SEX PHEROMONE OF SUGARCANE TIP BORER *Scirpophaga excerpalis* WALKER

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Sugarcane tip borer, *Scirpophaga excerpalis* Walker, is one of the destructive pests in sugarcane fields. It is distributed in Guangdong, Guangxi and Taiwan provinces of China, and also in Southeast Asia and South Asia. Attractant activities of the crude extract of abdominal tips of the female moth have been studied by REN Da-fang, one of the authors (unpublished data). It has shown that the extract after liquid chromatographic separation revealed attractant activities in field tests. This note describes the chemical structure and field tests of the sex pheromone of this species.

I. MATERIALS AND METHODS

(1) *Insects and extraction*

Overwintering larvae with sugarcane stalks collected from fields were put into a net box and allowed to pupate until to adult stage under natural light-dark cycle. The extraction was conducted at 2—3 o'clock in the morning. The abdomens were pressed lightly with fingers and the tips with last three segments were excised and extracted with heptane (chromatographic pure, 2 μl/tip). After 3 h the extracts were taken out and stored in a sealed ampoule at -25°C for analysis.

(2) *Chemicals*

The tested chemicals were synthesized by ourselves or collected from other sources with more than 98% purities.

(3) *Electroantennagram (EAG) analysis*

EAG data were measured following the method reported before[1]. The excised antenna were stimulated with synthetic monoenoic alcohols and its acetates, as well as monoenoic aldehydes. EAG-response (mV) was calculated in percentages. The response of Z-11-hexadecenal (Z11-16: ALD) was utilized as standard, its value was taken as 100%. The others were normalized in comparison with the standard and shown in %.

(4) *Microchemical reactions*

Microreduction[2] was conducted as follows: 2 μl of saturated sodium borohydride solution in isopropanol and 20 μl of heptane extracts of 20 tips were mixed and shaken for 3—5 min, then 5 μl of distilled water was added and agitated for 2 min. The water layer was taken out with a microsyringe and the heptane solution of reduction product was left for further analysis.

Microozonolysis was conducted with the extract of 10 tips and E-11-hexadecenal (E11-16: ALD) following the method described by Beroza and Bier[3].

(5) *Gas chromatographic (GC) analysis*

A Pye 204 Instrument equipped with SGE split/splitless injector was used for analysis with fused silica capillary columns on Grob injection mode. Hydrogen gas was used as carrier gas. Column A: BP-20 (SGE), 50 m × 0.2 mm (ID), carrier gas linear velocity was 50 cm/s and the temperature was programmed from 80°C to 180°C at 4°C/min. Column B: OV-225 (Petroleum Research Institute, Beijing), 18 m × 0.3 mm (ID), carrier gas linear velocity was 40 cm/s and the temperature was programmed from 70°C to 190°C at 4°C/min. A PE-990 instrument with direct injector was used for packed column analysis. Injector temperature was 200°C, detector temperature was 250°C, nitrogen was used as carrier gas. Column C: glass column with 5% PEG on chromosorb AW-DMCS (80—100 mesh), 2 m × 2 mm (ID). Column temperature was 170°C, velocity of carrier gas was 25 ml/min. Column D: stainless steel column with 4% OV-101 plus 1% FFAP on chromosorb W-AW DMCS, 2 m × 2 mm (ID). Column temperature was 160°C, velocity of carrier gas was 30 ml/min.

(6) *GC-mass spectrometry (GC-MS)*

Mass spectra were recorded on a JMS-D300S instrument conjunct with Hitachi GC 663-50, and a data system JAM-2000S using electronic ionization (70 eV). Fused silica capillary columns were used. Helium was used as carrier gas with its velocity at 2 ml/min. Temperature of ion source was 250°C. Column E: SE-52, 25 m × 0.2 mm (ID). Temperature was programmed from 120°C to 200°C at 4°C/min. Column F: OV-101, 30 m × 0.2 mm (ID). Temperature was programmed from 130°C to 220°C at 4°C/min. Column G: BP-1, 50 m × 0.2 mm (ID). Temperature was programmed from 80°C to 180°C at 4°C/min.

(7) *Field tests*

Trapping tests were conducted in sugarcane fields in suburbs of Zhanjiang, Guangdong Province in 1985—1987. Hexane solution of test chemicals was impregnated into rubber septa of sleeve type (Beijing Shunyi Rubber Factory) to make attractant lures. Water bowl type traps were used. Bowls were filled with water containing detergent. Lures were suspended in the center of the traps 1—2 cm above water level. Traps were set 1 m above the ground at 20 m intervals. 3 duplicates were set in every treatment. The virgin female traps were used as comparison. One virgin female was put into a net box of 6 cm × 3 cm (ID). The females were changed every 2—3 days.

