A sex attractant of the cabbage webworm, *Hellula undalis* Fabricius (Lepidoptera: Pyralidae)

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Abstract

Recently, crop damages by the cabbage webworm, *Hellula undalis* Fabricius (Lepidoptera: Pyralidae), has been frequently observed in Japan, and the development of a species-specific trap for monitoring of the field population is required. Unfortunately, the attraction activity of the known sex pheromone of this species, (*E,E*)-11,13-hexadecadienal, is too weak to be effective in a lure for this trap. Thus, we began field screening tests to find compounds that could enhance the attraction activity of (*E,E*)-11,13-hexadecadienal. In the tests, we found that a mixture of 5 µg of (*E,E*)-11,13-hexadecadienal and 15–500 ng of (*Z*)-11-hexadecenal showed the same attraction activity as virgin females. The existence of this compound was not confirmed in the extract of virgin females.

Key words: *Hellula undalis*; cabbage webworm; sex attractant; (*E,E*)-11,13-hexadecadienal; (*Z*)-11-hexadecenal

INTRODUCTION

The cabbage webworm, *Hellula undalis* Fabricius (Lepidoptera: Pyralidae), is distributed in Asia, Africa, and Europe, and is known as a pest of cruciferous vegetables (CAB, 1981). A sex pheromone component of this species has been reported to be (*E,E*)-11,13-hexadecadienal (Arai et al., 1982) and the attraction activity has been confirmed (Koshihara et al., 1985). Later, the attraction activity of this compound was found to be weak, and the addition of geometric isomers of the compound to the component did not increase the attraction activity in the field (Y. Shirai, unpublished). Recently, crop damage by this species has been frequently observed in Japan (Futai et al., 2000; Shibao and Tanaka, 2000), and the development of a species-specific trap is planned for monitoring of the field population. Thus, we began searching for an effective attractant to use as a lure for the trap.

MATERIALS AND METHODS

Insects. The larvae of *H. undalis* were collected in Kasai City, Hyogo Prefecture in August 1999, and were reared using germinating daikon (Japa-

nese white radish) seeds (Yamada and Koshihara, 1981) for successive generations at 23°C under 16L–8D. The pupae obtained were placed in plastic eclosion pots at 15°C under continuous light. The adults emerged were separated by sex and kept at 23°C under continuous light.

Extraction of pheromone. The 2 to 3-day-old female moths were moved to a dark place for 3 h before extraction. The crude pheromone was extracted from the whole body with 1 ml of hexane in a small glass vial. In a preliminary test, the crude pheromone extracted over night in a refrigerator did not show attraction activity in a field. Thus, it was decided to extract for 10 min. The extracts were stored in a refrigerator until use.

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

High pressure liquid chromatography (HPLC). A high pressure liquid chromatograph (HP 1050) with a Rheodyne injector was used. The absorbance at 210 nm was measured with a UV detector. A Lichrosorb Si-60 column (2.1 mm

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i.d.×250 mm) was used to purify the sample derived from virgin females and eluted with 5% ether in hexane at a flow rate of 1 ml/min. A Nucleosil 100-5SA column (4 mm i.d.×250 mm) treated with AgNO₃ (van Beck and Subrtova, 1995) was used to purify chemicals and eluted with 20% ether in hexane at a flow rate of 0.7 ml/min.

Gas chromatography (GC). A gas chromatograph (HP 5880) equipped with a splitless injector and a flame ionization detector, and a capillary column (FFAP, 0.25 mm i.d.×30 m, 0.25 µm film thickness) was used under a programmed oven temperature condition: 80°C for 0.5 min up to 220°C at 7°C/min. The purge delay time was 30 s. He gas was used as a carrier at a flow rate of 20 cm/s.

Gas chromatography-mass spectrometry (GC-MS). A gas chromatograph-mass spectrometer (JEOL SX102) was used. The temperature of the GC-MS interface was 220°C. The same kind of capillary column was used under the same conditions as GC. The ionization voltage was 70 eV in the electron impact mode.

