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Date: Jan. 1987-1990

TITLE: USE OF SEX PHEROMONES FOR MONITORING AND CONTROL OF HELIOTHIS ARMIGERA POPULATIONS IN COTTON

NAMES OF INVESTIGATORS:

Principal Investigators: 1. Moshe Kehat (Israel)

2. Sawang Wangeonkong (Thailand)

Cooperating investigators: 1. Shmuel Gothilf (Israel)

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DURATION OF PROJECT: Three years

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Table of contents Page

Abstract.....2

I. Heliothis armigera (Hubner)

IA. Behavioural responses of male H. armigera moths in a flight tunnel to combinations of components identified from female sex pheromone glands.....4

IB. Analysis of gland extracts of H. armigera from Thailand.....19

IC. Age structure and nocturnal behavior in cotton fields in Israel.....21

ID. Night observations in cotton fields in Thailand.....36

IE. Effects of pheromone application as mating disruptant.....52

II. Heliothis peltigera (Schiff)

IIA. Female sex pheromone components: chemical identification from gland extracts and male response.....58

IIB. Sexual behavior.....78

III. Heliothis assulta (Guenee)

IIIA. Sex pheromone components.....85

Abstract

The sex pheromone components of Heliothis armigera were identified from female sex pheormone glands. Two major components - Z11-16:Ald and Z9-16:Ald - were essential for attracting males. An additional component - Z7-16:Ald - was important only when very low dosages were used. The two major components, and at almost the same ratios, were detected also in gland extracts obtained from Thailand. A positive relationship between captures of males in pheromone traps and the actual population in the field was detected in field tests conducted in Israel and Thailand. This indicates the reliability of pheromone traps for monitoring H. armigera populations. The nocturnal behavior of adult moths and mating activity throughout the night were also studied. Peak activity of mating occurred between 02:00-04:00. Pheromone applications for mating disruption in closed small containers resulted in complete inhibition of mating, at high pheromone dosages. However, in small ventilted screen cages mating was not prevented but was somewhat delayed. In a field test using Mitsubishi ropes, over 97% mating disruption, as indicated by the reduction in trap captures, was achieved.

The sex pheromone components of Heliothis peltigera, a closely related species of H. armigera, which occurs in Israel, were identified. Z11-16:Ald and Z9-14:Ald (6:1) were identified as the major components. The addition of Z11-16:OH, another pheromonal component, to the binary mixture decreased the number of males of the sympatric H. armigera attracted. Sexual behavior tests indicated that most females called during the second half of the scotophase. Five to seven-days-old females called more frequently than 1-day-old females. Peak mating activity occurred between 5-8 hrs into scotophase. Peak flight activity of males was during the 6-8 hrs. into scotophase. No differences in flight activity between 3-8 days old males was detected.

The sex pheromone components of H. assulata, a species occurring in Thailand, were identified as Z9-16:Ald, Z11-16:Ald and 16:Ald (8:3:2). Field tests in Thailand confirmed these results.

I. Heliothis armigera (Hubner)

IA. Behavioral responses of male Heliothis armigera (Lepidoptera: Noctuidae) moths in a flight tunnel to combinations of components identified from female sex pheromone glands

INTRODUCTION

Compounds related to the sex pheromone of the cotton bollworm, Heliothis armigera (Hubner), have been identified in a number of studies. Five compounds were identified in gland extracts : (Z)-11-hexadecenal (Z11-16:Ald), (Z)-9-hexadecenal (Z9-16:Ald), hexadecenal (16:Ald), hexadecanol (16:OH), and (Z)-11-hexadecenol (Z11-16:OH) (Dunkelblum et al., 1980; Gothilf et al., 1978a, 1978b, 1979; Nesbitt et al., 1979, 1980, Piccardi et al., 1977, Rothschild 1978). In the present study, two additional compounds, namely, (Z)-7-hexadecenal (Z7-16:Ald) and (Z)-9-tetradecenal (Z9-14:Ald) were identified in our laboratory.

In field tests carried out in Israel, the combination of Z11-16:Ald and Z9-16:Ald (97:3) applied on rubber dispensers (2 mg/dispenser) effectively attracted H. armigera males (Kehat et al., 1980). The binary mixture caught more males than traps baited with live females; the other compounds had either no influence on trap catch or showed inhibitory properties. Therefore, this mixture is presently being used commercially in pheromone traps for monitoring H. armigera populations in Israel (Kehat et al., 1982). In this study we used a flight tunnel to complement the work that has been done in the field and to determine the role that each of these components plays in the sexual communication of H. armigera.

MATERIALS AND METHODS

Rearing

Heliothis armigera larvae were raised individually on a modified bean diet (Shorey and Hale, 1965). Pupae were segregated by sex, and the sexes were kept in separate chambers. Larvae and adults were maintained at 25±2°C in a L:D 14:10 regime. Adults always had access to a 10% sucrose solution.

Chemical Identification

Pheromone glands of 2- to 3-day-old virgin females were excised 6-7 h into scotophase and extracted with hexane for 15 min. Extracts (5-10 glands/sample) were concentrated to a small volume and analyzed by capillary GC on a Varian 3700 model equipped with a FID detector and a splitless injector system. A 30 m x 0.25 mm bonded cyanosilicone column (DB 225, J&W) was kept at 60°C for 2 min and then programmed to 150°C at the rate of 10°/min. The purge valve was opened after 1 min and the inlet helium pressure was 15 psi. Quantification of peak areas was performed with a Varian 4270 electronic integrator.

A combined extract of 25 glands was submitted to dimethyl disulfide (DMDS) derivatization (Buser et al., 1983; Dunkelblum et al., 1985). The DMDS adducts were analyzed by capillary GC-MS on Finnigan 5100 EI system at 70 eV, coupled with a Superincos data system. A 30 cm x 0.25 mm SE 54 column (J&W) was kept at 60°C for 2 min and then programmed to 230°C at the rate of 20°/min. The column was operated in the splitless mode, with a purge 30 sec after injection and a helium pressure of 10 psi.

Chemicals

All compounds used in the behavioral tests were from our stock and had an isomeric purity of 98-99% (as verified by capillary GC). Baits were prepared by loading rubber septa (Maavit, Tel Aviv, Israel) with a hexane solution

containing the synthetic compounds. The emission rate of Z11-16:Ald from the septa was measured with an airborne collection device. The system was built from a vertical charcoal filter, connected with a B24 joint to a horizontal chamber. The chamber was 10 cm long fitted with a B24 female-joint on one side and a B14 male-joint on the other side. The radius of the central part of the tube was 1 cm. The collection filter, filled with 0.7 g Porapak Q (50-80 Mesh), was connected with a B14 female-joint to the chamber. A pump (Charles Austen Pumps, Weybridge, England) connected through a Dweyer flow-meter, equipped with a valve for adjustment of the flow, was used to pull air through the system. Septa loaded with 0.5 mg, 1 mg and 2 mg of Z11-16:Ald were placed in the chamber and purified air was pulled through at a velocity of 0.3 m/sec. The emitted pheromone was collected for 1 h on the Porapak Q trap and then it was eluted with 4 ml ether. An internal standard (500 ng of Z8-13:Ac) was added and the solution was concentrated and analyzed by capillary GC.

Behavioral tests. Male moths were tested during the fifth through eighth hours of their 4th or 5th scotophase in a clear flight tunnel (0.5 m wide at the floor, 0.5 m high, 2.0 m long (0.4-0.5 m/sec wind velocity) modified after Miller and Roelofs (1978). Moths were kept in 30x30x30 cm cages in the tunnel room ($25\pm2^{o}C$, 0.3 lux light intensity) for at least 24 h prior to the start of the experiment. Five minutes prior to testing, individual moths were transferred into small galvanized mesh cages (6x6x6 cm) and placed on a tripod in the center of the pheromone plume, ca 20 cm upwind from the downwind end of the tunnel. The location of the plume was predetermined by a $TiCl_4$ smoke source. Baits were placed on a tripod in the center of the tunnel, ca 20 cm from the upwind side. At the beginning of the trial an impregnated septum was placed on the tripod and the cage (containing the male) was opened; the male was given 2 min to take

flight out of the cage. If found to be incapable of flying at all, the male was not scored and was replaced by a new moth. Males that were capable of sustained flight were observed until they left the pheromone plume and contacted the flight tunnel ceiling or flew out to the back of the flight tunnel. Those males capable of sustained flight were scored for the following behavior; (i) upwind flight in the pheromone plume but no closer than 50 cm from the pheromone source; (ii) flight to within 10 cm of the pheromone source; (iii) landing on the pheromone source; and (iv) after landing on the source, exhibition of copulatory response (curling of the abdomen). Each observation lasted for 2 min, after which the male was removed. In some tests only the copulatory response was recorded. All rubber septa were used within 2-5 days of initial loading. All statistical analyses were performed according to the method of adjusted significant levels for proportions (Ryan, 1960).

RESULTS

The composition of gland extracts was analyzed on a high resolution bonded capillary column capable of resolving most of the hexadecenals. The analysis confirmed the presence of the previously identified components (Dunkelblum et al., 1980, Nesbitt et al., 1979, 1980) and in addition revealed the presence of Z7-16:Ald and, in some extracts, Z9-14:Ald. DMDS derivatization of a pooled sample and subsequent GC-MS analysis established the identity of all monounsaturated compounds: Z9-14:Ald, Z7-16:Ald, Z9-16:Ald, Z11-16:Ald and Z11-16:OH. The diagnostic $RCHSCH_3^+$, $R'CHSCH_3^+$ and M^+ ions in the DMDS adducts were monitored by the multiple ion detection (MID) mode thus confirming the gross structure and location of the double bond in all components (Dunkelblum et al., 1985; Leonhardt and DeVilbiss, 1985). The relative composition of the gland extracts is presented in Table IA 1.

The emission rate of Z11-16:Ald, from rubber septa was measured in a flow system and the aldehyde was trapped on Porapak Q. There was no breakthrough of Z11-16:Ald as checked by connecting a second Porapak Q trap in line. The recovery of 200 ng Z11-16:Ald, deposited directly on the Porapak Q surface, was 69-74% (three replicas). The emission rate of each loading, 0.5, 1 and 2 mg of Z11-16:Ald, was measured three times and the rates were 1.94±0.19, 4.11±0.43 and 8.08±1.15 ng/min (X±SD) respectively.

