Reinvestigation of sex pheromone components and attractiveness of synthetic sex pheromone of the pink borer, *Sesamia inferens* Walker (Lepidoptera: Noctuidae) in Okinawa

Atsushi Nagayama,1,* Sadao Wakamura,2 Naoki Tanai1 and Norio Arakaki1

1 Okinawa Prefectural Agricultural Research Center; Itoman 901–0336, Japan
2 Laboratory of Insect Behavior, National Institute of Agrobiological Sciences; Tsukuba 305–8634, Japan

(Received 5 January 2006; Accepted 13 March 2006)

Abstract

Two EAG-active compounds were found in the solvent extract of abdominal tips of virgin females of the pink borer *Sesamia inferens*. They were identified as (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-11-hexadecen-1-ol (Z11-16:OH) at ca. 60 ng and ca. 20 ng per female, respectively, by means of GC-MS analysis and chemical derivatization. In field tests, a 75:25 blend of these compounds showed maximum attractions at 1.0 mg when loaded on rubber septa. The optimal dose appears to be between 1.0 and 2.0 mg septum. Addition of a possible minor component, (Z)-11-hexadecenal, showed neither synergistic nor inhibitory effects. The trap catches with the 75:25 blend of Z11-16:Ac and Z11-16:OH at 1.0 mg septum were greater than those with virgin females, but the difference was not significant. This indicates that the attractiveness of this blend is comparable with that of virgin females.

Key words: Synthetic sex pheromone; *Sesamia inferens*; (Z)-11-hexadecenyl acetate; (Z)-11-hexadecen-1-ol; field attraction

INTRODUCTION

The pink borer, *Sesamia inferens* (Walker), is one of the serious pests of *Gramineae* crops in many Asian countries (Azuma and Oshiro, 1969; Mia and Iwahashi, 1999). In Okinawa and Kagoshima, Japan, this species has been known as a borer of the sugarcane shoot and stalk. Larvae often kill the shoots. The first noticeable sign of damage is the appearance of ‘deadhearts’ that follows damage to the base of the spindle leaves. Larvae also attack in the internodes of the stalk, and tunnel through the nodes. Damaged stalks are weakened and more easily broken by strong winds. Moreover, borer tunnels expose the plants to infection by the red lot which drastically decreases plants’ sucrose contents. Since the larvae live inside of the plants, it is difficult to control this borer with contact insecticides. Application of a synthetic sex pheromone has potential for monitoring and control.

A sex pheromone compound of *S. inferens* was first identified as (Z)-11-hexadecenyl acetate (Z11-16:Ac) by Nesbitt et al. (1976), however, this lure was not effective in field trapping in China (Zhu et al., 1987). Takahashi (1983) reported that males of *S. inferens* were captured in traps baited with 9:1 or 4:1 mixtures of Z11-16:Ac and (Z)-11-hexadecen-1-ol (Z11-16:OH) in a lure for the rice armyworm, *Mythimna separata* (Walker). Wu and Cui (1986) identified second sex pheromone compound as Z11-16:OH and found that the lures containing Z11-16:Ac and Z11-16:OH at the ratios of 9:1, 8:1 and 5:5 were more attractive than Z11-16:Ac alone. Furthermore, Zhu et al. (1987) reported that in China Z11-16:Ac, Z11-16:OH and (Z)-11-hexadecenal (Z11-16:Ald) were pheromone components and the optimal ratio was 4:1:0.1.

Variation in the pheromone composition of several lepidopteran species from different geographic populations has been well documented (Cardé and Baker, 1984). Sex pheromone components of *S. inferens* were identified for the Chinese population (Wu and Cui, 1986; Zhu et al., 1987). For control-
ling this insect pest with synthetic sex pheromone in Okinawa, it is important to confirm whether the components of the Japanese population are identical to those of the Chinese population, since they are separated by 650 km of ocean. This paper deals with the reinvestigation of the sex pheromone components of *S. inferens* in Okinawa, and the subsequent optimization of synthetic sex pheromone formulation.

