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Biological Activity of Female Sex Pheromone of the Oriental Tobacco Budworm, *Helicoverpa assulta* (Guenee) (Lepidoptera: Noctuidae): Electroantennography, Wind Tunnel Observation and Field Trapping

담배나방 성페로몬의 생물학적 활성: 촉각전도, 풍동 및 야외 트랩시험

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ABSTRACT Electroantennography, wind tunnel observation, and field trapping experiments were carried out to investigate the biological activity of synthetic sex pheromone in the oriental tobacco budworm, *Helicoverpa assulta*. Two major sex pheromone components of *H. assulta*, Z9-16 · Ald and Z11-16 : Ald. elicited a big EAG response in male, but not in female. Their mixture ratios did not give much influence on EAG size. Female *H assulta* showed a great EAG response only to its host plant extract. EAG size also increased with the amount of mixture from 0.01 to 10 μg but rather decreased when the amount was 100 μg. *H. assulta* always revealed a series of stereotyped behavior in a wind tunnel. The behavioral response was different when the males were stimulated with the sex pheromone containing some minor components, 16 : Ald and Z9-16 : Ac, or being different in mixing ratios of the two major components. The best ratio of the sex pheromone components for attracting *H assulta* male adults was 20-25 : 1 between Z9-16 : Ald and Z11-16 : Ald in net house and red pepper field experiments in Korea. When the lure contained Z9-16 : OH, attracting power rapidly decreased. The synthetic sex pheromone showed a strong attraction when compared to virgin females.

KEY WORDS Wind tunnel, field trapping, Z9-16 : Ald, Z11-16 . Ald, Z9-16 : OH, *Helicoverpa assulta*, sex pheromone, electroantennography

초 록 합성 성페로몬의 담배나방(*Helicoverpa assulta* (G.))에 대한 생물학적 활성을 알아보기 위하여 촉각전도 측정, 반응행동 관찰 및 야외 유인실험을 하였다. 담배나방 성페로몬의 주성분인 Z9-16 · Ald와 Z11-16 : Ald는 모두 수컷에서 큰 촉각전도반응을 보였으며 이들의 혼합 비율은 촉각전도반응 크기에 영향을 주지 않았다. 담배나방 암컷은 두 페로몬 주성분에 대해서는 촉각전도반응을 나타내지 않았으며, 기주식물인 고추기름에만 큰 촉각전도반응을 보였다. 처리량이 0.01~10 μg 일 때는 페로몬의 양이 증가함에 따라서 촉각전도반응도 증가했으나 100 μg을 처리했을 때에는 오히려 반응의 크기가 줄어들거나 10 μg 처리시와 같았다. 풍동에서 담배나방 수컷은 일련의 규칙적인 반응행동들을 나타냈다. 이 반응행동들은 성페로몬의 두 주성분 비율에 따라 달랐으며, 미량성분인 16 · 1 Ald와 Z9-16 : Ac를 첨가했을 때에는 교미행동까지 나타냈다. 야외에서 트랩을 이용하여 유인력을 검정한 결과, Z9-16 : Ald와 Z11-16 : Ald의 비율이 20~25 : 1일 때 유인력이 가장 컸으며 여기에 Z9-16 : OH가 첨가되었을 때에는 다른 성분의 비율에 관계 없이 유인력이 아주 낮았다. 합성 성페로몬의 유인력은 미교미암컷의 유인력보다 강한 것으로 판명되었다.

검색어 담배나방, 성페로몬, 촉각전도, 풍동, 트랩, Z9-16 : Ald, Z11-16 . Ald, Z9-16 . OH

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The oriental tobacco budworm, *Helicoverpa assulta* (Guenee). is the major insect pest to tobacco leaves and especially to red pepper fruits in Korea. It is difficult to control *H. assulta* larvae with chemical insecticides in fields mainly due to their feeding habit within red pepper fruits. Use of physiologically active substances like sex pheromones may be a proper mean for controlling *H. assulta*. The sex pheromone of *H. assulta* is a blend of Z9-16 : Ald, Z11-16 : Ald, Z9-16 : Ac, and Z11-16 · Ac (Cork *et al.* 1992). However, the best ratio of two major components, Z9-16 : Ald and Z11-16 : Ald, for attracting male *H. assulta* and the role of minor components were reported to be different in Korea, China, and Thailand (Cork *et al.* 1992). In order to enhance the potential utilization of pheromones for pest management, a thorough understanding of the processes involved in odor detection is needed. Many integrated pest management systems, designed to maximize cost-efficiency and to minimize the side effects of pesticides, currently include pheromones (Grant & O'Connell 1986). In practice, sex pheromone is used as a monitoring agent of the population and/or a direct control mean, and the success of these applications depends on the competetive attractancy of synthetic lures against alive females. Therefore, the biological activity of synthetic pheromones should be elucidated for practical application. This study was carried out to investigate the biological activity of synthetic sex pheromone of *H. assulta*. This included electroantennography(EAG), wind-tunnel observation, and trapping experiments in a net house and fields