When each compound was tested singly, at a loading of 2 mg/dispenser, Z11-16:Ald elicited significant levels of upwind flight no different from those obtained with the two- or three-compound mixtures. However, Z11-16:Ald, at the 2 mg/dispenser rate, elicited significantly fewer approaches to within 10 cm of the source, source contacts and copulatory responses than some of the compound mixtures tested (Table IA 2). Of the other compounds, Z9-16:Ald elicited low levels of upwind flight (10% directed flights) and copulatory attempts (one moth out of 30) and the other components (16:Ald, Z7-16:Ald) did not elicit any upwind flight.

The binary mixture of Z11-16:Ald and Z9-16:Ald (97.5:2.5) evoked high response levels in all categories of behavior, with 73% of the males attempting to copulate with the lure. Addition of 16:Ald to the binary mixture caused no increase over that of the binary mixture. Addition of Z7-16:Ald, Z9-14:Ald or Z11-16:OH to the binary mixture reduced significantly the levels of copulatory response (Table IA 2).

When the dosage of the binary mixture was reduced to 200 ug/dispenser (Table IA 3), the level of copulatory response, as compared with the 2000 ug/dispenser, was not reduced. The addition of 16:Ald to the binary mixture again caused no decrease or increase over that of the binary mixture. However, at this loading Z7-16:Ald did not cause any reduction in level of copulatory

response. The combination of Z11-16:Ald and Z9-16:Ald evoked response levels not significantly different from the four-compound mixture (Table IA 3).

When dosage of the binary mixture was reduced further, to 10 ug/dispenser, the level of copulatory response was significantly reduced as compared with that obtained at 2000 ug or 200 ug loading/dispenser. Again, the addition of 16:Ald had no effect on behavioral activity. However, at this dosage, the addition of Z7-16:Ald to the binary mixture increased significantly the level of response (Table IA 3).

DISCUSSION

The reinvestigation of the composition of gland extracts revealed the presence of the compounds listed in Table IA 1. The behavioral tests in the flight tunnel were based mainly on the four C16 aldehydes.

The main component Z11-16:Ald alone, at a loading of 2 mg/dispenser was capable of eliciting upwind flight and sometimes even copulatory behavior. However, all behavioral responses were increased significantly by the addition of 2.5% Z9-16:Ald. The saturated aldehyde 16:Ald alone, or in combination with the binary mixture, was inert with regard to H. armigera sexual communication, as similarly reported for H. zea (Vetter & Baker, 1984).

The effect of Z7-16:Ald on the copulatory response depended on the emission rate of the blend. At a high concentration of the binary mixture (2000 ug/dispenser), the addition of Z7-16:Ald (1%) reduced the level of copulatory response; at moderate concentrations (200 ug/dispenser) it did not have any affect; but at low concentrations (10 ug/dispenser) the copulatory response was increased significantly. This may indicate that at low pheromone concentrations, such as those possibly released by the female, Z7-16:Ald is an essential pheromonal component. Recently, the importance of minor pheromone components in

the behavioral analysis of males in the flight tunnel was demonstrated also in another Heliothis species, namely, in H. virescens (Teal et al., 1986).

The addition of Z9-14:Ald or Z11-16:OH to the binary mixture reduced significantly the upwind flight activity. Although found in the gland extracts of H. armigera, it is doubtful whether these compounds are also released by the female. The alcohol Z11-16:OH is probably the biosynthetic precursor of Z11-16:Ald (Teal and Tumlinson, 1987). The presence of Z9-14:Ald is surprising although its biosynthesis can be rationalized by B-oxidation of a Z11-16:precursor (Roelofs and Bjostad, 1984). These two components are important for H. peltigera and thus are responsible for the isolation between these two sympatric species (Dunkelblum and Kehat, 1989).

Comparison of the release rates of Z11-16:Ald from septa loaded with 2000, 1000 and 500 ug emitting approximately 8, 4 and 2 ng/min, respectively, indicates that a loading of 200 ug released less than 2 ng/min. The emission rates used in our dose-response experiments (8 ng/min to less than 2 mg/min) were, therefore, similar to those reported for other Heliothis species: 1.3 ng/min from live females of H. virescens (Teal et al, 1986), 3.26 ng/min from glands of H. virescens (Pope et al. 1982) and 0.89 g ng/min from glands of H. zea (Pope et al. 1984). In H. armigera a low loading of 200 ug/dispenser resulted in a very high response from males. Even a loading of 10 ug, which certainly released far less than 2 ng/min, stimulated more than 50% of the males to reach the bait. These observations indicate a very high sensitivity of H. armigera males to females emitting extremely low quantities of pheromone. Still, the concentration of the mixtures had an effect on the level of upwind flight and on copulatory response. At a low loading of the binary mixture (10 ug), copulatory response was reduced as compared with 200 ug/dispenser.

In our experiments, variation among male's response to calling females was very large, similar to that reported by Vetter and Baker, 1984, for H. zea. Since our main goal was to investigate the role of the different pheromonal components by measuring the increase or decrease in behavioral responses and copulatory attempts of males, no comparison between pheromone blends and live females or gland extracts was made in this study. However, it has been shown in field tests that only Z11-16:Ald and Z9-16:Ald are important as pheromone components (Kehat et al., 1980). This was confirmed in the present work by using a flight tunnel. The only exception found in this study was that in addition to Z11-16:Ald and Z9-16:Ald, Z7-16:Ald may also be considered as a pheromone component of H. armigera. However, for practical purposes of capturing males in traps, Z7-16:Ald would not be important and at high concentrations may even interfere with capture. All the other compounds had either no influence on male response or showed inhibitory properties.

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Tab. IA 1. Relative proportions of compounds identified from sex pheromone gland extracts[a]

Compound	Relative proportion (%)	Range (%)
Z9-14:Ald	1.5[b]	0-3.0[b]
16:Ald	3.5	2.1-7.0
Z7-16:Ald	0.6	0.4-0.9
Z9-16:Ald	2.5	1.5-3.1
Z11-16:Ald	100	
Z11-16:OH	5.5	4-7.8

[a] Analysis of five samples, each one contained 5-10 glands.

[b] Present occasionally, however, identity confirmed in a pooled sample by the DMDS method.

Tab. IA 2. Response (%) of male Heliothis armigera in a wind tunnel to one or more compounds identified from the female sex pheromone gland.

Treatments (ug)[a]						Number[b] tested	% Response[c]			
Z11-16:Ald.	Z9-16:Ald	Z7-16:Ald	16:Ald	Z9-14:Ald	Z11-16:OH		Directed flight	Within 10 cm of source	Source contact	Copulation attempt
2000	-	-	-	-	-	30	85.0a	23.3bcd	23.3b	20.0b
-	2000	-	-	-	-	30	10.0b	3.3d	3.3b	3.3b
2000	50	-	-	-	-	37	100 a	94.6a	89.2a	73.0a
2000	50	-	100	-	-	40	100 a	95.0a	87.5a	77.5a
2000	50	20	-	-	-	36	91.6a	47.2b	19.4b	5.5b
2000	50	-	-	100	-	25	88.0a	12.0cd	4.0b	0 b
2000	50	-	-	-	100	54	83.3a	37.0bc	3.7b	0 b

[a]Z7-16:Ald or 16:Ald alone, at a rate of 2000 ug/dispenser, did not elicit any up wind flight.

[b]These numbers do not include moths that, for the entire 2-minute observation period, did not fly at all (0-15%).

[c]Percentages followed by the same letter are not significantly different ($P<0.05$) according to the method of adjusted significance levels for proportions (Ryan, 1960).

Tab. IA 3. Copulatory response of male Heliothis armigera in a wind tunnel to low dosages of mixtures of compounds identified from the female sex pheromone gland.

Treatments (ug)				Number[a] tested	% Response[b]
Z11-16:Ald	Z9-16:Ald	Z7-16:Ald	16:Ald		
200	5	-	-	35	85.7a
200	5	2	-	48	70.8ab
200	5	-	10	33	78.7a
200	5	2	10	48	70.8ab
10	0.25	-	-	50	4.0d
10	0.25	-	0.50	40	7.5d
10	0.25	0.10	-	38	36.8c
10	0.25	0.50	-	40	50.0bc
10	0.25	0.10	0.50	40	50.0bc

[a]These numbers do not include moths that, for the entire 2-minute observation period, did not fly at all (0-15%).

[b]Percentages followed by the same letter are not significantly different ($P<0.05$) according to the method of adjusted significance levels for proportions (Ryan, 1960).

IB. ANALYSIS OF GLAND EXTRACTS OF HELIOTHIS ARMIGERA FROM THAILAND

For comparison of pheromone gland content of Heliothis armigera females from Thailand, several gland extracts of 3-day-old females were prepared in Bangkok and shipped to Bet Dagan for chemical analysis.

Fourteen samples of pheromone gland extracts of Heliothis armigera from Thailand were analyzed. To each extract an internal standard (100 ng of Z8-13:Ac) was added and the samples were concentrated to a small volume for GL analysis. All samples were analyzed on a DB 225, 30 m x 0.25 mm column and the pheromone content was monitored with the main component Z11-16:Ald. Most samples had considerable amounts of unidentified compounds. The results are summarized in the Table IB 1.

Samples 5, 9, 10 and 13 were examined for additional components and they contained 5-7% of Z9-16:Ald which is the second essential component in the pheromone blend of H. armigera in addition to the main component Z11-16:Ald. This relative amount is larger than that of 2-3% in the H. armigera females in Israel. The additional minor component, Z7-16:Ald, was not observed.

The residues from the four samples were combined and subjected to DMDS derivatization and subsequent GL-MS analysis. the presence and identity of the two pheromone components Z9-16:Ald and Z11-16:Ald was confirmed. The minor component Z7-16:Ald was not detected.

