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Identification and Field Evaluation of the Sex Pheromone of *Synanthedon bicingulata* (Staudinger)

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Abstract The sex pheromone of *Synanthedon bicingulata* (Staudinger), a major pest of *Prunus* species in many regions of northeast Asia, was identified. Two major components from the pheromone gland extracts of female moths are (*E*,*Z*)-3,13-octadecadienyl acetate (*E*3,*Z*13-18:OAc) and (*Z*,*Z*)-3,13-octadecadienyl acetate (*Z*3,*Z*13-18:OAc), and the average ratio of these components is about 4:6, respectively. In addition to the major components, four minor components, (*Z*)-13-octadecenyl acetate (*Z*13-18:OAc), (*E*,*Z*)-2,13-octadecadienyl acetate (*E*2,*Z*13-18:OAc), (*E*,*Z*)-3,13-octadecadien-1-ol (*E*3,*Z*13-18:OH), and (*Z*,*Z*)-3,13-octadecadien-1-ol (*Z*3,*Z*13-18:OH) also were identified from pheromone gland extracts. Field tests showed that *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc are essential for attraction of male *S. bicingulata* moths, and males are optimally attracted to the blend ratio found in pheromone gland extracts of conspecific females. Addition of the minor glandular components (*Z*13-18:OAc, *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH) did not affect captures of males to the primary binary blend. Thus, the blend of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc at the natural ratio can be used for monitoring populations of this species.

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Introduction

Synanthedon bicingulata (Staudinger) (Lepidoptera: Sesiidae) is an economically important pest of *Prunus* species (Rosaceae), such as *P. persica*, *P. mume*, *P. salicina*, and *P. serrulatavar* in Korea, China, and the Russian Far East (Arita et al., 2004). Larvae feed on the cambial tissue within tree trunks of host plants, resulting in significant production losses. This insect can be difficult to control with conventional insecticides due to the sheltered larval development. Species-specific sex pheromone baited traps for *S. bicingulata* may be useful for monitoring populations and timing application of control measures. In addition, the pheromone is potentially useful for population control using mating disruption or mass trapping (e.g., Leskey et al., 2009). The sex pheromone of *S. bicingulata* has not been previously reported.

Although sex attractants for more than 110 sesiid moth species have been determined in field screening experiments, to date, sex pheromones of only 19 species have been identified (El-Sayed, 2010). Typically, sesiid moths use 3,13- and 2,13-octadecadien-1-ols and their corresponding acetates and aldehydes as sex pheromone components. In moths, species specificity of sex pheromone blends is responsible for pre-mating reproductive isolation between sympatric species (Löfstedt et al., 1991; Yang et al., 2009a). Therefore, it is important to examine the pheromone components produced by different species to understand the evolutionary diversification of pheromone signals in Lepidoptera (Roelofs and Brown, 1982). We report here the

chemical identification and field testing of the female-produced sex pheromone blend of *S. bicingulata*.

Methods and Materials

Insects Pupae of *S. bicingulata* were collected by excavating them from infested trunks of peach trees in May 2009 at Jeonju (35.4°N,127.0°E), Korea. They were placed individually into capped plastic cups (30 ml), and maintained at 25°C under a 14L:10D photoperiod. After emergence, the sexes were separated based on distinctive hair tufts. Moths were provided with a cotton pad soaked with a 10% sucrose solution as food.

Chemicals Synthetic pheromone standards used in this study were purchased from Pherobank (Wageningen, The Netherlands). Isomeric purity of these compounds exceeded 99%. Straight-chain hydrocarbons, dimethyldisulfide, iodine, and sodium thiosulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Pheromone Extraction Virgin females of *S. bicingulata* exhibited the characteristic calling posture by extruding the abdominal tip during photophase. Therefore, pheromone gland extracts were taken from 1- to 2-d-old females at 7 h into the photophase. Excised pheromone glands were placed in 10 μl hexane in a 0.3-ml conical glass vial (Wheaton, Millville, NJ, USA) for 10 min at room temperature. The supernatant was transferred to a clean vial and stored at −20°C until analysis.

