that *E*3-14:Ac is the main and possibly only component of the sex pheromone of *C. insularis*. Although the *Z* isomer itself was weakly attractive (Table 1), it does not appear to be a critical component of the pheromone because 100% *E*3-14:Ac was as attractive as the 95:5 naturally produced ratio. Alcohols, aldehydes, or acetates of 14-carbon compounds are known sex pheromones and attractants for many lepidopteran species (Mayer and McLaughlin, 1991). However, 14-carbon compounds with the double bond in position 3 are rare. *E*3-14:Ac has been identified only from *Symmetrischema tangolias* (Gelechiidae) (Griepink et al., 1995) and *H. hippophaecolus* (Cossidae) (Fang et al., 2005) as a sex pheromone component, and also has been reported as a sex attractant for two gelechiid moths (Priesner, 1987; Tòth and Doolittle, 1992) and a cossid moth (Doolittle and Solomon, 1986). *Z*3-14:Ac has been identified only from two cossid moths, *H. insularis* (Zhang et al., 2001) and *H. hippophaecolus* (Fang et al., 2005), and has been reported as a sex attractant for several gelechiid species (Priesner, 1987; Willemse et al., 1987).

A cossid moth, *H. insularis* Staudinger, occurs in China where the larvae inflict serious damage to broad-leaved trees (Wang and Zhang, 1993). The genus *Holcocerus* was established as a subgenus of *Cossus* Fabricius, 1793 (Fletcher and Nye, 1982). However, Inoue (1987) considered it doubtful whether to separate *Holcocerus* Staudinger, 1884 from *Cossus* Fabricius, 1793 as a different genus. He did not conclude that *H. insularis* was a synonym of *C. insularis* because he had not checked the type species, *Cossus nobilis* Staudinger, 1884. Because the male genital structures of *C. insularis* from Japan and *H. insularis* from China are nearly identical (Yoshimatsu, personal communication), these moths are probably the same species, but this remains to be conclusively demonstrated. At the present time, therefore, we take the position that *C. insularis* and *H. insularis* are different species, meaning that the sex pheromone of *C. insularis* has been identified for the first time in the present study.

Furthermore, the sex pheromone identified from *H. insularis* was a 51:39:10 blend of *Z*3-14:Ac, (*E*)-3-tetradecen-1-ol, and (*Z*)-3-tetradecen-1-ol, but *E*3-

14:Ac, the main pheromone component of *C. insularis*, was not positively identified (Zhang et al., 2001). *E*3-14:Ac has been identified as a sex pheromone component from another cossid moth, *H. hippophaecolus*, but it attracted male moths only when it was combined with (*Z*)-7-tetradecenyl acetate in a 1:1 ratio (Fang et al., 2005). Thus, our study is the first to demonstrate *E*3-14:Ac as an attractive pheromone component by itself. The Δ3-14:Ac motif has appeared in several closely related species in *Cossus* and *Holcocerus*, but the role of the isomers differed between species, as described above. It will be intriguing to study sex pheromones in other *Cossus* or *Holcocerus* species to understand the phylogenic relationships among these genera.

Because the larvae of many forest insect species, especially wood borers, have long life cycles and low emergence rates even when reared on artificial diet (e.g., Ohya and Ogura, 1993), it is not easy to obtain sufficient numbers of adults for pheromone research. *C. insularis* takes about 6 months to complete its life cycle when reared on artificial diet, and the survival rate is not high (Chen, unpublished), resulting in a limited number of adult moths emerging. Thus, conventional extraction methods used with other lepidopteran species were not practical. In the present study, female sex pheromone components could be collected repeatedly from the same individual by SPME, demonstrating the value of SPME for collecting volatiles repeatedly from organisms that are available only in limited quantities.

Agelopoulos and Pickett (1998) mentioned that the ratio of compounds such as pentan-3-ol, 4-penten-1-ol, hexan-1-ol, 6-methyl-5-hepten-2-one, hexyl acetate, and limonene in samples collected by SPME can deviate markedly from samples collected by syringe or porous polymers, and that for estimation of proportions, SPME should be used in conjunction with another sampling method. However, the results presented here from the live females and the standard synthetic compounds show that SPME sampling can be used in the determination of the relative mixture ratios of geometric isomers because the vapor pressures of the isomers and their absorption to polydimethylsiloxane do not differ.