Tab. IB 1: Amount and ratio of pheromone components in Heliothis armigera gland extracts from Thailand (the results of only 7 samples were presented)

Sample	No. ♀	Date	Z11-16:Ald/♀	Z9-16:Ald*	Z11-16:OH*
5	18	20.9	21 ng	7%	2%
6	14	19.8	8 ng		
7	12	18.8	10 ng		
9	20	18.8	38 ng	7%	7%
10	12	17.8	58 ng	5%	5%
13	20	18.8	66 ng	5%	2.5%
14	9	16.9	7 ng		

*Relative amounts (%) with respect to Z11-16:Ald

IC. Age structure and nocturnal behavior of adult *Heliothis armigera* (Lepidoptera: Noctuidae) in cotton fields in Israel

Introduction

The cotton bollworm, *Heliothis armigera* (Hubner), is a polyphagous pest of economic importance in Asia, Africa, Europe and Australia (Avidov & Harpaz, 1969). Current methods to control this pest are not always effective and alternative control measures are needed. The use of sex pheromones may supplement or provide an alternative to existing control practices. The sex pheromone components of *H. armigera* have been identified in a number of studies (Dunkelblum et al., 1980; Gothilf et al., 1978a, 1978b, 1979; Kehat et al., 1980, 1982; Nesbitt et al., 1979, 1980; Piccardi et al., 1977; Rothschild, 1978). Nevertheless, knowledge on additional aspects of the pest's nocturnal behavior, i.e., emergence, copulation, oviposition and flight activity, is needed before control methods employing sex pheromones can be used successfully. Other factors that may affect the performance of pheromones as mating disruptants are age structure (sex ratio and mating status of females) and size of the population.

Studies describing the noctural behavior of *H. armigera* adults under field (Roome, 1975; Topper, 1987) or laboratory (Hackett and Gatehouse, 1982; Kou and Chow, 1987a, 1987b) conditions are very limited. Information regarding age structure of *H. armigera* populations is also restricted. The present study attempted therefore to clarify, by direct night observations, the following: (a) age structure of adult *H. armigera* populations in cotton during early and mid season; (b) nocturnal behavior of *H. armigera* native adults in the field; and

(c) mating activity of laboratory reared adults (confined in small cages) under outdoor conditions and the effect of pheromone applications on activity.

Materials and Methods

The nocturnal observations were conducted in cotton fields at Yizre'el, between 4 June and 2 July 1984, and at HaBonim between 14 Aug. and 31 Aug. 1986. Using head lanterns (6V), three or four observers walked through the observation plots (2 ha each) and collected single insects and mating pairs of H. armigera. Observations started at 21:00 h and continued until 04:00 h during 1984; each observation lasted for 30 min and six such observations were carried out throughout the night. Only two observations, each lasting 30-60 min, were performed per night during 1986. All collected females were dissected to determine their mating status (the presence of a spermatophore indicating that mating had occurred). For male catches, five traps baited with synthetic pheromone (Kehat et al., 1982) were positioned in each plot.

To determine mating of laboratory-reared H. armigera, males and females were kept together in ventilated screened cages (30 x 30 x 30 cm) under outdoor conditions. The moths used were reared on a modified bean diet (Shorey and Hale, 1965) and the sexes maintained separately at 25±1°C in a LD 14:10 regime. Adults always had access to a 15% sucrose solution. The moths used in the experiments were of different ages, 1-5 days old. Fifteen to twenty pairs were confined in each screen cage, and four to six cages were usually observed together on the same night. The cages were observed twice every hour and the mating pairs were removed. Sometimes these pairs were kept under continuous observation (25±1°C) to determine the duration of mating. Observations were continued throughout the night.

The effect of pheromone applications on mating activity of H. armigera was studied in males and females placed together (10 pairs in a cage) in screened cages (30 x 30 x 30 cm) under outdoor conditions. Pheromone applications were 20, 40 or 300 mg/cage (loaded on dental rolls and hung in the center of the cage). Mating activity was observed directly twice every hour throughout the night.

Results and Discussion

(a) Age structure of populations during early and mid season

The cotton bollworm, H. armigera, is a polyphagous pest. Its host plants in Israel differ according to the season. During early summer, until late June, it attacks wild plants, tomatoes, peppers, cabbage, kidney beans, chickpeas, safflower and other plants (Avidov and Harpaz, 1969). Nevertheless, eggs, larvae or pupae of H. armigera have never been found prior to late May in cotton fields. This already suggests that larval infestations occurring in cotton during mid June - mid July (most H. armigera infestations in cotton in Israel occur during that period) are the result of H. armigera females moving into cotton fields from other host plants, and laying their eggs on cotton plants.

The adult population density at Yizre'el during June 1984 was relatively low, as indicated by the small numbers of males and females collected on plants. Trap captures of males, however, were quite high. A considerable number of collected females were already mated (Table IC 1). The relatively low level of virginity and the preponderance of single females that were multiple-mated indicate an old adult population. Since the number of sexually attractive females in the population was relatively small compared with the number of active males that could mate, captures of males in pheromone traps were high. This also indicates an old adult population which probably had moved into the

field from other areas. Moreover, since the egg and larval infestations that followed were very low, and there was no need for any chemical applications, it seems that most females moved into the Yizre'el cotton field as mated females after having laid most of their eggs somewhere else. Therefore, it appears that the level of infestation in different cotton fields during mid June - mid July will depend, among other factors, on the density and age structure of the adults (young or old) invading the field. At that period pheromonal applications for mating disruption will be effective only if most of the invading females are still virgin and ineffective if most are already mated.

The adult population density in a cotton field at HaBonim during August 1986 (Table IC 2) was higher than that detected at Yizre'el during June 1984 (Table 1). Trap captures of males, however, were quite low. Most females collected on Aug. 14 and Aug. 19 were virgin, whereas a few days later (Aug. 25) most females were already mated (Table IC 2). The adult population at HaBonim during mid August 1986 was young, as indicated by the large number of virgin females in the population. Since the number of sexually attractive females in the population was relatively high, captures of males in pheromone traps were relatively low, which indicates, again, a young adult population. Many of the observed females were docile and some of them with their wings still extended directly overhead, typical of newly emerging adults. It appears, therefore, that while in June H. armigera young or old females are moving into cotton fields, laying eggs there and thus beginning the build up of a new population, the adults detected in cotton during August are mostly the result of local larval populations which have developed already in cotton.

(b) Nocturnal behavior of *H. armigera* adults

Diel patterns of nocturnal behavior of *H. armigera* adults during June 1984 were recorded systematically and are presented in Table IC 3. Relatively few males were collected on plants before 22:00 h, indicating that most males were then flying probably in search of food or mating partners. More males were collected on plants between 22:00 and 24:00 h indicating that many males had settled down on the plants. This is inferred also from the relatively small numbers of males captured then in traps. During 24:00-04:00 h, flight activity of males increased again, as indicated by the reduction in the percent of males collected on plants and by the increased numbers of males captured in traps.

No females were collected on plants before 22:00 h, indicating increased flight activity of females during the early evening, as detected also for the males. Later on (22:00-24:00 h), females settled down on the plants and relatively high numbers were collected between 24:00 and 03:00 h. Mating pairs were observed only after 23:00 h and peak copulation occurred between 02:00-03:00 h.

These results indicate early night activities for the search of food and oviposition sites and late night activities for sexual communication and copulation. The present findings are in general agreement with those reported by Roome (1975) and Topper (1987) for *H. armigera* and by Lingren et al. (1982) for *H. virescens*. However, results obtained in Aug. 1986 (Table IC 2) indicate that most matings (of the second generation) took place within the first half of the night, which is in contrast to the results obtained by us during June 1984 (first generation). Mating activity in the field throughout the cotton season should therefore be studied further.

(c) Mating activity of laboratory reared *H. armigera* adults

Results regarding mating activity of laboratory-reared *H. armigera* adults (kept in small ventilated screen cages outdoors) are presented in Tables IC 4 and IC 5. Results indicate the following: (i) In 2- to 4-day-old moths most matings occurred during the second half of the night; similar results were reported by Roome (1975) and by Topper (1987). Mean duration of mating (for 40 2- to 3-day-old pairs) was 81.3 min (40-150 min); similar durations were reported by Kou and Chow (1987a). (ii) Percent mating increased with age, and was 6.0, 26.3 and 66.7 in 1-, 2- and five-day-old moths, respectively. (iii) Five-day-old moths began to mate soon after darkness and continued to mate throughout the night; Kou and Chow (1987a) also reported that as moths aged, mating commenced earlier in the scotophase.

The effect of pheromone applications on mating activity of 5-day-old laboratory-reared adults (kept in small ventilated screen cages under outdoor conditions) is shown in Table IC 6. Pheromone applications, even at high dosages (300 mg/cage), were ineffective as mating disruptants. This indicates that once males and females are close together (=high population densities), pheromone applications are no longer effective in preventing mating. However, in a pheromone-permeated atmosphere, matings were somewhat delayed during the night.

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Table IC 1. Density, and age structure of adult Heliothis armigera population in cotton (Yizre'el, June 1984) (Data reflect ten nights of observation)

Date	No. of singles collected* ♂	♀	% Virgin females	Mean no. of spermato-phores	No. of mating pairs collected	Mean No. of ♂♂ collected/ observer/ 30 min	Total No. ♂ captured trap/night
1.VI.84	0	0	-	-	0	0	4.8
4.VI.84	1	1	0	1.0	0	0.1	7.4
10.VI.84	7	1	0	1.0	1(4.0)**	0.5	14.4
12.VI.84	5	6	16.6	1.8	1(2.0)	0.7	21.0
14.VI.84	3	7	14.3	2.1	1(1.0)	0.7	44.0
19.VI.84	10	8	50.0	0.9	3(2.0)	1.3	27.0
21.VI.84	9	6	33.3	1.2	3(1.7)	1.2	41.1
25.VI.84	2	9	22.2	1.3	5(2.0)	1.2	54.1
28.VI.84	0	6	33.3	2.0	0	0.3	29.1
2.VII.84	0	1	100.0	0	1(1.0)	0.1	4.3

*Three observers for 6x30 min; observations were performed from 9 pm to 4 am.