**MATERIALS AND METHODS**

**Insect.** *S. inferens* females used for extraction of pheromone glands were from larvae collected in sugarcane fields on Ihei Island, Okinawa, Japan in March 2002, and reared on sugarcane stems in the laboratory at 25°C and 14L10D photoregime. Pupae were sexed and placed in separate containers under the same conditions (scotophase: 10:00 to 20:00) until emergence. Females used for field tests originated from larvae collected at Ihei Island in November 2004.

**Extraction.** Solvent extracts of sex pheromone glands were obtained from 2 to 4-d-old females after 5 h after lights off, since females were most frequently observed to take a calling posture at this time (Nagayama et al., 2004). The tip containing the pheromone gland was excised from the female abdominal tip with fine tweezers and soaked in hexane (10 tips/0.5 ml) for ca. 15 min. The tips were filtered off through a small wad of absorbent cotton to obtain an extract. They were rinsed twice with the same volume of hexane and the rinses were added to the extract. The extracts from 54 females were accumulated and stored below -20°C until use.

**Florisil chromatography.** Female gland extracts from 54 females were poured onto 0.2 g of Florisil (100–200 mesh, Floridin Co., U.S.A.) in a Pasteur pipette. One milliliter each of hexane, 0.2%, 0.5%, 1%, 2%, 5%, 15%, 50% ether in hexane, and ether were used as the eluting solvents.

**Gas chromatography and electroantennographic detection (GC-EAD).** GC-EAD analyses were conducted with a Hewlett-Packard (HP) 5890II gas chromatograph (GC) equipped with a flame ionization detector (FID, operation temperature: 270°C) and EAD. An HP-1 fused silica column (15 m×0.25 mm (ID)×0.25 μm film thickness) was used at a column head pressure of 55 kPa. Injection was made directly into the capillary column through an on-column injector held at 53°C, and the temperature was controlled at oven temperature plus 3°C after injection. The temperature program for the column oven was 50°C for 1 min, 50°C to 150°C at 25°C/min, 150°C to 240°C at 5°C/min and then held at the final temperature for 5 min. An electroantennographic detector was used as in Struble and Arn (1984).

For complete separation of geometric isomers, a DB-23 column (30 m×0.25 mm (ID)×0.25 μm film thickness, J & W Scientific, Folsom, CA, U.S.A) was used under the same conditions as above but column head pressure was 110 kPa. For calculation of retention indices (*k*; Kováts, 1965), a hexane solution of dodecane, tetradecane, hexadecane, and octadecane was added to injection materials as internal standard.

**Coupled gas chromatography-mass spectrometry (GC-MS),** GC-MS analyses were conducted with a JEOL JMS SX-102A mass spectrometer (EI mode, 70 eV) connected with an HP6890 GC. GC was equipped with the HP-1 column and operated in the same conditions as above but column head pressure was 35 kPa.

**Dimethyl disulfide (DMDS) adducts.** Twenty microliters of DMDS and a very small crystal of iodine were added to ca. 5 μl of hexane solution of test material in a 100-μl capillary tube with one end sealed, as in Buser et al. (1983). The other end of the capillary was then sealed and kept at 55°C for 2 h. After pouring the contents into 100 μl of 5% Na₂S₂O₃ solution in distilled water, the products were extracted three times with 100 μl of hexane. The extracts were combined and then concentrated to ca. 5 μl and 0.5 μl was injected into the GC-MS.

**Chemicals.** Z11-16:Ac and (E)-11-hexadecenyl acetates (E11-16:Ac) were originally provided by Shin-Etsu Chem. Co., Ltd. and used after purification by column chromatography on silica gel impregnated with 16.7% silver nitrate. The corresponding alcohols (Z11-16:OH and E11-16:OH) were obtained by hydrolysis of the acetates. The purity of all the compounds was more than 99.5% in respect to positional and geometric isomerism.