Chemicals. (Z,Z)-11,13-Hexadecadienal, and (Z)-9-, (E)-9-, (Z)-11-, (E)-11-, (Z)-13-, and (E)-13-hexadecenals were synthesized by Shin-Etsu Chem. Co. (E,E)-11,13-Hexadecadienal was converted from the (Z,Z)-isomer by the method of Sgoutas and Kummerow (1968). All compounds were purified by HPLC using the AgNO₃ coated Nucleosil 100-5SA column, and the purity was confirmed by capillary GC to be more than 99%. Each solvent was distilled before use.

Field tests. The field tests were carried out at Tsukuba, Ibaraki Prefecture from 20th to 24th September in 2000 and from 8th to 17th October in 2001. Sticky traps (23×30 cm) were placed in a grass plot along the road about 0.4 m above the ground at intervals of more than 7 m. A hexane solution sample was loaded on a cotton rope (4 mm o.d.×3 cm) and attached to the trap after 21:00. Field trapping was carried out using one trap for each test sample and three traps baited with three 1-day-old virgin females.

RESULTS AND DISCUSSION

Attraction activity of fractions from Florisil column chromatography

The crude extract (3,600 female equivalents

(FE)) of virgin female moths was dried on anhydrous sodium sulfate and concentrated with an evaporator at 35°C. This crude extract was fractionated with the Florisil column and 5 fractions eluted successively by hexane (abbreviated as H, hereafter), 5% ether in hexane (abbreviated as E/H, hereafter), 15% E/H, 25% E/H and 50% E/H were obtained. The attraction activity of the 5% E/H fraction and the mixture of fractions of H, 15% E/H, 25% E/H, and 50% E/H were compared at the dose level of 100 FE. The activity was observed in the 5% E/H fraction, and no effect of the addition of other fractions was seen (Table 1). Therefore, the attraction activity was considered to be in the 5% E/H fraction.

Analysis of the 5% E/H fraction

The 5% E/H fraction was analyzed by GC-MS. The GC retention times of (E,E)- and (Z,Z)-11,13-hexadecadienals were 19.12 min and 18.75 min, respectively. The GC retention times of the other two geometric isomers were between 17.5–18.5 min. The GC retention time of the tallest peak (19.12 min) in the analysis of the 5% E/H fraction fit well with that of authentic (E,E)-11,13-hexadecadienal, and the MS spectrum of the tallest peak was also the same as that of (E,E)-11,13-hexadecadienal. The amount of (E,E)-11,13-hexadecadienal in 1 FE was calculated to be 0.2 ng.

Attraction activity of related compounds

In order to detect compounds which increase the attraction activity of (E,E)-11,13-hexadecadienal, the effect of the addition of some compounds related to (E,E)-11,13-hexadecadienal was evaluated.

Table 1. Attraction activity of the eluates from the Florisil column chromatography. Test was carried out from 20th to 24th September, 2000.

Samples	No. males caught/trap/day ^a (mean±SE)
5% ether/hexane	12.4±8.5 a
All	12.4±9.3 a
All except 5% ether/hexane	0 b
Blank	0 b

^a Data (*X*) were transformed to the square root of (*X*+0.5) and compared by variance analysis. Means followed by the same letter are not significantly different at the 5% level by Tukey's test.

Table 2. Attraction activity of the mixtures of 5 μ g of (*E,E*)-11,13-hexadecadienal and 50 ng of some compounds

Samples	No. males caught/trap/day ^a (mean $\pm$ SE)
A from 8th to 10th October, 2001	
EE	0.7 $\pm$ 0.3 b
EE+Z11	7.0 $\pm$ 2.1 a
EE+E11	1.0 $\pm$ 1.0 b
EE+Z13	0 b
EE+E13	0.3 $\pm$ 0.3 b
EE+Z11+Z13	0.7 $\pm$ 0.7 b
3 females	1.1 $\pm$ 0.6 b
B from 11th to 13th October, 2001	
EE+Z9	0 c
EE+E9	0.3 $\pm$ 0.3 bc
EE+Z11	5.0 $\pm$ 0.8 a
EE+Z11+E11	5.0 $\pm$ 1.5 a
EE+Z11+Z13	3.3 $\pm$ 1.2 ab
EE+Z11+E13	5.0 $\pm$ 1.2 a
Z11	0 c
3 females	1.4 $\pm$ 0.9 bc