**Numbers in parentheses indicate mean number of spermatophores in a copulating female.

Table IC 2. Density, sex ratio, virginity of females, and mating of the adult Heliothis armigera population in cotton (HaBonim, 1986) (Data reflect four nights of observations)

Date	Hours of observation	No. of singles collected* ♂	♀	% Virgin ♀	Mean no. of spermatophores	No. of pairs collected*	Mean No. collected/ observer/ 30 min	Total No. ♂ trap/night
14.VIII	23:20-23:50	21	15	86.6	0.2	4	11.0	7.2
	01:30-02:30	-	-	-	-	2	-	
19.VIII	23:15-24:15	14	16	81.2	0.3	12	6.7	14.0
	02:15-02:45	12	11	90.9	0.1	1	6.2	
25.VIII	22:20-23:20	12	12	25.0	0.9	12**	6.0	5.2
	01:40-02:40	4	9	11.1	1.4	0	1.6	
31.VIII	21:15-22:15	0	1	0	1.0	0	0.1	1.4
	23:00-24:00	1	1	0	1.0	0	0.5	
	Total	64	65					

*By four observers. On Aug. 14 between 01:30 and 02:30, only pairs were collected.

**Mean no. of spermatophores = 1.7 (1-6).

Table IC 3. Diel patterns of nocturnal behavior of adult Heliothis armigera in cotton, Yizre'el, 1984 (Data reflect ten nights of observation (1.VI-2.VII.84).

Status of adults collected	Total collected*	% Collected at different hours of the night							
		20 22	22 23	23 24	00 01	01 02	02 03	03 04	04 05
♂ on plants	37	8.1	18.9	27.0	16.2	16.2	10.9	0	2.7
♂ in traps	716	2.3	9.6	9.6	18.7	18.7	13.2	17.3	10.6
♀ on plants	45	0	4.4	13.3	28.9	28.9	20.0	4.5	0
Mating pairs	15	0	0	13.3	13.3	13.3	46.7	6.7	6.7

*Three observers for 6x30 min.

Table IC 4. Mating of laboratory-bred Heliothis armigera adults in screened cages under outdoor conditions (21 July, 11 and 14 Sept. 1986, Bet Dagan)

No. of pairs tested*	91
Total no. of pairs mated	52
No. of pairs mated at 0-3 h into scotophase	0 (0)**
3-4 h into scotophase	2 (3.8)
4-5 h into scotophase	3 (5.8)
5-6 h into scotophase	6 (11.5)
6-7 h into scotophase	8 (15.4)
7-8 h into scotophase	12 (23.1)
8-9 h into scotophase	13 (25.0)
9-10h into scotophase	8 (15.4)
% Mated (total)	57.1
% Mated during first 4 h of darkness	3.8
% Mated during last 6 h of darkness	96.2

*3-4 days old

**Numbers in parentheses indicate percent mating of the total.

Table IC 5. Mating of laboratory bred Heliothis armigera adults in screen cages under outdoor conditions (Bet Dagan, 1987).

Dates of Experiments:	1,4 and 7 June; 9 July					16,17 and 18 Aug.
Age of moths (days):	1	2	3	4	5	5
Total No. of pairs tested	100	144	70	114	90	110
Total No. of pairs mated	6	38	29	61	60	71
No. mated at 0-2 h into scotophase	1	5	5	10	19	32
2-3 h into scotophase	0	2	1	2	6	12
3-4 h into scotophase	1	6	4	5	9	8
4-5 h into scotophase	2	7	4	12	11	5
5-6 h into scotophase	0	6	7	13	8	6
6-7 h into scotophase	0	5	4	8	5	6
7-8 h into scotophase	1	6	4	10	2	2
8-9 h into scotophase	1	1	0	1	0	0
% Mated (total)	6.0	26.3	41.4	53.5	66.7	64.5
% Mated during first 4 h	33.3	34.2	34.5	27.8	56.7	73.2
% Mated during last 5 h	66.4	65.8	65.5	72.2	43.3	26.8

Table IC 6. Effect of pheromonal application on mating of Heliothis armigera adults (in small screened cages, 30x30x30 cm; Bet Dagan, 1987)

Date	Pheromone (mg)	No. of pairs tested*	% Mated	
			Total**	During first 3 h
16.VIII.87	20	10x3	40.0	16.7
	40	10x3	43.3	7.7
	Control	10x4	77.5	67.7
17.VIII.87	300	10x4	50.0	10.0
	Control	10x3	70.0	61.9

*Number of pairs in a screen cage x number of cages.

**No significant differenences according to the χ^2 test of independence.

ID. Night observations in cotton fields in Thailand

(a) 1987 experiments

The present work attempted to clarify, by direct observations relationships between trap captures of males and the actual adult field population. Night observations on the cotton bollworm, *H. armigera* were conducted in cotton plots in which males and females on plants and males caught in traps were collected. These observations were carried out at Nakon Sawan Field Crop Research Center, Thailand, between 10 Sep. to 17 Dec. 1987. Using head lanterns, 6 observers walked through the plot (1.92 ha) and collected single insects and mating pairs of *H. armigera*. Observations were carried out at 19:00, 20:30, 22:00 and 23:30. Each observation per observer lasted for 30 mins. All collected females were dissected to determine their mating status (the presence of a spermatophore indicating that mating had occurred). Five traps were placed in the plot and counting of captured males were taken every 3-4 days.

Field readings of single insects collected on plants, and of males captured in traps are shown in Fig. ID 1 and Tab. ID 1. Adults were scarce in the field and only few males were caught in traps until 1 Oct. This indicates a positive relationship between captures in traps and the actual population in the field. During Oct. and Nov. many moths were collected on plants and captures of males in traps increased indicating, again, the general trend of a positive relationship between captures in traps and the actual population in the field. However, results also indicated that increase in female population in the field was accompanied with a decrease in captures of males in traps (mid Sep., mid Oct., early Nov., mid Dec.). Apparently competition for males by attractive females reduced the efficacy of pheromone traps. As the density of attracting

females decreased, more males responded to the pheromone trap. This was in particular obvious during late Nov. - early Dec. when captures of males were very high while the actual population in the field was low. This indicates that there may be no correlation between male captures and infestation by adults or larvae. Therefore, pheromone traps can be used as a warning device indicating potential attack, but larval counts are required before decissions can be made regarding control procedures.

Percent of virgin females and mean number of spermatophore/females (Tab. ID 1) indicate the existence of mature population in the experimental field throughout the observation period. Probably mated females were repeatedly invading the field thus indicating that mating disruption should be applied on a wide scale and never be confined to single small plots. On 15 Oct, however, percentage of virgin females in the experimental plot increased (Tab. ID 1) indicating, probably, the emergence of new females and a beginning of a new generation, originating from the experimental field.

Results in Tab. ID 2 and Fig. ID 2 indicate peak flight activity during early evening. At 20:30 h activity declined considerably and reached a minimum at 23:30 h. Activity during later hrs of the night was not observed at this study. Anyway, the present results indicate that feeding and probably egg laying activities occurs just after onset of darkness whereas mating activity occurs later at night (after 02:00).

(b) <u>1988 Experiments</u>

Night observations were continued also in 1988 (2 Sep. to 8 Dec.) and were performed in the same plots and with the same methods as those described for 1987 experiments. However, in 1988 observations were carried out throughout the night (from 19:00-04:30 h) and were not stopped at midnight. Unfortunately,

captures in pheromone traps throughout the 1988 season were very low, probably due to some deficiency in the pheromonal components, and results regarding trap captures were therefore discarded.

Field readings of eggs, single insects and mating pairs collected on plants are shown in Tab. ID 3 and Fig. ID 3. Positive relationship between number of eggs, females and mating pairs collected on plants was detected (Fig. ID 3). Adult population was low up to late Sep-early Oct, and increased during Oct. and Nov. (similarly to the results obtained in 1987); three distinct generations were detected.

Percent of virgin females and mean number of spermatophores/female (Tab. ID 3). indicate the existence of mature population in the experimental field up to 18 Nov. Probably mated females were repeatedly invading the field as found also in 1987. From 18 Nov., however, percentage of virgin females in the experimental plot increased (Tab. ID 3) indicating the emergence of new females and the beginning of a new generation.

Diel patterns of nocturnal behavior of H. armigera adults (Tab. ID 4, Fig. ID 4) indicate early night activities for the search of food and oviposition sites and late night activities for sexual communication and copulation. Thus, activity of males and females was highest at 19:00-19:30 h and declined considerably later on. This decline in activity was particularly obvious in mated females (that searched only for food and oviposition sites) and less in males or virgin females (Fig. ID 4). The percentage of virgin females collected, out of the total number of females collected, increased towards the end of the night paralell to the increase in the number of mating pairs collected (Tab. ID 4).

(c) 1989 experiments

Results obtained in 1989 confirm, generally, most results obtained during the past two years: Positive relationship was detected between number of eggs, females, males and mating pairs (Tab. ID 5, Fig. ID 5). An early generation was detected also in August. Mating activity took place during the second half of the night and reached its peak between 02^{00}-04^{00} (Table ID 6, Fig. ID 6).

Table ID 1. Number of Heliothis armigera single insects (and mating status of females) collected on cotton plants and number of males captured in traps. (Nakon Sawan, Thailand, 1987).

Date	Total* moths collected	♂	♀	ratio ♂ ♀	% virgin	Mean no. spermato-phore/ ♀	♂/trap night
10.9	55	10	45	1:4.5	6.6	2.3	
14	58	15	43	1:2.8	9.3	1.9	4.5
19	73	23	50	1:2.1	22.0	1.5	5.8
24	16	6	10	1:1.6	20.0	1.3	3.2
1.10	28	9	19	1:2.1	21.0	1.4	0.7
8	108	41	67	1:1.6	16.4	1.9	17.3
15	90	40	50	1:1.2	58.0	0.6	18.0
22	232	131	101	1:0.8	20.8	1.3	6.5
29	211	93	118	1:1.2	20.3	1.5	27.6
5.11	378	168	210	1:1.2	16.6	1.6	11.9
12.11	125	40	85	1:1.9	12.9	2.3	39.7
19	126	44	82	1:1.8	21.9	1.5	5.0
26	165	63	102	1:1.6	19.6	1.4	33.8
4.12	114	20	94	1:4.7	8.5	2.1	76.5
9	27	7	20	1:2.8	10.0	1.9	67.5
17	18	5	13	1:2.6	15.3	2.0	54.5

*Total collection time (between 19:00 and 23:00) was 80 mins, (4x30) per observer. (6 observers)

Table ID 2. Number of Heliothis armigera males and females collected (between 19:00 h and 23:30 h) in the experimental field. (Nakon Sawan, Thailand, 1987).