MATERIALS AND METHODS

Insects

The oriental tobacco budworm, *Helicoverpa assulta*, larvae were reared on an artificial diet (Park 1991). Larvae were maintained in a controlled environmental room, at a temperature of 25± 1℃, 60± 10% relative humidity under a 16 hr light/10 hr dark regime. Pupae were collected daily, sexed, and placed in separate holding cages provided with an 8% solution of sugar. Adult moths were harvested daily and used for study.

Pheromone Synthesis

Monounsaturated acetates, alcohols, and aldehydes were obtained by standard Wittig and acetylenic coupling reactions (Henrick 1977) or purchased from Shin Etsu Chemical Co. (Tokyo, Japan). Compounds used for field testing were further purified by argentation chromatography (Houx *et al.* 1974) to give material at least 99.9% isomerically and chemically pure by GC analysis.

Pheromone dispensers were white rubber septa (Aldrich, catalog No. Z10, 072-2) impregnated with 0.1 ml of hexane solution containing the synthetic pheromone blend and an equivalent weight of 2,6-di-tert-butyl-4-methylphenol as antioxidant.

Electroantennography

A conventional recording method was used (Schneider 1957, Pouzat & Ibeas 1989), except for the following details. The antenna was not separated from the head, and the whole insect was fixed on a slide glass with a sticky tape A tungsten wire was introduced into dorsal abdomen and acted as an indifferent electrode. This avoided leakage of haemolymph. The antenna was stuck on a tape in a position A few flagellar subsegment were cut from the distal tip of the antenna and a recording electrode (a micropipette 100 μm in diameter filled with 0.1 M KCl) was immediately positioned to contact the tip of the antenna. A chlorinated silver wire was used to connect the pipette to the input probe of the preamplifier (Kenwood CS-8100 input resistance $10^{12}\Omega$) and the remainder of the recording apparatus was conventional. EAGs were displayed on a screen of a digital storage oscilloscope (Nihon-Koden) and recorded.

Air from air compressor was passed through activated charcoal, over distilled water. The air (180 ml/min) was then sent to the inlet of the glass stimulator. The diameter of the output tube of the stimulator was 5 mm. Serial dilutions were made in liquid paraffin or n-hexane for all of the compounds tested One μl of the appropriate dilution was applied with a disposable pipette to a piece of filter paper (5 mm diameter) held in a glass syringe. All the insects used in these experiments were 2- or 3-day-old, virgin, sexually active adults. In each ex-

Table 1. Relative EAG response of both sexes of *Helicoverpa assulta* to individual sex pheromone component and host plant extract

Sex	Relative ratio of EAG in each treatment						
	Air	Paraffin oil	Z9-16 : Ald	Z11-16 : Ald	Z11-16 Ac	Z11-14 : Ac	Hot pepper oil
Male	0.90c	1c	1.64a	1.45ab	1.08c	1.07c	1.18bc
Female	1.02c	1c	1.04c	1.05c	1.24b	1.12c	1.76a

†Figures followed by different letters are significantly different by Duncan's multiple range test ($p=0.01$); $\bar{x}\pm s$ 5 replication.

periment, results given are averages for at least five different antennae recordings. All experiments were performed at room temperature.

Wind-tunnel Observation

The wind tunnel used in these experiments was a 2 m long cylindrical transparent polystyrene tunnel, 60 cm in diameter. Air speed was fixed at 30 cm /sec. Air was blown into an end of the wind tunnel by an electrical fan (diameter 40 cm) through a layer of thin plastic tubes. Air from the tunnel was vented to the outside through an aluminum tube (diamater 15 cm). The pheromone sources were mounted in the center of the tunnel at the upwind end on a metal wire. Virgin female moths were housed in small screen cages. Synthetic pheromone was incorporated into white rubber septa.