II. Results and Discussions

(1) *EAG responses*

EAG responses of 48 synthetic chemicals were recorded on 14 male antenna. Among them 6 chemicals revealed more than 50% response. They are E-11-hexadecenal (E-11-16: ALD) (125%), Z-11-16: ALD (100%), Z-11-hexadecenyl acetate (95%), E-10-hexadecenal (85%), Z-10-hexadecenal (80%) and Z-11-hexadecenol (55%). E-11-16: ALD showed the highest EAG response in tests with 12 antenna. This fact was similar to that as in sugarcane stem borer, *Chilo punctiferelis* Snellen[4].

(2) *GC analysis*

When heptane extract of 2 tips was injected into Column A, 2 major peaks appeared. Their retention times were 26′35″ and 26′53″ separately, and identical with synthetic E11-16: ALD and Z11-16: ALD. When extract of 2 tips was injected into column B, two major peaks appeared, which was identical with synthetic E11-16: ALD and Z11-16: ALD. Their retention times were 32′29″ and 32′45″ respectively. The mean ratio of the two components was 78:22, calculated on analysis of multiple tips with Column A. When the reduction product of the sex pheromone extract was injected into Column C, a peak with retention time of 5′20″ appeared, which was identical with the retention time of E-11-hexadecen-1-ol. The product of ozonolysis was analyzed on Column D. One major peak was recorded. Its retention time was identical with that of the product of ozonolysis of E11-16: ALD.

(3) *GC-MS analysis on Column E*

Mass spectra of the two major peak were identical with the mass spectra of synthetic E11-16: ALD and Z11-16: ALD, which showed M/Z 238 (M^+) and 220 (M^+-18). Mass spectrum of the reduction product showed a characteristic fragment, M/Z 222(M^+-18), which was identical with synthetic E11-16: OH. From the above data a conclusion was drawn that the major peak was hexadecenal. Analysis of the product of ozonolysis only showed one major peak, with fragments of E/Z 166(M^+-18) and E/Z 156, which was identical with 1, 11-undecanedial, one of the products of ozonolysis of E11-16: ALD. This fact confirmed that the double bond was at the position of 11 and 12 carbon atoms. Because of high volatility, pentanal, another product of ozonolysis, came out along with the solvent and could not be identified in these conditions[5]. From the data obtained by EAG, GC and GC-MS analysis we could draw conclusion that the two major components of sugarcane tip borer moth were E11-16: ALD and Z11-16: ALD.

(4) *Discussions*

Field tests indicated that not all the EAG active compounds have attractant activities. Among the different ratios of the synthetic compounds only the mixtures of E11-16: ALD and Z11-16: ALD showed attractant activities to the male moths. The best ratio of E to Z-isomers was 85: 15, although the mixtures with different ratios between 5 : 5 and 9: 1 of E to Z-isomers showed certain degree of attractant activities to male moths (Table 1). The attractant activities of the synthetic lures exceeded the live virgin female moth in the overwintering generation and second generation. But in the 3rd and 4th generations the activities of the synthetic lures were less than the live female moths. The reasons were not clear yet. Possibly there may be still other minor components which remain to be identified.

Table 1

Field Attraction Tests of the Sex Pheromone of Sugarcane Tip Moth, *Scirpophaga excerpalis* Walker

Date	Blend of Chemicals[a)]	Dosage (μg)	No. of Moths in 3 Traps
March 7—8, April 6—7, 1985	E11-16: ALD	300	0
	E11-16: ALD+ Z11-16: ALD	270+ 30	15
	E11-16: ALD+ Z11-16: OH	297+ 3	0
	E11-16: ALD+ Z11-16: AC	297+ 3	0
	live virgin female(1)		3
July 10—18, 1985	E11-16: ALD+ Z11-16: ALD	196+ 4	0
	E11-16: ALD+ Z11-16: ALD	193+ 7	2
	E11-16: ALD+ Z11-16: ALD	160+ 40	78
	E11-16: ALD+ Z11-16: ALD	100+ 100	40
	E11-16: ALD+ Z11-16: ALD	40+ 60	0
	live virgin female(1)		179
March 18—April 2, 1986	E11-16: ALD+ Z11-16: ALD	170+ 30	55
	E11-16: ALD+ Z11-16: ALD	160+ 40	33
	E11-16: ALD+ Z11-16: ALD	156+ 44	19
	E11-16: ALD+ Z11-16: ALD	152+ 48	31
	live virgin female (1)		2
July 9—18, 1987	E11-16: ALD+ Z11-16: ALD	240+ 60	144
	E11-16: ALD+ Z11-16: ALD + Z9-16: ALD	240+ 60+ 15	135
	E11-16: ALD+ Z11-16: ALD + Z9-16: ALD	240+ 60+ 30	88
	live virgin female (1)		320

a) E11-16: ALD, Z11-16: ALD, Z9-16: ALD, Z11-16: OH and Z11-16: AC were symbolic names of the following chemicals separately: E-11-hexadecenal, Z-11-hexadecenal, Z-9-hexadecenal, Z-11-hexadecenol and Z-11-hexadecenyl acetate.

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