Identification of Sex Pheromone of the Semi-Looper, *Autographa nigrisigna* WALKER (Lepidoptera: Noctuidae)

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Sex pheromone of the semi-looper, *Autographa nigrisigna*, was extracted from abdominal tips of virgin females. The crude extract was purified by Florisil column chromatography. The pheromonal activity of samples was evaluated in a flight tunnel. (Z)-5-, (Z)-7-, (E)-7-, 11-Dodecenyl acetates, (Z)-7-dodecenol and (Z)-7-tetradecenyl acetate were identified in the virgin female extract through analysis by capillary GC and GC-MS. A rubber septum loaded with 1.68 mg of a mixture of the (Z)-5-, (Z)-7-dodecenyl acetates, (Z)-7-dodecenol and (Z)-7-tetradecenyl acetate in a ratio of 100:2:62:4 attracted male moths under field conditions. These 4 compounds were therefore determined to be the sex pheromone components of *A. nigrisigna*.

Key words: identification, sex pheromone, *Autographa nigrisigna*

INTRODUCTION

The semi-looper, *Autographa nigrisigna* (WALKER), is distributed in Japan and known as one of the major pests of cabbage, chinese cabbage, and other cruciferous vegetables. Knowledge of the distribution and population dynamics of this pest is important for its control. The sex pheromone trap may be useful to monitor population trends because adult *A. nigrisigna* is taxonomically similar to other Plusiinae moths. Therefore we began to isolate and identify the sex pheromone of *A. nigrisigna*.

MATERIALS AND METHODS

Insects. *A. nigrisigna* larvae were collected in cabbage fields in Saitama (Sep. 23, 1988) and reared on an artificial diet (KAWASAKI et al., 1987) for successive generations at 25°C and 16L-8D. Pupae were sexed and placed into plastic eclosion pots (21 cm dia. 12 cm high) in continuous light at 20°C. Sucrose solution (10%) in a piece of absorbent cotton was supplied as food.

Extraction. Females were kept under continuous light condition for 3 days after emergence. The abdominal tips of females were removed about 2 hr after light-off

and extracted with hexane in a glass bottle. Finally, 630 female equivalent (FE) of the crude extract was collected.

Bioassay. A flight tunnel ($1 \times 1 \times 3$ m) was used to detect the activity of extracts (KAWASAKI and SUGIE, 1990a). Male moths were moved to dark condition five hr before this assay. A sample was loaded on a filter paper disk (6 mm dia.). A water-pan trap (12 cm dia.) was set at the upwind part of the flight tunnel and male moths were released from the downwind part. Frequency of orientation flight and approaching within 50 cm from the lure was counted for 10 min. After that, number of trapped male moths were counted. The wind velocity was controlled at 0.5 m/sec.

Column chromatography (LC). Column chromatography on Florisil (100/200 mesh, 30 g) was carried out according to a class-separating method for lipids (CARROLL, 1961). The column was eluted with 50 ml of hexane, 120 ml of 5% ether in hexane, 150 ml each of 15%, 25% and 50% ether in hexane, 2% methanol in ether and 4% glacial acetic acid in ether, successively.

Gas chromatography (GC). HP 5880 GC equipped with a splitless injector, a flame ionization detector and a capillary column (Silar 10C, 0.25 mm i.d. $\times$ 30 m, Gasukuro Kogyo Co.) were used under a programmed oven temperature condition of 80°C for 0.5 min increased to 160°C at a 10°C/min rate. The samples were injected into the column in the splitless mode (purge delay time, 30 sec) and nitrogen was released in the column at a rate of 18 cm/sec.

Gas chromatograph-mass spectrometry. A HP 5970 gas chromatograph-mass spectrometer (GC-MS) was used. The temperature of the separator and the ion source were 210°C and 250°C, respectively. A capillary column (DB-23, 0.25 mm i.d. $\times$ 30 m, J & W Scientific) was used under a programmed oven temperature condition of 60°C for 1 min and increased to 180°C at a 4°C/min rate. The samples were injected into the column in the splitless mode (purge delay time, 1 min) and helium was released at a rate of 20 cm/sec.

Derivatization with dimethyl disulfide. A sample was derivatized with dimethyl disulfide (DMDS) according to the method of BUSER (1983). The hexane solutions of the products were introduced into the GC-MS.