All the tests were conducted with males in hours 2 and 4 of their second or third scotophase; males were used only once in an assay. Environmental conditions of the assays were as follows : temperature, 25± 1℃, 40~60% RH; 10 W red light. Behavioral categories reported by Cho & Boo (1988) were used to evaluate male responses to stimuli in the flight tunnel. The experimental design for the flight tunnel assay was a randomized complete block with 5 replicates. Analysis of behavioral response was followed the criteria for mating behavior of *H assulta* (Cho & Boo 1988).

Net House and Field Test

Field tests were conducted in red pepper fields in Suwon, Korea. Cone traps or universal traps were used for field test in a net house or red pepper field. Test compounds in n-hexane were impregnated into white rubber septa. Cone traps were fixed approximately 1.5 m above ground level. Rubber septa dispensers impregnated with sex pheromone component(s) were used as lures and one or two virgin female(s) were used as a control. Control traps were baited with the female *H. assulta* held in a wire cage (5 cm high by 4 cm in diameter) that was centered inside the cone traps. Virgin female was replaced at two-day intervals, and supplied with 8% sucrose solution. Traps were at least 7 m from other traps. The lure positions were arranged by a randomized complete block design and rotated clockwise daily. Five to twenty virgin males, one or two days old, were daily released in the net house. Traps were checked daily, the number of *H. assulta* males trapped was recorded, and the moths were removed. Trap catch data were submitted to analysis of variance by the Duncan's multiple range test.

RESULTS AND DISCUSSION

H. assulta male and female antennal sensitivity to their own female sex pheromone or red pepper oil was investigated electrophysiologically. The EAG configuration evoked by Z9- and Z11-16 : Ald was charicterized by a relatively fast initial fall followed by a slow plateau during the time of stimulation, and then the EAG response gradually returns to the initial level with a half-decay time of 1~2 sec. There was a variability in EAG response amplitudes between preparations. The mean responses of *H. assulta* antennae to the air puffing or paraffin oil stimulation were not significantly different between males and females (Table 1). However, there was a great difference in EAG response to sex pheromone component or host plant extract between male and female *H. assulta*. Only Z9-16 : Ald and

Table 2. EAG response of male *H. assulta* to different amount of Z9-16 : Ald or Z11-16 : Ald

Treatment (μg)	Peak value (−mV)	Treatment (μg)	Peak value (−mV)
Air	0	Hexane	0.1± 0.05
Z9-16 : Ald		Z11-16 . Ald	
100	1.2± 0.04	100	0 6± 0.05
10	1.4± 0.09	10	1.0± 0.03
1	0.9± 0.08	1	0.6± 0 09
0 1	0.5± 0 03	0 1	0.3± 0.05
0.01	0.4± 0.05	0.01	0.2± 0.00

† 4 replication; ⁻⁺ Mean ± S.D.

Table 3. EAG peak value(mV) in male *H. assulta* responding to different blends (total amount, 100 μg) of Z9-16 : Ald and Z11-16 : Ald (The antennal tip was excised)

Ratio Z9-16 : Ald Z11-16 : Ald	A[a]	B[a]
100:0	2 1± 0.25	1.2± 0.04
100:2	1 8± 0.15	1.3± 0.28
100:10	1.9± 0.17	1 3± 0 14
100·100	1.4± 0.08	1.2± 0.07
10:100	1.8± 0.19	1 2± 0 05
0·100	1.5± 0.10	0.6± 0.05

[a] Each pheromone blend was applied at the right angle to the air flow (A) or through the air flow (B) † 4 replication; ⁻⁺ Mean± S.D.

Z11-16 : Ald, the two major sex pheromone components (Cork *et al.* 1992), elicited significantly greater EAG responses from male *H. assulta* than any other treatment (Table 1). But, the female was insensitive to the pheromone components and her antenna seemed to be specialized for plant volatile reception (Table 1). Males showed a much smaller response to host plant extract than females. The existance of receptors for two major sex pheromone components, Z9-16 : Ald and Z11-16 : Ald, was not shown in the antennae of females by these EAGs.

These sexual dimorphism in responding to sex pheromone and plant odour might be useful to *H. assulta*, by increasing the power carrying out its reproductive role in each sex Scanning electron microscopic examination of antennae of *H. assulta* also revealed morphological differences between both sexes (Park & Boo, unpublished observation)

The pheromone-sensitive neurones are specialists responding to a particular component in the pheromone mixture (Kaissling *et al.* 1989). However, *H. virescens* and *H. zea* were reported to have no specific neuron responding to Z9-16 : Ald, a major component in their sex pheromone (Almaas & Mustaparta 1990) Z11-16 : Ac was reported as a minor component of sex pheromone in *H. assulta* (Cork *et al.* 1992). But, in EAG screenings, Z11-16 : Ac gave no difference in response size to male *H. assulta* from control (Table 1). This suggested a possibility that Z11-16 : Ac might not have a behavioral role in sex pheromonal communication in *H. assulta*.