Time*	19:00			20:30			22:00			23:30		
	♂	♀	total	♂	♀	total	♂	♀	total	♂	♀	total
10.9	2	12	14	3	12	15	5	13	18	0	8	8
14	2	17	19	3	8	11	3	7	10	7	11	18
19	4	15	19	4	8	12	8	14	22	7	13	20
24	1	6	7	0	4	4	1	0	1	4	0	4
1.10	4	13	17	0	1	1	3	3	6	2	2	4
8	18	27	45	5	19	24	10	10	20	8	11	19
15	18	30	48	10	10	20	8	3	11	4	7	11
22	67	37	104	25	38	63	20	16	36	19	10	29
29	49	51	100	19	35	54	10	21	31	15	11	26
5.11	70	73	143	18	45	63	48	66	114	32	28	60
12	24	50	74	6	11	17	7	14	21	3	10	13
19	5	25	30	12	15	27	16	26	42	11	16	27
26	7	39	46	16	17	33	12	26	38	28	20	48
4.12	12	67	79	3	14	17	1	10	11	4	3	7
9	5	15	20	1	2	3	1	2	3	0	1	1
17	2	9	11	0	2	2	2	1	3	1	1	2
Total	290	486	776	125	241	366	155	232	387	145	152	297

*On each observation 6 observers were scouting the field for 30 mins.

Table ID 3. Number of Heliothis armigera single insects (and mating status of females) and mating pairs collected on cotton plants (Nakon Sawan, Thailand, 1988)

Date	Moths collected*			Ratio		% Virgin	Mean sperma-tophore/♀	No. pairs collected
	Total	♂	♀	♂	♀			
18.8	105	47	63	1	1.2	20.7	1.4	3
26.8	67	9	58	1	6.4	12.0	1.8	6
2.9	18	5	13	1	2.6	0	1.7	0
9	25	7	20	1	2.6	10.0	2.3	0
16	43	10	33	1	3.3	21.2	1.3	2
23	117	37	80	1	2.1	21.2	1.9	4
28	286	139	147	1	1.0	23.1	1.6	5
6.10	38	10	28	1	2.8	25.0	1.8	1
14	195	55	140	1	2.5	43.5	0.8	5
21	498	231	267	1	1.1	33.7	1.3	18
27	418	209	209	1	1.0	23.4	1.8	7
4.11	18	7	11	1	1.5	45.4	1.4	1
11	13	7	6	1	0.8	14.3	1.5	0
18	50	31	19	1	0.6	78.9	0.3	2
24	373	147	226	1	1.5	90.2	0.1	13
1.12	123	82	41	1	0.5	75.6	0.4	5
8	50	30	20	1	0.7	80.0	0.3	4
Total	2444	1063	1381	1	1.3	42.0	1.2	46

*6 observers; total collection time per observer was 7x30 min

Table ID 4. Density, sex ratio, virginity of females and mating of adult *Heliothis armigera* at different times throughout the night (Data reflect 17 nights of observations in a cotton field at Nakon Sawan, Thailand, between 18.8-8.12.1988).

Time (h) of observation	19:00 19:00	20:30 21:00	22:00 22:30	23:30 24:00	01:00 01:30	02:30 03:00	04:00 04:30	19:00-04:30
Number of moths collected*	801	341	253	284	321	259	185	2444
" " ♂ "	323	130	89	143	157	133	88	1063
" " ♀ "	478	211	164	141	164	126	97	1381
" " virgin ♀ "	127	64	61	72	96	84	77	581
" " pairs "	0	0	5	0	11	24	36	76
Ratio ♀/♂	1.5	1.6	1.8	1.0	1.0	0.9	1.1	1.3
% virgin ♀ (out of ♀)	26.5	30.3	37.2	51.0	58.5	66.6	79.4	42.0
% of paris (of total pairs)	0	0	6.6	0	14.5	31.5	47.4	100.0

*By six observers

Table ID 5. Number of Heliothis armigera single insects (and mating status of females) and mating pairs collected on cotton plants (Thailand 1989)

Date		Total*	Males	Females	Ratio M/F	% Virgin	No. of pairs	Eggs
Aug.	3	173	57	116	0.49	16.7	6	12
	11	793	274	519	0.53	14.7	18	48
	18	1139	311	828	0.38	7.1	12	296
	26	1716	518	1198	0.43	7.1	52	264
Sep.	2	503	222	281	0.79	7.7	22	65
	9	384	108	276	0.39	9.4	17	63
	15	650	210	440	0.48	8.1	19	11
	22	161	67	94	0.71	4.3	8	33
	28	164	77	87	0.89	11.6	5	23
Oct.	6	856	378	478	0.79	32.8	11	9
	13	910	398	512	0.78	12.0	39	142
	19	170	71	99	0.72	7.1	3	59
	27	288	142	146	0.97	14.9	3	6
Nov.	3	116	44	72	0.61	25.8	5	23
	10	262	128	134	0.96	31.7	3	21
	17	214	116	98	1.18	27.6	2	5
	24	178	101	77	1.31	12.9	4	5
Total		8677	3222	5455	0.59		226	

*Collected by six observers within 3.5 hr. (7 times x 30 min).

Table ID 6. % collected out of total collected

Time	Male	Female	Pair	% M	% F	%P
19.00	597	1442	0	18.5	26.4	0.0
20.30	380	884	2	11.8	16.2	0.9
22.00	357	735	6	11.1	13.5	2.7
23.30	392	700	14	12.2	12.8	6.2
1.00	480	647	28	14.9	11.9	12.4
2.00	453	523	80	14.1	9.6	35.4
4.00	560	527	96	17.4	9.7	42.5
Total	3219	5458	226	100.0	100.0	100.0

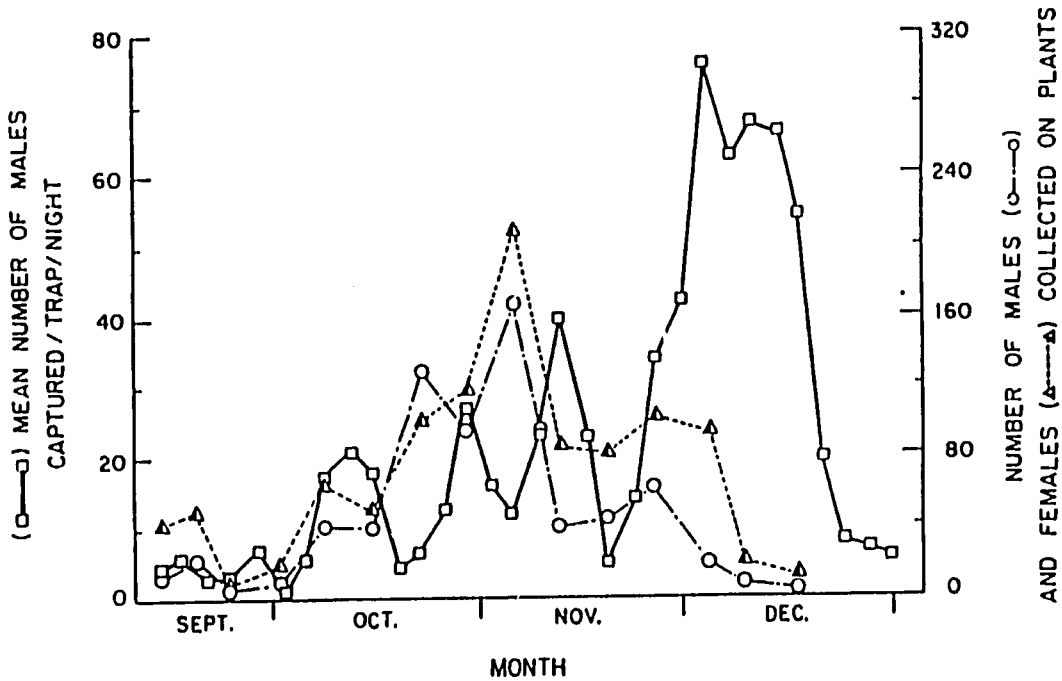

Fig. I D 1. Relationship between number of H. armigera males captured in traps and males and females collected on cotton plants (Thailand 1987).