Dimethyldisulfide Derivatizations Dimethyldisulfide (DMDS) derivatives of pheromone gland extracts and synthetic standards were prepared according to procedures by Buser et al. (1983). Approximately 50 μl DMDS and 5 μl iodine solution (60 mg of I_2 in 1 ml of diethyl ether) were added to an extract (10 μl) of 18 female equivalents of *S. bicingulata* pheromone, and held at 60°C for 48 h. After addition of 50 μl of sodium thiosulfate (5%), the organic layer was transferred to a clean vial and concentrated to ca. 10 μl for GC-MS analysis. The same procedures described above were followed for hexane solutions (10 ng in 10 μl n-hexane) of synthetic *Z*3-18:OAc, *E*3,*Z*13-18:OAc, *Z*3,*Z*13-18:OAc, *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH.

Chemical Analysis Pheromone gland extracts and synthetic standards were analyzed on an Agilent 6890N GC interfaced to an Agilent 5975C mass-selective detector. Samples were run on DB-Wax and DB-23 columns (30 m×0.25 mm ID, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The oven temperature was maintained at 80°C for 1 min, increased to 180°C at 10°C/min, then to 220°C at 5°C/min, and held at the final temperature for 10 min. Helium was the carrier gas (1 ml/min), and injections were splitless. Injector temperature was 250°C with the purge valve opening 0.75 min after a manual injection. The ionization voltage was 70 eV, and the ion source temperature was 230°C. Compounds from pheromone gland extracts were identified tentatively by comparison of their mass spectra with the mass spectra library (Wiley-NIST, Hoboken, NJ, USA); and identifications were subsequently confirmed by comparison of retention indices (RI; relative to alkane standards, Van den Dool and Kratz, 1963), and mass spectra with those of authentic standards on two different columns. DMDS derivatives were analyzed with a DB-1 column (30 m× 0.25 mm ID, 0.25 μm film thickness), using a temperature program of 100°C to 300°C at 10°C/min. The GC conditions were the same as those described above.

Field Experiments Experiments were conducted in June 2009 at Jeonju, Korea. Sticky delta traps (Green Agro Tech, Korea) baited with rubber septa (Aldrich Chemical Co., Milwaukee, WI, USA) impregnated with candidate pheromone components in hexane were hung in peach orchards at a height of 1.5 m. Field tests employed a complete randomized block design with four replicate blocks of each treatment. The distance between traps within a block was about 10 m. Captured moths were counted at intervals of 3–4 d.

Experiment 1 compared attraction of male *S. bicingulata* to *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc alone or in various combinations. Experiment 2 tested whether attractiveness of standard baits of two major components could be enhanced by addition of four minor components identified in female gland extracts; *Z*13-18:OAc, *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH. Trap catch data (x) were transformed to log (x+1) and submitted to one-way analysis of variance (ANOVA). Treatments that failed to capture males were not included in the analyses to avoid violating assumptions of ANOVA. Means were compared by Tukey's test at α=0.05 (SAS Institute Inc. 2008).

Results

Chemical Analysis Analysis of pheromone gland extracts of female *S. bicingulata* by GC-MS revealed the presence of two major components and four minor components, as well as normal alkanes including pentacosane and heptacosane (Fig. 1). The mass spectrum of compound 1 showed a molecular ion *m/z* 310, and diagnostic fragment ions *m/z* 250 (M-60), 222, and 61 (indicative of an acetate functionality), which suggested an octadecenyl acetate. The DMDS adduct of compound 1 showed a molecular ion *m/z* 404 and diagnostic ions *m/z* 287, 227, and 117,