EAG size increased with the amount of mixture from 0.01 to 10 μg but rather decreased when the amount was 100 μg (Table 2). It is not clear why, but could be related to the fact, reported in other insects, that insect behavior may be suppressed at the high concentration of pheromone (Baker & Roelofs 1981).

The mixture ratios of Z9-16 : Ald and Z11-16 : Ald did not give much influence on EAG size in male *H assulta* (Table 3). But, in all cases, the mixture containing Z9-16 : Ald elicited greater EAG response than Z11-16 : Ald alone regardless of the mixing ratios (Table 3). Z9-16 : Ald is the major sex pheromone of *H. assulta* (Cork *et al* 1992) and it was likely that there were much more receptor neurones responding to Z9-16 : Ald than to any other sex pheromone components in *H. assulta* males.

Male *H. assulta* maintained a resting position without any locomotive activity during photophase, and showed no response to any physical or chemical stimuli such as vibration, light, touch, and chemical odorants. However, during scotophase, it lasted the position of wing-spread and antennal stretch, so became sensitive to various physical or chemical stimulation. Most behavioral response of *H. assulta* to its sex pheromone was shown within 6 hrs after onset of dark period. *H. assulta* showed some behavioral patterns of wing fanning, anemotaxis, and upwind flight without chemical stimulant. Therefore, these behaviors were excluded from the criteria for discriminating behavioral effect of synthetic sex pheromone to *H. assulta*. After upwind flight, male *H.*

Table 4. Behavioral response of male *H. assulta* to different blends of Z9-16 : Ald and Z11-16 : Ald in a wind tunnel. Positive(+) or no(−) behavioral response

Lure(Z9-16 : Ald : Z11-16 : Ald)	Wing spread	Antennal spread	Wing vibration	Start of flying	Upwind flying	Arrival at[a] the source	Abdominal expansion
0:0	+	+	+	+	−	−	−
50:1	+	+	+	+	+	−	−
25:1	+	+	+	+	+	+	−
10:1	+	+	+	+	+	−	−
5:1	+	+	+	+	−	−	−
16:1[b]	+	+	+	+	+	+	+

[a]It was considered a positive response when the moth was staying within 3 cm range from the rubber septum for more than 3 seconds; [b]This lure contains two additional components, 16 . Ald and Z9-16 : Ac. †3 replication

assulta showed no characteristic behavioral pattern without applying sex pheromone. But, synthetic sex pheromone lure resulted in a series of behavioral responses, approaching, abdominal extrusion, landing, and copulation trial (Table 4). So, these behaviors were used as criteria for behavioral response to sex pheromone.

Behavioral response of male *H. assulta* to synthetic lure was varied with the ratio of Z9-16 : Ald and Z11-16 : Ald (Table 4). Especially, when the ratio of two chemicals is 25 : 1 male *H. assulta* approached and hovered around the lure within 3 cm range for 20-30 seconds, though no copulational trial was observed. But, it showed copulational behavior such as landing on and hairpencil expansion to the lure containing some minor components, 16 : Ald and Z9-16 : Ac (Table 4). These behavioral responses suggested that the ratio of two major components, Z9-16 : Ald and Z11-16 : Ald, has a role of a long-distance attraction, and minor component(s), 16 : Ald and/or Z9-16 : Ac, a copulational excitant.

Insect behavior responding to sex pheromone has been an interest to many scientists from the early days in pheromone research. Typical insect behavioral responses to pheromone stimulation are manifested in the form of taxis, flight, hovering, landing, mating attemt, etc. However, insect behavior is influenced by, besides the pheromone components, many biological and environmental factors, such as age, mating, sensory adaptation, circadian rhythm, photoperiod, temperature, etc. Some investigators claim that each component or partial composition may elicit different aspest of insect sex behavior, when a multicomponent sex pheromone is used. For example, in *Spodoptera eridania*, a sex pheromone component is claimed to play as a long-distance attractant and the other as a sexual exitant (Jacobson *et al.* 1970). Roelofs *et al.* (1975) called them as the primary and secondary components.