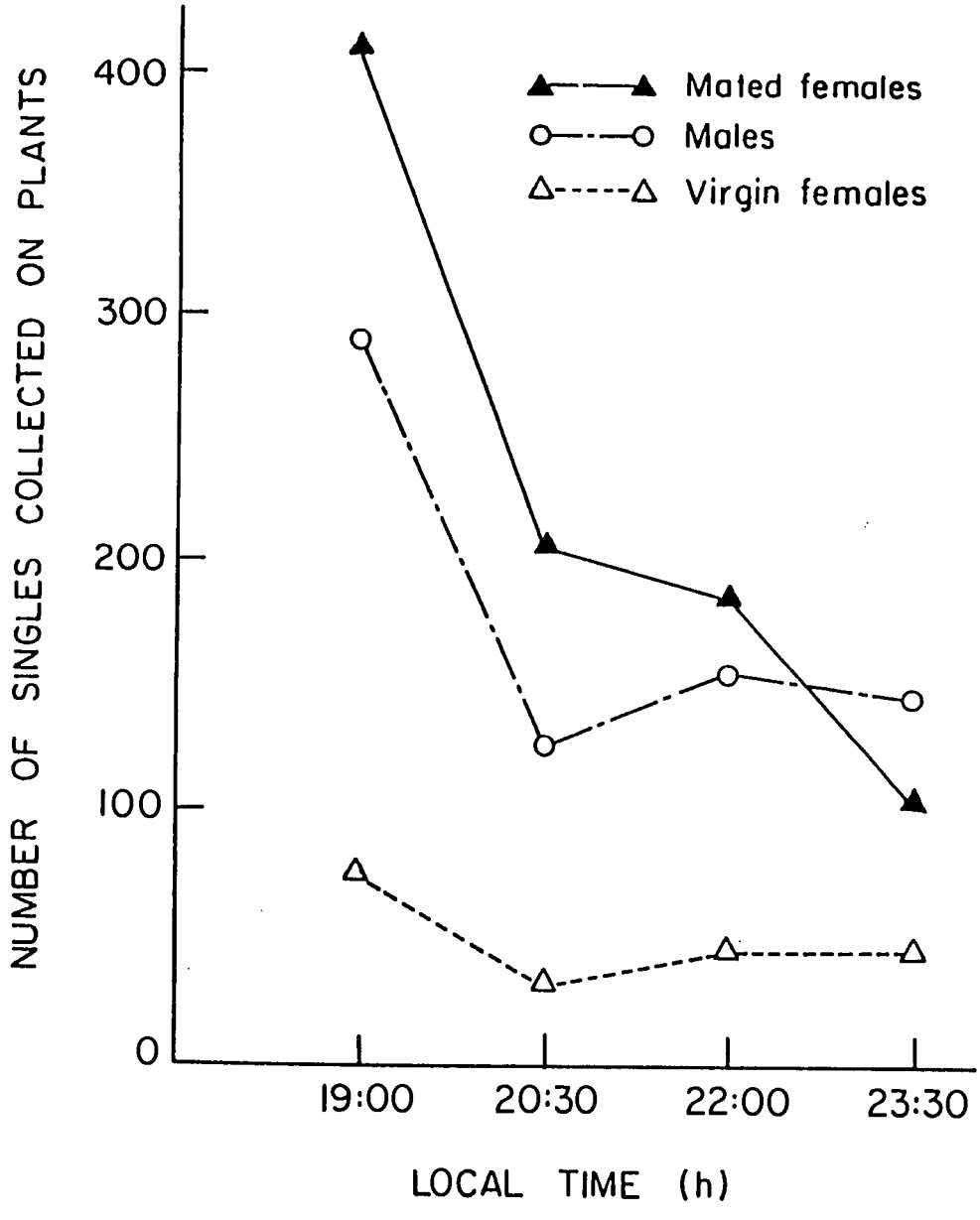

Fig. I D 2. Nocturnal activity of H. armigera males and females in the field (Thailand 1987).

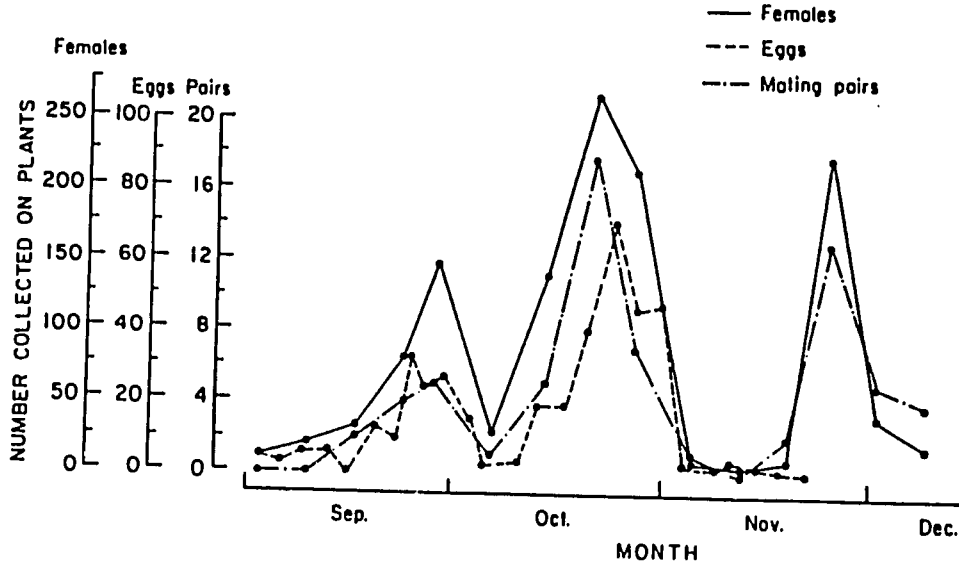

Fig. I D 3. Relationship between number of H. armigera eggs, females and mating pairs collected on cotton plants (Thailand 1988).

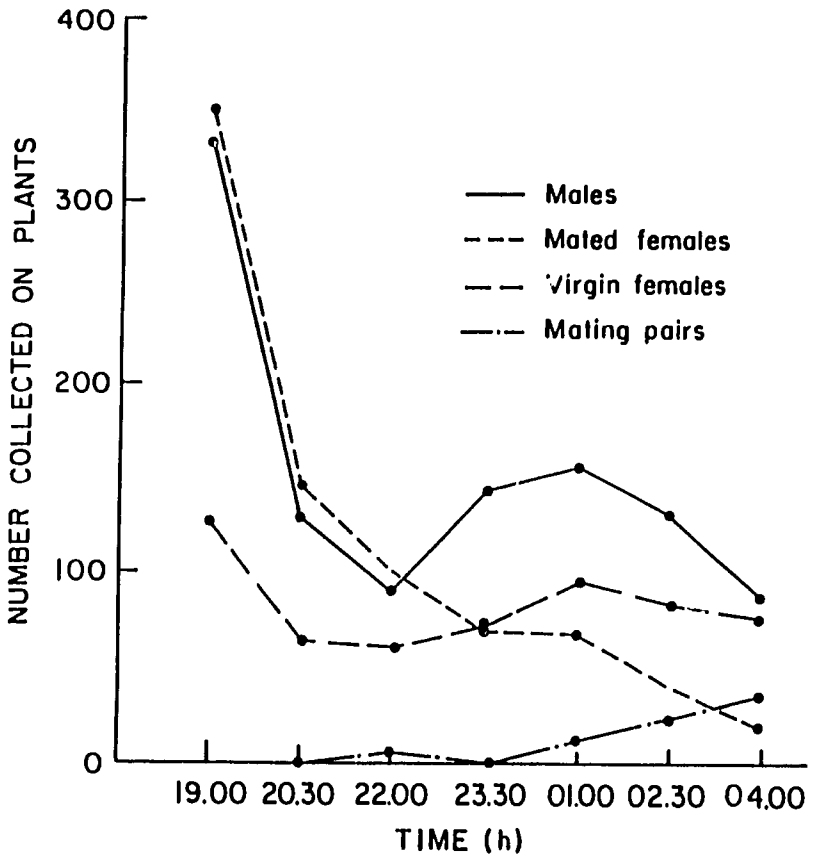

Fig. I D 4. Nocturnal activity of H. armigera males, mated females, virgin females and mating pairs in a cotton field (Thailand 1988).

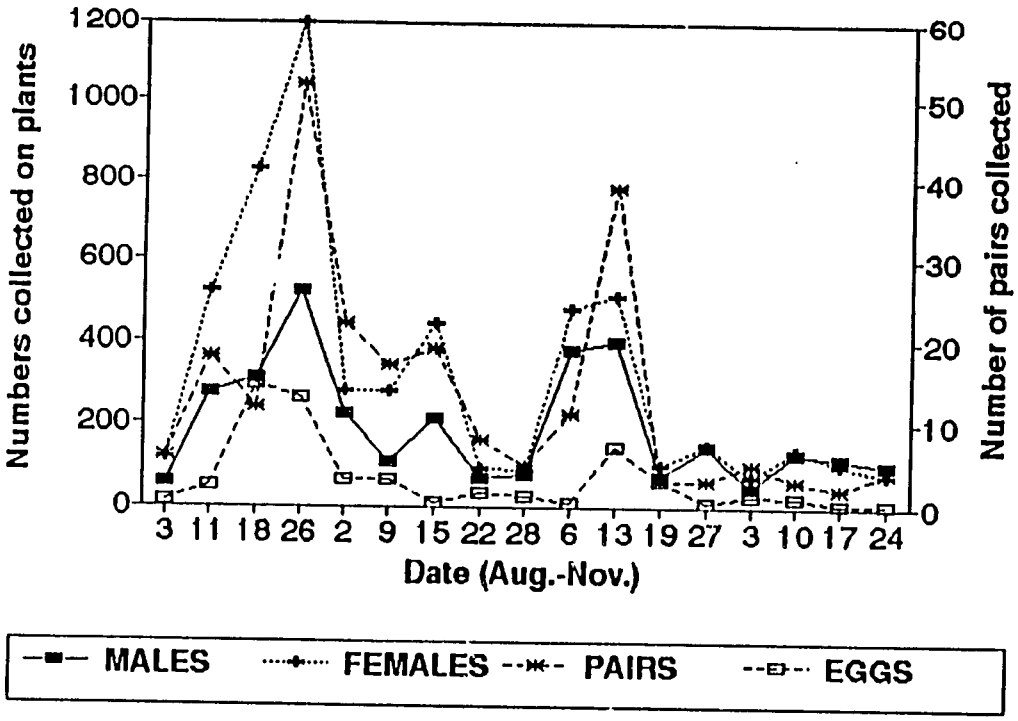

Fig. I D 5. Relationship between numbers of H. armigera eggs, males, females and mating pairs collected on cotton plants (Thailand 1989).