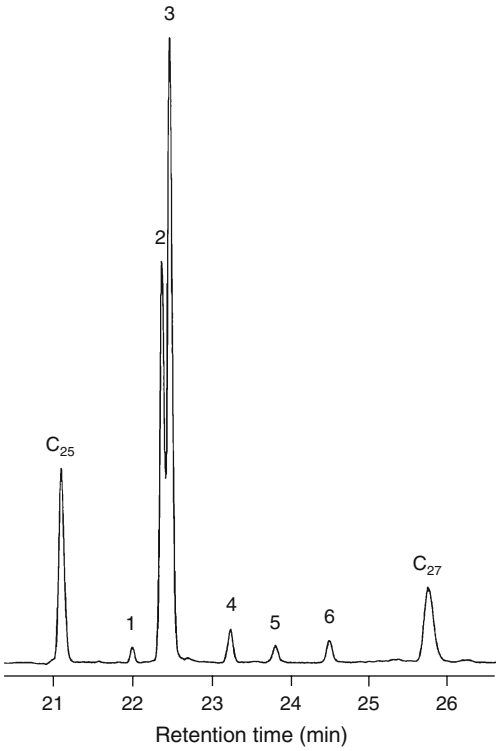

Fig. 1 Total ion chromatogram of GC-MS analysis of pheromone gland extract from female *Synanthedon bicingulata* on DB-Wax column. 1 (*Z*)-13-octadecenyl acetate, 2 (*E*,*Z*)-3,13-octadecadienyl acetate, 3 (*Z*,*Z*)-3,13-octadecadienyl acetate, 4 (*E*,*Z*)-2,13-octadecadienyl acetate, 5 (*E*,*Z*)-3,13-octadecadien-1-ol, 6 (*Z*,*Z*)-3,13-octadecadien-1-ol, C_{25} pentacosane, C_{27} heptacosane

indicating the original double bond at position 13 of the monounsaturated acetate (Buser et al., 1983). The calculated retention indices (RIs) of compound 1 coincided with *Z*13-18:OAc standard on two columns (KIs of 2548 on the DB-Wax column, and 2625 on the DB-23 column). The mass spectra of compounds 2 and 3 were similar and had diagnostic ions at *m/z* 308 (M^+), 248 (M-60), 219, 205, 191, and 61 suggestive of octadecadienyl acetates. The mass spectrum of the DMDS adduct of compounds in the pheromone extract showed a molecular ion *m/z* 496 (308+ 2MeSSMe) and diagnostic ions *m/z* 301, 255, 147, and 117, indicating the original double bonds at the 3- and 13-positions of octadecadienyl acetate (Vincenti et al. 1987). However, the geometry of the dienes was not confirmed by retention time comparisons because DMDS adducts of the isomers were poorly resolved on the DB-1 column. Comparison of RIs of the natural compounds with the 3,13 isomers of octadecadienyl acetates on the DB-Wax and DB-23 columns confirmed that compounds 2 and 3 were *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc, respectively (Table 1).

The mass spectrum of compound 4 showed diagnostic fragment ions of octadecadienyl acetate at *m/z* 248 (M-60), 219, and 61. Compounds 5 and 6 had a molecular ion *m/z* 266, and diagnostic fragment ions *m/z* 248 (M-18), 219, 205, and 191 suggestive of an octadecadien-1-ol. Whereas it was not possible to verify the structure of compounds 4, 5, and 6 using DMDS derivatization because of coelution with the large amount of unidentified products and/or the small amount of material in the derivatized gland extract, we were able to tentatively identify compounds 4, 5, and 6 as *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH, respectively, by comparison of their RIs with those of synthetic standards on two columns. The relative ratio of compounds 1–6 in gland extracts was 2:58:100:6:3:4 (N=10).

Field Experiments In field experiment 1, a total of 181 male *S. bicingulata* were caught, with no catch in controls or traps baited with single components. The maximum number of males was attracted to a 4:6 mixture of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc, although it was not significantly better than the 3:7 and 2:8 blends (Fig. 2). A total of 267 males were captured in field experiment 2. Trap catches of males to the binary blend of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc were unaffected by the addition of the minor glandular components, *Z*13-18:OAc, *E*2,*Z*13-18:

Table 1 Retention indices of octadecadienyl compounds (compounds 2, 3, 4, 5 and 6) present in gland extracts of female *Synanthedon bicingulata* and synthetic 3,13- and 2,13-octadecadienyl compounds on DB-Wax and DB-23 columns