EE: (*E,E*)-11,13-hexadecadienal, Z9: (*Z*)-9-hexadecenal, E9: (*E*)-9-hexadecenal, Z11: (*Z*)-11-hexadecenal, E11: (*E*)-11-hexadecenal, Z13: (*Z*)-13-hexadecenal, E13: (*E*)-13-hexadecenal.

^aData (*X*) were transformed to the square root of (*X*+0.5) and compared by variance analysis. Means followed by the same letter in each test are not significantly different at the 5% level by Tukey's test.

Fifty nanogram of each of the six compounds, (*Z*)-9-, (*E*)-9-, (*Z*)-11-, (*E*)-11-, (*Z*)-13- and (*E*)-13-hexadecenals, was added to 5 μ g of (*E,E*)-11,13-hexadecadienal. The mixtures containing (*Z*)-11-hexadecenal showed the strongest attraction activity (Table 2). When another compound was added to the mixture of (*Z*)-11-hexadecenal and (*E,E*)-11,13-hexadecadienal, no effect of the addition was observed (Table 2B). (*Z*)-11-Hexadecenal (0 to 500 ng) was added to 5 μ g of (*E,E*)-11,13-hexadecadienal to determine the optimum proportion of (*Z*)-11-hexadecenal. Mixtures of 15 to 500 ng additions showed the same level of attraction activity as virgin females (Table 3).

Examination of the existence of (*Z*)-11-hexadecenal in the 5% E/H fraction

In order to examine the existence of (*Z*)-11-hexadecenal in the 5% E/H fraction, the 5% E/H fraction was purified by HPLC using a Lichrosorb Si-60 column. Authentic (*E,E*)-11,13-hexadecadi-

Table 3. Attraction activity of the mixtures of 5 μ g of (*E,E*)-11,13-hexadecadienal and 0 to 500 ng of (*Z*)-11-hexadecenal. Test was carried out from 14th to 17th October, 2001.

Samples	No. males caught/trap/day ^a (mean $\pm$ SE)
EE	0 c
EE+5 ng Z11	0.3 $\pm$ 0.3 bc
EE+15 ng Z11	0.5 $\pm$ 0.3 abc
EE+50 ng Z11	4.0 $\pm$ 1.2 a
EE+150 ng Z11	4.5 $\pm$ 1.2 a
EE+500 ng Z11	2.8 $\pm$ 1.9 ab
3 females	2.9 $\pm$ 1.2 a

EE: (*E,E*)-11,13-hexadecadienal, Z11: (*Z*)-11-hexadecenal.

^aData (*X*) were transformed to the square root of (*X*+0.5) and compared by variance analysis. Means followed by the same letter are not significantly different at the 5% level by Tukey's test.

enal was eluted at 8 min, and the three fractions eluted between 0–7, 7–10, and 10–15 min were collected. Five hundred FE of the fractionated samples were analyzed by GC-MS for the presence of (*Z*)-11-hexadecenal but this compound was not detected. At these analyses, the limit of detection of this compound was 0.5% of the amount of (*E,E*)-11,13-hexadecadienal.

With *H. undalis*, just one or two larvae can cause severe damage to many cruciferous crops, because the larvae infest the growing points or shoots of young plants (Sivapragasam and Chua, 1997). Therefore, it is important to be able to detect the occurrence of this species at a low larval density level (Shirai and Yano, 1994). The mixture of (*Z*)-11-hexadecenal and (*E,E*)-11,13-hexadecadienal tested in the present study was shown to be useful as an attractant agent of *H. undalis* in the field.

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