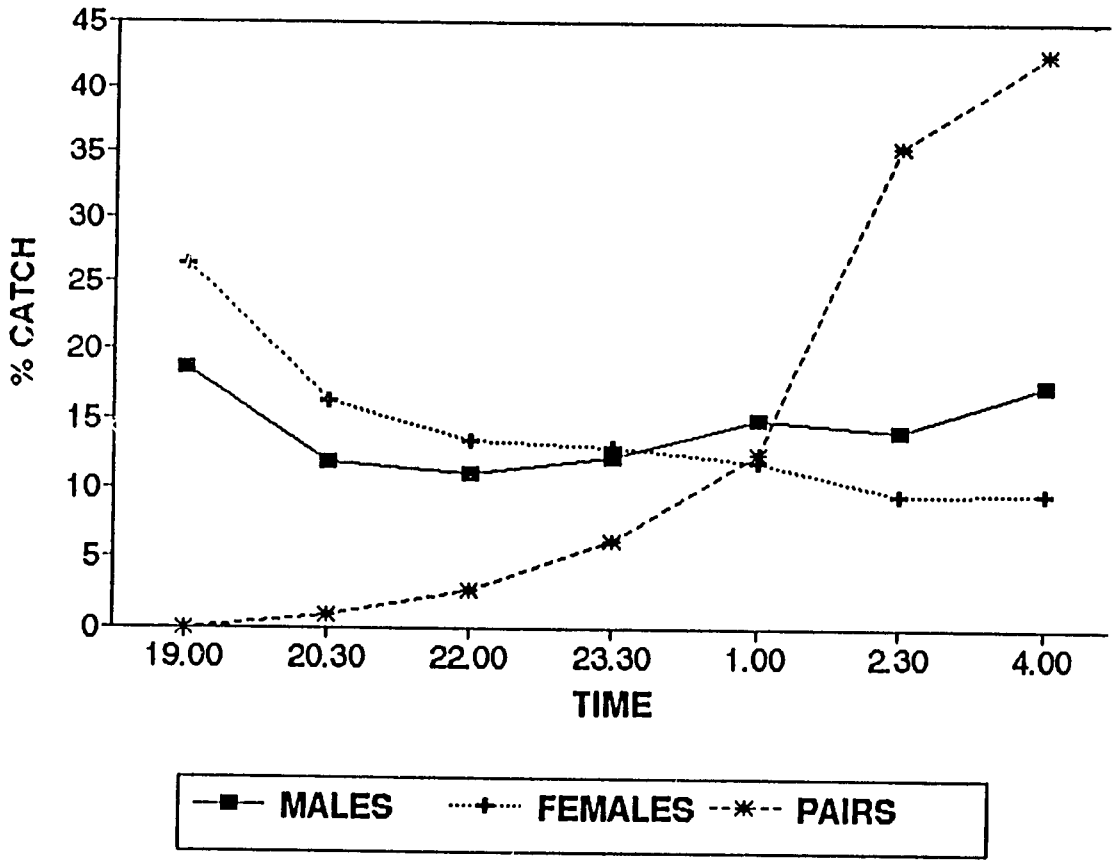

Fig. I D 6. Percent of H. armigera males, females or mating pair (out of the total of each stage) collected at different times during the night (Thailand 1989).

IE. Effects of pheromone application as mating disruptant of Heliothis armigera

(a) When evaporated in closed spacings.

Males and females were put together in 5 liters containers for 24 hrs. under laboratory conditions (25°C, 14:10 hrs. light:dark photoperiod). Females were then dissected (for spermatophore presence) to determine percent mating. Ten males and 10 females were placed in a container and one to six containers per treatment were used in the different experiments. The pheromone [Z11-16:Ald + Z9-16:Ald (97:3)] in concentrations (loaded on dental cotton wool) ranging between 5-60 mg/container.

Results indicated (Tab IE 1) that - when the pheromone concentration was increased to 60 mg/container mating was prevented. Since males unde these conditions could locate the females easily (confined in small spacing) and still mating did not occur; it seems that high concentrations of pheromone, directly affect the capability of males or females to copulate.

(b) When evaporated in outdoor small screened cages (30x30x30 cm).

Males and females (10 individuals of each sex) were placed in screened cages (30x30x30 cm) under outdoor conditions. Pheromone concentrations (loaded on dental-rolls) ranged between 20-600 mg/cage. Mating activity was directly observed twice every hour throughout the night and mating pairs were collected.

Results indicate (Tab. IE 2) that in small ventilated screen cages high pheromone concentrations did not prevent mating. Actually, in ventilated small cages pheromone, even at high concentrations (300 mg/cage) was ineffective as

mating disruptant. This probably indicates that once males and females are close together (=high population densities) pheromone applications are no longer effective in preventing mating. It should be pointed, however, that under pheromone permeated atmosphere matings were somewhat delayed.

(c) When evaporated in the field using Ropes (product of Shin-Etsu).

A preliminary test for disruption of *Heliothis armigera* with Shin-Etsu, Mitsubishi ropes was conducted in the Bet Dagan farm. A square of 1 dunam (1000 m^2) was chosen in an unsprayed cotton plot (Fig. IE 1). Ropes, containing each 100 mg of pheromone (Z11-16:Ald + 3% Z9-16:Ald), were tied to young plants at a distance of 3 m, altogether 117 units. Two pheromone traps were located in the test-plot and four control traps, 25 m away from each side of the treated plot, were located around the test field. Males in the traps were counted every 2-3 days during 2 months from May 16 till July 16; after this date the moth population declined sharply and counting was terminated. The average catch in the plot during 2 months was 2 males/trap whereas in the control 55 males/trap were caught. This result is encouraging, indicating that disruption might be achieved. The total population in 1989 was low, therefore the test must be repeated.

The release rate of Z11-16:Ald from the ropes was measured by hanging 3 dispensers in the open, at the time of the field test, and weighing them every 3-4 days. The results (Fig. IE 2) indicate that about 45% of the pheromone is released during two months. Approximately 50% of the pheormone is trapped in the dispenser. The residue became yellow and very viscuous, probably due to polymerization.

Tab. IE 1. Efficacy of pheromonal application as mating disruptant of Heliothis armigera when evaporated in closed containers (5 liters) (Bet Dagan, 1986, 1987)

Date	No. of pairs tested*	Pheromone (mg)	% mating
19.9.86	10x3**	60	0
	10x3	20	0
	10x3	Control	33.3
21.9.86	10x1	20	20.0
	10x1	10	10.0
	10x1	5	30.0
	10x3	Control	36.6
23.9.86	10x2	20	15.0
	10x2	10	20.0
	10x2	5	30.0
	10x3	Control	63.3
18.8.87	10x4	60	0
	10x4	Control	22.5
21.8.87	10x6	60	0
	10x6	Control	11.7
23.8.87	10x2	60	0
	10x2	Control	10.0
26.8.87	10x3	60	3.3
	10x3	Control	16.7

*3-5 days old

**number of pairs in a container x number of containers

Tab. IE 2. Efficacy of pheromonal application as mating disruptant of Heliothis armigera when evaporated in small screened cages (30x30x30 cm) (Bet Dagan 1987)

Date	Pheromone (mg)	No. of pairs* tested	% mated Total	within first 3 hrs
16.8.87	20	10x3**	40.0	16.7
	40	10x3	43.3	7.7
	Control	10x4	77.5	67.7
17.8.87	300	10x4	50.0	10.0
	Control	10x3	70.0	61.9

*4-5 days old

**number of pairs in a screen cage x number of cages

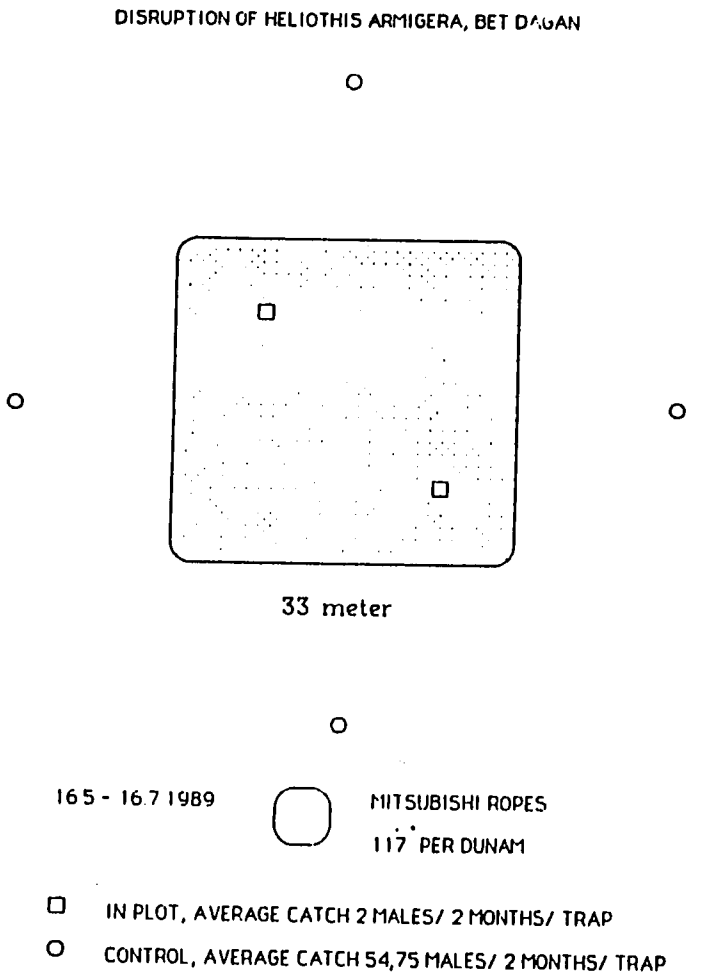

Fig. I E 1. Experimental design of the disruption test using Mitsubishi ropes.

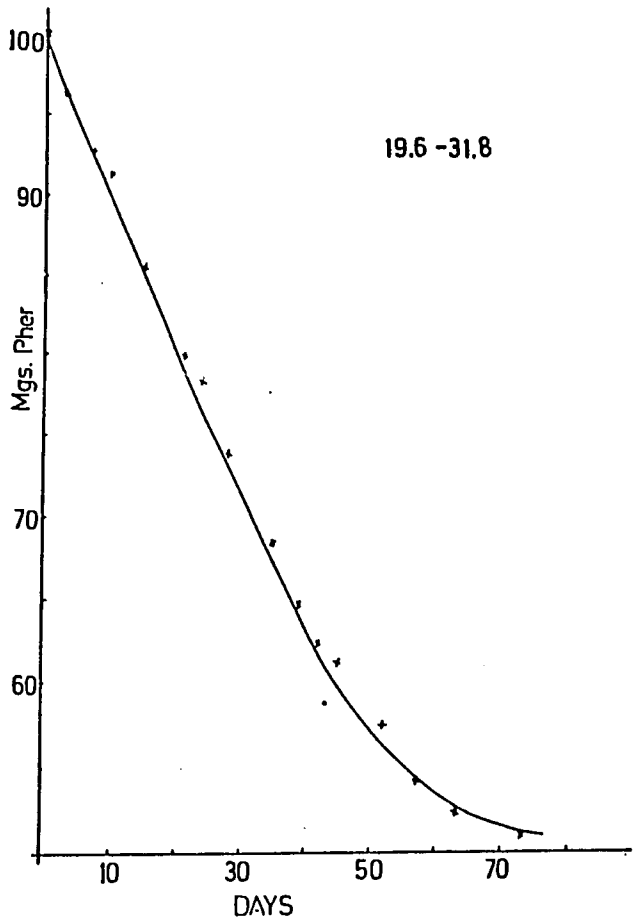

Fig. I E 2. Release rate of Z11-16:Ald from Mitsubishi ropes; initial loading was 100 mg/unit.