**Field tests.** A sticky trap (white type, SE Trap, Sankei Chem. Co., Kagoshima) was used for field attraction. Synthetic sex pheromones were impregnated into rubber septa (8 mm OD, 19 mm ht.,
Gray, West Co., Singapore) by applying 0.3 ml of hexane solution in the depression. Each septum was placed in a draft chamber for ca. 12 h at room temperature to allow the solvents to evaporate. The septa were stored at below -20°C until use. Each septum was hung with wire about 3 cm above the stick plate in a trap. Traps were set 1.0-1.2 m above the ground along the edge of sugarcane fields at Miyagi Island and at Shuri, Naha. Trap catches were checked, and trap locations were changed every five or seven days. For a control, traps were used with a rubber septa treated with equal amounts of hexane. All tests were replicated three times.

In certain experiments conducted at Itoman, traps baited with virgin females were added for comparison of attractiveness. A net cage (6 cm dia. x 6 cm high) containing 1- to 2-old females and a small piece of cotton wick impregnated with water was placed in a sticky trap. Trap catches and virgin females were checked every day. Trap catches were pooled for 3 d per trap.

Trap data (X) were subjected to square-root transformation, \((X + 0.5)^{1/2}\), before analysis and submitted to two-way layout ANOVA, where zero data (mean = 0) were omitted. The means were ranked by Tukey’s methods when ANOVA was significant at the 5% level. In the figures, the mean accompanied by the same letter are not significantly different, and the same letter was given to the zero data when the confidence range of the mean contained zero \([-0.5, 0.5]\). Before applying certain dose-response data to regression analysis, the doses (mg/septum) were transformed to logarithms.

RESULTS

Identification of EAG-active compounds in extracts of female pheromone glands

When 0.5 female equivalent (FE) of the crude extract was injected into the GC-EAD loaded with a male antenna, two distinct EAG signals were observed at \(t_R = 19.09\) min (\(K_I = 1842\); compound B) and \(t_R = 21.74\) min (\(K_I = 1974\); compound A). The amounts of compounds A and B were estimated to be ca. 60 ng/FE and ca. 20 ng/FE, respectively, by comparison with the FID peak sizes of known amounts of the hydrocarbon standards.

Compound A was eluted in the 1% and 2% ether-in-hexane fractions and compound B was eluted in the 15% fraction. GC-MS analyses of the 1% and 2% fractions showed a single FID peak at \(t_R = 12.34\) min (\(K_I = 1974\)) and the mass spectrum indicated hexadecenyl acetate \([m/z 282, (M^+, \text{relative intensity: 0.5})], 222 (M-60, 46%), 82 (base, 100%), 61 ([\text{CH}_3\text{C(OH)}_2]^+, 10.4\%), \text{etc.} \). DMDS adduct of compound A showed a molecular ion at \(m/z 376\) (23%) and diagnostic fragment ions were observed at \(m/z 259\) \([([\text{CH}_3\text{SCH(CH}_2)_6\text{OCOCH}_3]^+, \text{base})\) and \(m/z 117\) \([([\text{CH}_3\text{C(CH}_2)_4\text{SCHCH}_3]^+, 47\%)\]. This indicated that the double bond locates at the 11-position. In order to determine geometric isomerism, compound A was injected the GC equipped with the polar DB-23 column. Retention value for compound A \((t_R = 14.59\) min, \(K_I = 2407\)) was identical to that for Z11-16:Ac but different from that for the \((E)-\)isomer \((t_R = 14.34\) min, \(K_I = 2387\)). Thus, compound A was confirmed to be Z11-16:Ac. In these GC analysis, the geometric isomer was not detected (<10 pg/FE).