Chemicals. DMDS was purchased from Tokyo Kasei Co. and (Z)-7- and (Z)-5-, 11-dodecenyl acetates, (Z)-7-dodecenol and (Z)-7-tetradecenyl acetates were supplied by Shin-Etsu Chem. Co. Ltd. These compounds were purified with column chromatography on silicic acid impregnated with 16.7% silver nitrate. The isomeric purity of all compounds was more than 99%. Each solvent were distilled before use.

Field tests. Field tests were carried out at Maebashi (Gumma Prefecture), Ami and Tsukuba (Ibaraki Prefecture). Chemicals were loaded on rubber septa (Aldrich, Z10,072-2) as hexane solution and the septum was baited in a cone-trap (KAWASAKI and SUGIE, 1990 b). Several traps were placed in fields at about 1.2 m above the ground at intervals greater than 5 m. The taxonomy of the male moths captured were examined by comparison of genitalia (ICHINOSE, 1962).

A field screen cage ($4.3 \times 7.3 \times 2.6$ m) was also used for field tests. In the cage, 4 traps were set at the same time and a total of 290 male moths were released before sunset.

RESULTS AND DISCUSSION

Purification

The crude extracts of abdominal tips of 630 virgin females was fractionated with the Florisil column. The activity was checked in a flight tunnel at 0.06 female equivalent (FE). Each fraction showed no biological activity when individually presented to male moths, but a mixture of the fractions eluted with 5% ether in hexane (5% fraction) and 25% ether in hexane (25% fraction) showed a potent attractant activity (Table 1).

Capillary gas chromatography

The active LC fractions were analyzed with capillary GC. From the 5% fraction, 4 peaks were found: 20.77 min (peak A), 21.11 min (peak B), 21.44 min (peak C) and 28.37 min (peak D). The ratio of the areas of the 4 peaks was 2:100:2:4. These retention times were similar to those of authentic (*Z*)-5-dodecenyl acetate (Z5-12: Ac, 20.80 min), (*Z*)-7-dodecenyl acetate (Z7-12: Ac, 21.13 min), 11-dodecenyl acetate (11-12: Ac, 21.45 min) and (*Z*)-7-tetradecenyl acetate (Z7-14: Ac, 28.39 min). From the 25% fraction, one peak was found: 21.48 min (peak E). This retention time was identical to that of authentic (*Z*)-7-dodecenol (Z7-12: OH, 21.48 min). The ratio of peak B to peak E was quantified to be 100:62. Under the same conditions, the retention times of (*E*)-5-dodecenyl acetate, (*E*)-7-dodecenyl acetate (E7-12: Ac), (*E*)-7-tetradecenyl acetate and (*E*)-7-dodecenol were 20.46, 20.76, 27.81 and 20.08 min, respectively.

Gas chromatograph-mass spectrometry

From the mass chromatogram of the 5% fraction, peaks A, B and C were estimated to be dodecenyl acetates. Peak D was expected to be a tetradecenyl acetate. To confirm the position of double bond, GC-MS analysis of DMDS derivatives of a portion of the 5% fraction was carried out. Diagnostic M^+ , $RCHSCH^3+$ and $AcORCHSCH^3+$ ions were selected for monitoring. Five DMDS adducts were detected with a selected ion monitor (SIM). The retention times were 43.32 min (m/z 320, 145, 175), 44.68 min (m/z 320, 117, 203), 45.24 min (m/z 320, 117, 203), 52.17 min (m/z 348, 145, 203) and 56.91 min (m/z 320, 61, 259). These were identical to those of the DMDS adducts of Z5-12: Ac, Z7-12: Ac, E7-12: Ac, Z7-14: Ac and 11-12: Ac, respectively. One DMDS

Table 1. Attractiveness of the mixture of the 5% ether in hexane fraction from Florisil LC and another fraction in the flight tunnel bioassay

Added fraction	Frequency of male orientation	Male captures
hexane	0	0
15% ether/hexane	0	0
25% ether/hexane	23	7
50% ether/hexane	3	0
2% methanol/ether	0	0
4% acetic acid/ether	0	0

Fifteen males were used for each test. One replication.

adduct of peak E was detected with SIM (m/z 278, 117, 161) at 46.36 min. The profile was identical to the derivatives of Z7-12:OH. The chemical structures of peaks A, B, C, D and E were identified to be Z5-12:Ac, Z7-12:Ac, 11-12:Ac, Z7-14:Ac and Z7-12:OH, respectively. Because of its small amount, E7-12:Ac (1% of Z7-12:Ac) was not detected with capillary GC, but was found in the extracts of female moths with the more sensitive SIM.