Compound	DB-Wax	DB-23
Female extract		
Compound 2	2569	2626
Compound 3	2574	2639
Compound 4	2610	2660
Compound 5	2633	2648
Compound 6	2660	2683
Authentic standard		
(*E*,*E*)-3,13-octadecadienyl acetate	2563	2606
(*E*,*Z*)-3,13-octadecadienyl acetate	2569	2626
(*Z*,*E*)-3,13-octadecadienyl acetate	2567	2618
(*Z*,*Z*)-3,13-octadecadienyl acetate	2574	2639
(*E*,*E*)-2,13-octadecadienyl acetate	2603	2636
(*E*,*Z*)-2,13-octadecadienyl acetate	2610	2660
(*Z*,*E*)-2,13-octadecadienyl acetate	2574	2618
(*Z*,*Z*)-2,13-octadecadienyl acetate	2581	2639
(*E*,*E*)-3,13-octadecadien-1-ol	2626	2627
(*E*,*Z*)-3,13-octadecadien-1-ol	2633	2648
(*Z*,*E*)-3,13-octadecadien-1-ol	2652	2661
(*Z*,*Z*)-3,13-octadecadien-1-ol	2660	2683
(*E*,*E*)-2,13-octadecadien-1-ol	2684	2674
(*E*,*Z*)-2,13-octadecadien-1-ol	2693	2698
(*Z*,*E*)-2,13-octadecadien-1-ol	2684	2693
(*Z*,*Z*)-2,13-octadecadien-1-ol	2693	2716

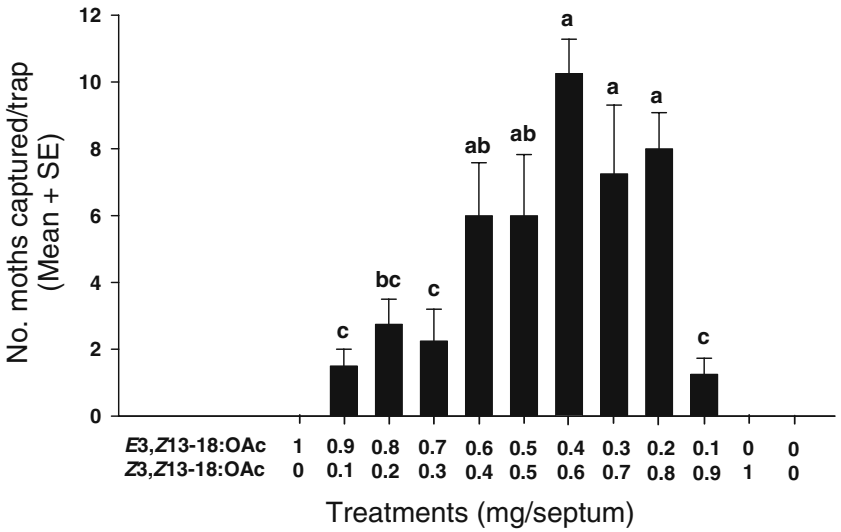

Fig. 2 Number of *Synanthedon bicingulata* males captured in traps baited with lures containing different ratios of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc at peach orchards in Jeonju, Korea, 10–18 June 2009 (*N*=4). Bars with the same letter are not significantly different (Tukey's test: $P>0.05$)

OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH (Fig. 3). Moreover, the full six-component blend mimicking the blend found in a female gland extract did not increase trap catches compared with traps baited with the primary binary blend.

Discussion

Chemical analyses and field trials indicate that the sex pheromone of *S. bicingulata* consists of two major synergistic components, *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc, at a ratio of 4:6, respectively. In our previous study on the pheromone of the congeneric *S. haitangvora*, GC-MS analyses of the DMDS-derivatized extract conducted on a polar DB-Wax column did not show the presence of DMDS adducts (*m/z* 496) of octadecadienyl acetate derivatives (Yang et al., 2009b). The failure of the previous attempt probably was because the tetrasubstituted linear high-boiling derivatives are not eluted on the polar column (Vincenti et al., 1987). In this study, we found that DMDS adducts of octadecadienyl acetates eluted on the nonpolar DB-1 column. Thus, DMDS adducts can be useful in pheromone identification of sesiid species.