Compound B in the 15% fraction showed a mass spectrum that was similar to those of hexane-1-ol: \([m/z 240, (M^+, \text{not observed})], 222 (M-60, 22\%), 55 \text{ (base, etc.)}\]. Compound B appeared to have a double bond at the same position as compound A by homology of pheromone components (see Arnt et al., 1992, 1997). Compound B was then comparatively analyzed with \((Z)-\) and \((E)-\)hexadecan-1-ol by using GC equipped with the DB-23 column. GC retention value for compound B \(t_R = 14.93\) min (\(K_I = 2434\), which was identical to that for Z11-16:OH but different from that for the \((E)-\)isomer \((t_R = 14.68\) min, \(K_I = 2414\)). Thus compound B was determined to be Z11-16:OH. The geometric isomer was not detected (<10 pg/FE) in the 15% fraction.

Zhu et al. (1987) had found Z11-16:Ald, tetrade- can-1-ol (14:OH) and hexadecan-1-ol (16:OH) in the extract of female pheromone glands. Therefore, all the fractions were carefully analyzed by GC and GC-MS but none of these compounds was detected (<10 pg/FE).

Field attraction

When Z11-16:Ac and Z11-16:OH were blended in different ratios between 100 and 0 at 1.0 mg/septum, maximum catch was observed at a 75:25 ratio (Fig. 1A). To examine whether maximum attraction might occur between 100:0 and 75:25, a follow-up test was conducted. Maximum catch was
observed again at the 75:25 ratio (Fig. 1B).

Male catch with the 75:25 blend significantly increased in correspondence to the amounts between 0.1 and 2.0 mg/ septum ($r^2=0.64$, $p<0.001$; Fig. 2A), but significant regression was not observed between 0.2 mg/ septum and 5.0 mg/ septum ($r^2=0.15$, $p=0.16$; Fig. 2B). These finding indicated that the optimal dose should be between 1.0 and 2.0 mg/ septum.

In order to examine the effect of a possible minor component, Z11-16:Ald was added at the dose range of 0.1 to 5% of total amount (1.0 mg). No significant regressions were observed in the repeated field tests ($r^2=0.003$, $p=0.80$; Fig. 3A, $r^2=0.01$, $p=0.17$; Fig. 3B). This indicated that addition of Z11-16:Ald has neither synergistic nor inhibitory effects.

The trap catches with the blend of Z11-16:Ac and Z11-16:OH with 75:25 ratio (1.0 mg/ septum) was greater than that of virgin females, but the difference was not significant ($p=0.20$; Fig. 4). This indicates that the attractiveness of this blend was comparable to that comprised of virgin females.

**DISCUSSION**

Two EAG active compounds Z11-16:Ac and Z11-16:OH were identified in an Okinawan popu-
This difference of minor component between two populations may be geographic variations in the sex pheromone components of this species between Chinese and Okinawan populations. In several noctuid species, differences in the pheromone blends of different geographic populations have been well documented (Cardé and Baker, 1984). Zhu et al. (1987) also found 14:OH and 16:OH in China. The difference in saturated alcohols may be due to different duration of extraction: 15 min in our study vs. 8 h (1 h plus 7 h) in Zhu et al. (1987). In our procedure, only surface materials were considered to be extracted from pheromone glands. In the Zhu et al. (1987) procedure, materials in the tissues could also be extracted from tissue by long soaking times. Saturated alcohols are probably precursors of sex pheromone components.

The trap catches with the 75:25 blend of Z11-16:Ac and Z11-16:OH were comparable with that of virgin females. Sex pheromone components of the Okinawan population were therefore confirmed to be Z11-16:Ac and Z11-16:OH at the ratio of 75:25. Synthetic pheromone would be useful for population monitoring and mating disruption of this pest.

ACKNOWLEDGEMENTS

We thank Mieko Kinjo of our Experiment Station for her valuable suggestions on rearing methods of the insects. We also thank Ryou Akamine, Masayo Kinjo, Yuichi Kawatake, Chio Hayashi, Naruhito Higa, Hidekazu Tamashiro, Yasunobu Toyozato and Kazue Miyahira of our laboratory for their assistance with rearing of the insect. Thanks are also due to S.
REFERENCES