Field tests

As shown in Table 2, Z7-12:OH combined with the 5% fraction attracted and caught more males than a mixture of the 5% and 25% fractions in the flight tunnel bioassay. This result indicates that the activity of the 25% fraction is caused solely by Z7-12:OH. In another flight tunnel assay, a 4-component mixture of Z7-12:Ac, Z7-12:OH, Z5-12:Ac and Z7-14:Ac showed more frequent orientation flight and male trap catch (151 orientation flights, 9 captures, $n=45$) than a 2-component mixture of Z7-12:Ac and Z7-12:OH (64 orientation flights, 3 captures, $n=45$). As the next step, the attractiveness of a 6-component mixture (the 4-component mixture plus E7-12:Ac and 11-12:Ac) and that of the 4-component mixture were compared in the field. The activity of the 4-component mixture was as strong as that of the 6-component mixture (Table 3), and both E7-12:Ac and 11-12:Ac were concluded not to be essential

Table 2. Comparison of attractiveness of (Z)-7-dodecenol with that of the 25% ether in hexane fraction from Florisil LC when combined with the 5% ether in hexane fraction in the flight tunnel bioassay^a

Lures	Frequency of male orientation ^b	Male captures
25% ether/hexane +5% ether/hexane (Z)-7-dodecenol +5% ether/hexane	18.0±3.6 20.0±3.5	0.7±0.6 7.7±1.5

^a Fifteen males and 0.06 FE were used for each test.

^b Mean±SD ($n=3$).

Table 3. Comparison of attractiveness of the 6-component mixture with that of the 4-component mixture in the field (1989)

Lures	Total male captures ^a	
	Maebashi	Tsukuba
	June 15–July 24	June 24–Aug. 9
6-component ^b	34	97
4-component ^c	57	83

^a Two traps were used for each test.

^b (E)-7-Dodecenyl acetate (10 µg) and 11-dodecenyl acetate (19 µg) were added to the 4-component mixture.

^c A mixture of (Z)-5-dodecenyl acetate (19 µg), (Z)-7-dodecenyl acetate (1 mg), (Z)-7-tetradecenyl acetate (38 µg) and (Z)-7-dodecenol (618 µg).

Table 4. Comparison of attractiveness of the 4-component mixture with those of 3-component mixtures in the field (Tsukuba, June 24–July 17, 1989)

Lures	Total number of male captures ^a
4-component ^b	36
(Z)-5-dodecenyl acetate + (Z)-7-tertadecenyl acetate + (Z)-7-dodecenol	0
(Z)-5-dodecenyl acetate + (Z)-7-dodecenyl acetate + (Z)-7-dodecenol	0
(Z)-7-dodecenyl acetate + (Z)-7-tetradecenyl acetate + (Z)-7-dodecenol	7
(Z)-5-dodecenyl acetate + (Z)-7-dodecenyl acetate + (Z)-7-tetradecenyl acetate	0

^a Two traps were used for each test.

^b A mixture of (Z)-5-dodecenyl acetate (19 µg), (Z)-7-dodecenyl acetate (1 mg), (Z)-7-tetradecenyl acetate (38 µg) and (Z)-7-dodecenol (618 µg).

Table 5. Comparison of the attractiveness of 5 virgin females with those of the 4-component mixture in a field cage (Tsukuba, Oct. 7–14, 1989)

Lures	Total number of male captures ^a
4-component ^b	97
5 virgin females	120

^a Two traps were used for each test.

^b Same as in Table 4.

attraction components. One of the components was then deleted from the 4-component mixture. As shown in Table 4, Z7-12: Ac, Z7-12: OH and Z7-14: Ac were the essential components for male attraction and the 4-component mixture was the best combination. The attractiveness of the 4-component mixture was almost equal to that of the virgin female moths (Table 5). From these data, the 4-component mixture was identified to be sex pheromone of *A. nigrisigna*. As sex pheromone of *Autographa*, sex pheromone of the *Autographa gamma* had been reported to be the mixture of Z7-12: Ac and Z7-12: OH (TÓTH et al., 1983). The attractiveness of this mixture has already been confirmed in Japan (KITAMURA et al., 1989). Two components were common between these 2 species.

The 4-component mixture also attracted male moths of *Macdounnoughia purissima* in field tests.

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