*E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc have been identified as constituents of a sex pheromone in several other sesiid species (El-Sayed, 2010). However, *Synanthedon hector* is the only previously reported sesiid species that uses a mixture of these two dienes as its sex pheromone. *Synanthedon hector* is distributed only in Japan, and is an important economic pest of cultivated *Prunus* spp. This species can be distinguished easily from *S. bicingulata* based upon the color patterns of the adult abdominal

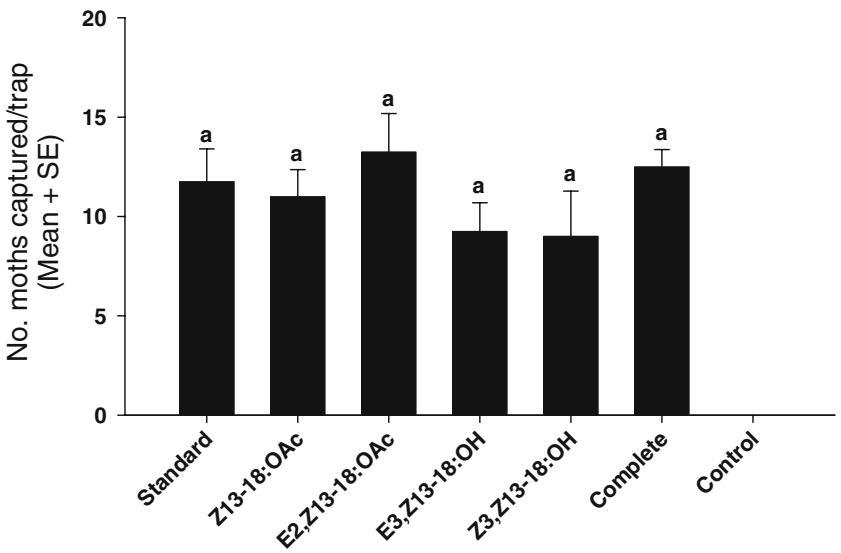

Fig. 3 The effect of adding 3% of different minor components to standard baits with 1 mg/septum of a 4:6 blend of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc on captures of male *Synanthedon bicingulata* at peach orchards in Jeonju, Korea, 19–28 June 2009 (*N*=4). The complete blend consisted of all six components in a ratio found in the pheromone gland extract. Bars with the same letter are not significantly different (Tukey's test: $P>0.05$)

sternites (Arita et al., 2004). The pheromone glands of female *S. hector* contain *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc at 38:62 ratio (Naka et al., 2008), and conspecific males are optimally attracted to 5:5 blend (Yaginuma et al., 1976; Naka et al., 2008). These data show that allopatric *S. bicingulata* and *S. hector* utilize the same sex pheromone and host plants.

*Z*13-18:OAc, *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OH, and *Z*3,*Z*13-18:OH were identified as minor components of female *S. bicingulata* gland extracts, but addition of these components to the two major components had no significant effect on attraction of conspecific males. These minor components may serve an antagonistic function to suppress attraction of sympatric species. However, it is not known whether they are actually released from the female glands during calling. Previous studies with other sesiid species have shown that geometrical isomers or biosynthetic precursors of the major components are present in female pheromone glands, but the biological significance of these components remain unclear (Klun et al., 1990; Francke et al., 2004; Mozûraitis et al., 2006; Yang et al., 2009b). Hence, *Z*13-18:OAc and *E*2,*Z*13-18:OAc may just be by-products of the pheromone biosynthetic pathway, and *E*3,*Z*13-18:OH and *Z*3,*Z*13-18:OH are presumed to be biosynthetic precursors of the two major components, *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc, respectively.

During the course of this study, we found that no other sesiid species were attracted to a 4:6 mixture of *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc. In Korea, the genus *Synanthedon* is composed of 12 species (Arita et al. 2004), but sex pheromones or attractants have been reported for only three species, *S. haitangvora, S. tenuis* and *S. quercus* (Tamaki et al. 1977; Yang et al. 2009b). As shown in Fig. 2, *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc were essential for attraction of male *S. bicingulata*. This result suggests that *S. bicingulata* males may not be attracted to *S. haitangvora* females that emit *Z*3,*Z*13-18:OAc and *E*2,*Z*13-18:OAc as its pheromone (Yang et al., 2009b). In field screening trials for *S. tenuis*, Tamaki et al. (1977) found an antagonistic effect of *E*3,*Z*13-18:OAc on male attraction when it was mixed with the major component, *Z*3,*Z*13-18:OAc, in a very low ratio. Therefore, *E*3,*Z*13-18:OAc may prevent cross-attraction between *S. bicingulata* and *S. tenuis* where they occur sympatrically. They also confirmed that *E*3,*Z*13-18:OAc and *Z*3,*Z*13-18:OAc attracted few *S. quercus* males when tested separately or in binary mixture. We, therefore, suggest that specific chemical signals contribute strongly to the maintenance of premating reproductive isolation between sympatric populations of *Synanthedon* species.