Three species of *Cossus* (*C. cossus orientalis*, *C. ezoensis*, and *C. insularis*) occur in Japan (Tadauchi and Inoue). The larvae of these species feed on broad-leaved trees including poplar and willows. However, the previous reports on the genus *Cossus* in Japan may have confused these three species because of a poor understanding of their life histories, distributions, and host plants (Enda, 1994). It is now possible to document the distribution, phenology, and abundance of *C. insularis* using the sex pheromone identified in the present study.

*Acknowledgments*—We thank Dr. Shin-ichi Yoshimatsu of the National Institute of Agro-Environmental Sciences, Tsukuba, Japan, for identification of *C. insularis* and Dr. Zhang Zhen of the Chinese Academy of Forestry, Beijing, China, for specimens of *H. insularis*. We also thank

Dr. Joseph Patt of USDA-ARS for his review of the manuscript and two anonymous reviewers for invaluable comments. One of the authors (X. C.) is grateful to the former Science and Technology Agency (current Ministry of Education, Culture, Sports, Science, and Technology) of Japan for financial support.

## REFERENCES

AGELOPOULOS, N. G. and PICKETT, J. A., 1998. Headspace analysis in chemical ecology: Effects of different sampling methods on ratios of volatile compounds present in headspace samples. *J. Chem. Ecol.* 24:1161–1172.

BUSER, H. R., ARN, H., GUERIN, P., and RAUSCHER, S. 1983. Determination of double bond position in mono-unsaturated acetates by mass spectrometry of dimethyldisulfide adducts. *Anal. Chem.* 55:818–822.

CAPIZZI, A., TONINI, C., ARSURA, E., GUGLIELMETTI, G., MASSARDO, P., and PICCARDI, P. 1983. Sex pheromone components of the European goat moth, *Cossus cossus*. *J. Chem. Ecol.* 9:191–200.

DOOLITTLE, R. E. and SOLOMON, J. D. 1986. Stereoselective synthesis of (*Z,E*)-3,5-tetradecadienyl acetate: Sex attractant for carpenterworm moth, *Prionoxystus robiniae* (Peck) (Lepidoptera: Cossidae) and effect of isomers and monounsaturated acetates on its attractiveness. *J. Chem. Ecol.* 12:619–633.

DOOLITTLE, R. E., TAGESTAD, A., and MCKNIGHT, M. E. 1976. Trapping carpenterworms and aspen carpenterworms with sex attractants in North Dakota. *Environ. Entomol.* 5:267–269.

ENDA, N. 1994. Cossid moths, pp. 244–245, *in* F. Kobayashi and A. Taketani (eds.). Forest Insects (Shinrin Konchu). Yokendo, Tokyo, Japan (in Japanese).

FANG, Y. L., SUN, J. H., ZHAO, C. H., and ZHANG, Z. N. 2005. Sex pheromone components of the sandthorn carpenterworm, *Holcocerus hippophaecolus*. *J. Chem. Ecol.* 31:39–48.

FLETCHER, D. S. and NYE, I. W. B. 1982. *in* I. W. B. Nye (ed.). The Generic Names of Moths of the World, Vol. 4. British Museum, London.

FUKOMOTO, T., HIROKAWA, K., and SUZUKI, H. 1999. Purification of higher unsaturated aliphatic ester, Japan Patent A11-049723. http://www.ipdl.ncipi.go.jp/homepg_e.ipdl.

GRIEPINK, F. C., VAN BEEK, T. A., VISSER, J. H., VOERMAN, S., and DE GROOT, A. 1995. Isolation and identification of sex pheromone of *Symmetrischema tangolais* (Gyen) (Lepidoptera: Gelechiidae). *J. Chem. Ecol.* 21:2003–2013.

INOUE, H. 1987. On some species-groups of Japanese moth. *Yugato* 108:37–46 (in Japanese).