When a sex pheromone is composed of multicomponents, species specificity is possible due to their specific composition ratio. This kind of situation can be observed in physiologically close species such as 7 *Heliothis* (and *Helicoverpa*) species. The best ratio of the two major components, Z9-16 : Ald and Z11-16 : Ald, for attracting *H. assulta* male adults was 20~25 : 1 (Table 5). When the lure contained Z9-16 : OH, attracting power rapidly decreased (Table 8). And, the synthetic sex pheromone showed a very strong attraction when compared to virgin females (Table 6, 7, 8). The practical application of synthetic sex pheromone is based on the relative attractancy of synthetic sex pheromone to virgin female(s). Therefore. it will be possible that the synthetic sex pheromone be used for practical monitoring and/or control in *H. assulta*.

There was a minor peak of attraction at the mixture ratio of 5-7.5 : 1 (Table 7). This is interesting since field trapping experiments showed that the best ratio was 7.5 : 1 in Thailand but that there were no significant differences in attraction between 7 5 : 1 and 25 : 1 in China (Cork *et al.* 1992). In some

Table 5. Catches of male *H. assulta* moths in cone traps baited with four different blends of two major sex pheromone components, Z9-16 : Ald an Z11-16 : Ald, in a net house

Set	Ratio Z9-:Z11-16:Ald[a]	No. males/trap/night[b]
I	5:1	0.3b
	10:1	0.2b
	15:1	1.0b
	20:1	2.8a
II	5:1	2 5b
	10:1	2 3b
	25:1	9.8a
	50:1	4.0b
III	15:1	0.2c
	20:1	3.2a
	25.1	1.6b
	50:1.	0.8bc

[a]Total amount of components loaded in each rubber septum is 1 mg all; [b]Figures followed by different letters are significantly different by Duncan's multiple range test (p=0.01); †Experimental date : set I: July 16-22, 1990, set II: August 29-September 4, 1990; set III: July 23-31, 1990.

insect species sex pheromone composition is different depending on their distribution area Such examples were found in *Ostrinia nubilalis* in USA (Kochansky *et al.* 1975), *O. furnacalis* in Japan, Taiwan and China (Ando *et al.* 1980, Klun *et al.* 1980, Cheng *et al.* 1981, Kou *et al.* 1990), and *Adoxophyes* sp. in Japan and Taiwan (Tamaki *et al.* 1980).

Table 6. Catches of male *H. assulta* in cone traps baited with different blends of Z9-16 : Ald and Z11-16 : Ald and 2 virgin females in a net house

Ratio Z9- : Z11-16 · Ald[a]	No males/trap/night[b]
5:1	4.3ab
15:1	6.3a
25:1	10.0a
2 females	0.3b

[a]Total amount of components loaded in each rubber septum is 1 mg all; [b]Figures followed by same letters are not significantly different by Duncan's multiple range test (p=0.01). †Experimental date : September 5-15, 1990

Table 7. Catches of male *H. assulta* in universal traps baited with different blends of Z9-16 : Ald and Z11-16 : Ald or 2 virgin females in a red pepper field

Ratio Z9- . Z11-16 : Ald[a]	No males/trap/night[b]
1:1	0c
5:1	0 4bc
7.5:1	0.8ab
10·1	0.4bc
25:1	1.4a
50:1	0.2bc
2 females	0c

[a]Total amount of components loaded in each rubber septum is 1 mg all.
[b]Figures followed by same letters are not significantly different by Duncan's multiple range test (p=0.01)
†Experimental date· September 13 - October 11, 1988.

Table 8. Catches of male *H. assulta* in cone traps baited with the two major components, Z9- and Z11-16 : Ald, and some other minor components in a red pepper field

Ratio[a]								No. males trap/night[b]
Aldehyde			Acetate			Alcohol		
Sat.	Z9-	Z11-	Sat.	Z9-	Z11-	Sat	Z9-	
14	65	4	–	15	–	–	1	0c
14	65	4	–	0	–	–	1	0c
14	65	4	–	15	–	–	0	1 6a
0	65	4	–	15	–	–	1	0.1bc
	2 females							0 9ab
	1 female							0c

[a]Total amount of components loaded in each rubber septum is 1 mg all. The ratio of Z9- and Z11-16 : Ald is 20 : 1; [b]Figures followed by different letters are significantly different by Duncan's multiple range test (p=0 01); †Experimental date: September 19-28 and October 10-14, 1987 with 3 replication

All three species above use two components at different ratios as their sex pheromone. If such variations are widespread in insect world, we must more carefully look at the species specificity of the sex pheromone and this may appear as another hindrance to the application of sex pheromone in insect pest control.

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