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References

ARITA, Y., BAE, Y. S., LEE, C. M., and IKEDA, M. 2004. Sesiidae (Lepidoptera) of Korea. *Trans. Lepid. Soc. Japan* 55:1–12.

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

EL-SAYED, A. M. 2010. Internet database: http://www.pherobase.com/.

FRANCKE, W., KARALIUS, V., PLASS, E., LEHMANN, L., DOS SANTOS, A., BŮDA, V., BORG-KARLSON, A. K., and MOZŮRAITIS, R. 2004. New type of Sesiidae sex pheromone identified from the hornet moth *Sesia apiformis*. *J. Chem. Ecol.* 30:805–817.

KLUN, J. A., SCHWARZ, M., LEONHARDT, B. A., and CANTELO, W. W. 1990. Sex pheromone of the female squash vine borer (Lepidoptera: Sesiidae). *J. Entomol. Sci.* 25:64–72.

LESKEY, T. C., BERGH, J. C., WALGENBACH, J. F., and ZHANG, A. 2009. Evaluation of pheromone-based management strategies for dogwood borer (Lepidoptera: Sesiidae) in commercial apple orchards. *J. Econ. Entomol*. 102:1085–1093.

LÖFSTEDT, C., HERREBOUT, W. M., and MENKEN, S. B. J. 1991. Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology* 2:20–28.

MOZŮRAITIS, R., KARALIUS, V., BUDA, V., and BORG-KARLSON, A. K. 2006. Inter- and intraspecific activities of compounds derived from sex pheromone glands of currant borer, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae). *Z. Naturforsch.* 61c:278–284.

NAKA, H., HORIE, Y., MOCHIZUKI, F., VANG, L. V., YAMAMOTO, M., SAITO, T., WATARAI, T., TSUCHIDA, K., ARITA, Y., and ANDO, T. 2008. Identification of the sex pheromone secreted by *Synanthedon hector* (Lepidoptera: Sesiidae). *Appl. Entomol. Zool*. 43:467–474.

ROELOFS, W. L. and BROWN, R. L. 1982. Pheromones and evolutionary relationships of Tortricidae. *Annu. Rev. Ecol. Evol. Syst.* 13:395–422.

SAS Institute. 2008. Statview. SAS Institute Inc., Cary, NC, USA.

TAMAKI, Y., YUSHIMA, T., ODA, M., KIDA, K., KITAMURA, K., YABUKI, S., and TUMLINSON, H. H. 1977. Attractiveness of 3,13-octadecadienyl acetates for males of clearwing moths. *Jap. J. Appl. Entomol. Zool*. 21:106–107.

VAN DEN DOOL, H. 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.

VINCENTI, M., GUGLIELMETTI, G., CASSANI, G., and TONINNI, C. 1987. Determination of double-bond position in diunsaturated compounds by mass-spectrometry of dimethyl disulfide derivatives. *Anal. Chem.* 59:694–699.

YAGINUMA, K., KUMAKURA, M., TAMAKI, Y., YUSHIMA, T., and TUMLINSON, J. H. 1976. Sex attractant for the cherry tree borer, *Synanthedon hector* Butler (Lepidoptera: Sesiidae). *Appl. Entomol. Zool.* 11: 266–268.

YANG, C. Y., HAN, K. S., and BOO, K. S. 2009a. Sex pheromones and reproductive isolation of three species in genus *Adoxophyes*. *J. Chem. Ecol.* 35:342–348.

YANG, C. Y., KIM, J., KANG, T. J., and JEON, H. Y. 2009b. Identification and field bioassays of the sex pheromone of *Synanthedon haitangvora*. *J. Chem. Ecol.* 35:1197–1201.