KITAJIMA, H., MAKIHARA, H., and HASEGAWA, M. 1998. Morphology and injury forms of larvae of *Cossus* spp. (Lepidoptera: Cossidae). *Trans. Kanto. Jpn. For. Soc.* 49:65–68 (in Japanese).

LANDOLT, P. J., SOLOMON, J. D., and DOOLITTLE, R. E. 1985. Attraction of male *Prionoxystus piger* (Lepidoptera: Cossidae) to isomers of 3,5-tetradecadien-1-ol acetate. *Fla. Entomol.* 68:228–230.

MAYER, M. S. and MCLAUGHLIN, J. R. 1991. Handbook of Insect Pheromones and Sex Attractants. CRC Press, Boca Raton.

NAKANISHI, T. 2005. First report of occurrence of *Cossus insularis* (Staudinger) on the Japanese pear. *Jpn. J. Appl. Entomol. Zool.* 49:23–26 (in Japanese with an English summary).

OHYA, E. and OGURA, N. 1993. Rearing of the Cypress bark moth, *Epinotia granitalis* (Butler) (Lepidoptera: Tortricidae) on artificial diets. *J. Jpn. For. Soc.* 75:561–563.

PRIESNER, E. 1987. (*Z*)-3-Tetradecenyl acetate as sex attractant component in Gelechiinae and Anomologinae (Lepidoptera: Gelechiidae). *Z. Naturforsch.* 42c:1352–1355.

QI, Y., FU, W., XU, S., FANG, D., CHEN, S. L., WANG, X., LI, Z., LOU, S., and JAING, S. 1985. Studies on the synthetic attractants of *Cossus mongolicus* Ersch.—EAG responses and trapping effects. *Sci. Silvae Sinica* 21:383–388.

SOLOMON, J. D., DIX, M. E., and DOOLITTLE, R. E. 1978. Attractiveness of the synthetic carpenterworm sex attractant increased by isomeric mixtures and prolonged by preservatives. *Environ. Entomol.* 7:39–41.

STRUBLE, D. L. and ARN, H. 1984. Combined gas chromatography and electroantennogram recording of insect olfactory responses, pp. 161–178, *in* H. E. Hummel and T. A. Miller (eds.). Techniques in Pheromone Research. Springer-Verlag, New York.

TADAUCHI, O. and INOUE, H. 2000. On Dictionary of Japanese Insect Names (DJI) on INTERNET. Esakia. 40:85–86. http://konchudb.agr.agr.kyushu-u.ac.jp/dic/.

TERAUCHI, T., SAKURADA, T., FUKUMOTO, T., and SUZUKI, H. 2002. Isomerization of *cis*-alkenyl compound. Japan Patent B-3340517. http://www.ipdl.ncipi.go.jp/homepg_e.ipdl.

TONINI, C., CASSANI, G., MASSARDO, P., GUGLIELMETTI, G., and CASTELLARI, P. L. 1986. Study of female sex pheromone of leopard moth, *Zeuzera pyrina* L. Isolation and identification of three components. *J. Chem. Ecol.* 12:1545–1558.

TÒTH, M. and DOOLITTLE, R. E. 1992. Sex attractants for the white-barred groundling moth (*Recurvaria leucatella* Clerck) and the lesser bud moth (*R. nannela* Hübner) (Lepidoptera: Gelechiidae). *J. Chem. Ecol.* 18:1093–1105.

WANG, X. and ZHANG, Z. 1993. The research and control of woodboring insects in China. *J. Beijing For. Univ. (Engl. Ed.)* 2:53–60.

WILLEMSE, L. P. M., BOOIJ, C. J. H., and VOERMAN, S. 1987. New sex attractants for male Lepidoptera (Coleophoridae, Gelechiidae, Momphidae, Oecophoridae, and Yponomeutidae) found by field screening in the Netherlands. *J. Appl. Entomol.* 103:508–515.

ZHANG, J. T., HAN, Y., and MENG, X. 2001. Sex pheromone of the carpenterworm, *Holcocerus insularis* (Lepidoptera, Cossidae). *Z. Naturforsch.* 56c:423–429.