II. Heliothis peltigera (Schiff)

IIA. FEMALE SEX PHEROMONE COMPONENTS OF HELIOTHIS PELTIGERA (LEPIDOPTERA: NOCTUIDAE): CHEMICAL IDENTIFICATION FROM GLAND EXTRACTS AND MALE RESPONSE

INTRODUCTION

Heliothis peltigera (Schiff) is a polyphagous insect distributed over central and southern Europe, the Canary Islands, Asia Minor, Syria, Iraq and Iran. It infests ornamental plants, fodder crops, medicinal crops, safflower, tobacco, grapevine, stone fruit trees and citrus (Avidov and Harpaz, 1969; Yathom, 1971). In Israel it is also sometimes responsible for damage inflicted to young cotton plants and is abundant on chickpea and safflower. In order to monitor its population, particularly when adults and larvae resemble those of the sympatric H. armigera, work was undertaken to find attractants to be used in traps. There has been only one observation, from Sudan, on captures of H. peltigera in traps baited with pheromonal compounds (Outram, personal communication, in Nesbitt et al., 1979).

The purpose of the present study was to develop a specific sex pheromone blend for monitoring H. peltigera. To accomplish this aim, the chemical identification of the pheromonal compounds in gland extracts was performed and the major components, which evoked male response in the flight tunnel, were tested in the field. The emphasis in this work was to obtain a bait, as good or better than a virgin female, that would be able to capture efficiently and specifically H. peltigera males. Despite the fact that ten pheromone-like compounds were identified in the glands, there was no intention to perform a behavioural study of all the compounds. The development of sex pheromone traps

for H. peltigera will enable monitoring and improvement of practical control procedures for this pest.

MATERIALS AND METHODS

Insects and Extracts. Females of H. peltigera were captured in light traps and kept for egg laying. Larvae were individually reared on a modified diet medium (Shorey and Hale, 1975). Pupae were sexed and placed in separate rooms with a dark:light regime of 10h:14h, at 25°±2°C. The insects used in the study were from this colony, which was 6-12 months old.

All ovipositors were excised from 2- to 3-day-old females, 7-8 h into scotophase, which is the time of maximal mating in the laboratory. The ovipositors were immersed in redistilled hexane for 10-15 min and the supernatant solution was pipetted into conical vials sealed with a Teflon-lined screw cap. Four pooled samples, each consisting of 20-30 glands, were prepared and 200 ng of Z8-13:Ac was added to each one as internal standard. The extracts were concentrated by letting them stand in a fume hood; they were stored at -18°C until use.

Chemical Identification. Capillary GC analysis was conducted using a Varian 3700 model equipped with a FID detector and a splitless injector system. A 30 m x 0.25 mm DB 225 cyanosilicone column (J&W) was kept at 60°C for 2 min and then programmed to 150°C at 10°/min. The purge valve was opened 1 min after injection and the helium (carrier gas) pressure was maintained at 15 psi. Quantification of peak areas was performed by a Varian 4270 electronic integrator.

Capillary GC-MS analysis was conducted on a Finnigan 5100 EI machine at 70eV, coupled with a Superincoss data system and interfaced with a Finnigan MAT 9610 GC. A 30 m x 0.25 mm SE 54 (J&W) column was kept at 60°C for 2 min and then

programmed from 60° to $250^{o}C$ at 12°/min for pheromone components; for the dimethyl disulfide (DMDS) adducts the column was kept at $60^{o}C$ for 4 min and then programmed at 20°/min to $230^{o}C$. The column was operated in the splitless mode, the purge valve was opened 0.5 min after injection and the helium (carrier gas) pressure was maintained at 10 psi.

DMDS adducts were prepared from several standards (Z9-14:Ald, Z9-14:Ac, Z11-16:Ald, Z11-16:OH and Z11-16:Ac) and from a portion of the gland extracts. Samples in hexane were treated with DMDS and etheral iodine as described by Buser et al. (1983) and Dunkelblum et al. (1985). The EI mass spectrometric analysis was conducted in the multiple ion detection (MID) mode searching for the characteristic $RCHSCH_3^+$ and molecular ions.

Flight-Tunnel bioassays. The flight tunnel used (2 m x 0.5 m x 0.5 m) in these tests was of the same design as described by Snir et al. (1986). All tests were conducted with 4-day-old males, 6-8 h into scotophase. The insects were kept in screen cages (30 x 30 x 30 cm) in the tunnel room. Individual males were transferred into small cylindrical cages (6 cm diameter, 6 cm height) and placed on a tripod in the center of the tunnel, approximately 20 cm from the downwind end. The tests were performed at $24^{o}\pm2^{o}C$, with a light intenstiy of 0.3 lux and a wind speed of 40-45 cm/sec.

Baits were prepared by impregnating rubber septa (Maavit Products, Tel Aviv, Israel) with hexane solutions of the test compounds and they were aged for 48 h on a laboratory bench before use. The baits were kept in glass vials (2 cm diameter), fixed on top of a cork with a cut paper clip. The cork served as a stand for the bait in the tunnel and also enabled storage of the septa in the glass vials. They were stored in a refrigerator and kept at room temperature for 24 h before use. Baits were positioned in the wind tunnel on a tripod approximately 20 cm from the upwind side. Ovipositor extracts from one female (1

FE), in hexane, were placed on a 1 x 1 cm piece of filter paper. After evaporation of the hexane, the paper lures were fixed on a cork with a pin. Virgin females were tested as a group. Attempts to use one virgin female as a lure failed, and therefore five females were placed in a screen cage and put in the tunnel. The number of calling females at a specific moment was not recorded.

Individual males were used for testing the attractancy of different blends, extracts and live females. Four behavioural categories were recorded: (i) upwind flight (approximately half the length of the tunnel), (ii) flight close to source (within 10 cm of the bait and hovering around it), (iii) source contact, and (iv) copulation attempts. Results were analysed by the X^2 2x2 test of independence using the actual number of insects, and are presented as % of response. Only moths which responded within 2 min of release were scored.

Field Work. Tests were conducted in cotton, chickpea and safflower fields in the Bet Shean Valley, and at Mai Ami and Bet Dagan during the 1987 and 1988 seasons. Rubber septa dispensers were loaded with hexane solutions of test compounds containing 10% of butylated hydroxytoluene (BHT) as antioxidant. The traps used consisted of a plastic ring (9 cm diameter) with a plastic roof fixed 3-5 cm above the opening; the dispenser containing the synthetic chemicals was hung under the protecting roof. A nylon bag was attached to the plastic ring to collect the trapped insects. Attractancy of virgin females was tested by hanging a small screen cage containing two virgin females under the protecting roof. Talcum powder was inserted into the nylon bag to prevent the escape of captured moths. The traps were fixed at a height of ca 1.5 m above the ground; adjacent traps were located at least 30 m apart. Captured moths were collected and counted every 1-3 days. Positional bias was reduced by moving the different treatments one position whenever the traps were examined. In all experiments

three to five traps were used for each treatment. Duncan's New Multiple Range Test was used to analyse significant differences between treatments.

RESULTS

Chemical Identification Capillary GC analysis of the ovipositor extracts revealed the presence of a large number of peaks (Fig. IIA 1). Comparison of the retention times with synthetic compounds enabled the tentative identification of ten components: tetradecanal (14:Ald), (Z)-9-tetradecenal (Z9-14:Ald), (Z)-9-tetradecenol (Z9-14:OH), (Z)-9-tetradecenyl acetate (Z9-14:Ac), hexadecanal (16:Ald), (Z)-7-hexadecenal (Z7-16:Ald), (Z)-9-hexadecenal (Z9-16:Ald), (Z)-11-hexadecenal (Z11-16:Ald), (Z)-11-hexadecenol (Z11-16:OH) and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Table IIA 1). The amount of the major component Z11-16:Ald in the pooled extracts was 23-42 ng per female. The polar DB 225 capillary column gave a fair separation of Z7-16:Ald, Z9-16:Ald and Z11-16:Ald.

Additional information on the identity of the compounds present in the gland extracts was obtained from the GC-MS analysis on a SE 54 capillary column. The amount of some of the trace components, such as 14:Ald, was insufficient for a mass spectrum. Two of the minor unsaturated aldehydes, Z7-16:Ald and Z9-16:Ald eluted on the SE 54 column as one peak. The mass spectrometric data were in accordance with the proposed structures. Derivatization of a portion of the extracts with DMDS and subsequent GC-MS analysis enabled the separation of all unsaturated components. It confirmed the presence and established the position of the double bond in all these components. The mass spectrometric analysis in the MID mode enhanced the sensitivity of the analysis and enabled the detection of all the predicted DMDS adducts. The intense $RCHSCH_3^+$ ions as well as the

molecular M^+ ions were diagnostic for the structure of all the adducts. The mass spectrometic data are summarized in Table IIA 2.

Flight-Tunnel Bioassays. The flight tunnel tests reported here concentrated on the components present in largest amounts: Z11-16:Ald, Z9-14:Ald and Z11-16:OH. The best blends were compared with ovipositor extracts and with virgin females. In all flight tests, most males which reached the bait performed copulation attempts. This behavioural stage was readily recognized by the exposure of the hairpencils and the bending of the abdomen. The flight tests preceded, and served as a basis for the subsequent field tests.

In the first behavioural tests (Table IIA 3) the activity of the two major components Z11-16:Ald (HP1) and Z9-14:Ald (HP2) was tested individually and compared with that of several binary blends consisting of these two components in different ratios (HP3, HP4, HP5). Subsequently, a blend of Z11-16:Ald + Z11-16:OH (HP6) and a ternary blend of the two aldehydes and the alcohol were examined. The results showed that both Z11-16:Ald and Z9-14:Ald induced only upwind flight. Addition of varying amounts of Z9-14:Ald to Z11-16:Ald caused a significant increase in attractancy of the blends. Mixtures containing 5% of Z9-14:Ald (HP4) or 50% of Z9-14:Ald (HP5) performed similarly, with approximately 60% of the males completing the whole courtship sequence. Addition of Z11-16:OH to Z11-16:Ald (HP6) had no influence on the performance of the bait. In further tests the binary blend, HP5, was compared with a ternary blend of Z11-16:Ald + Z9-14:Ald + Z11-16:OH (HP7). A slight increase in activity was observed, but the difference was nonsignificant. The ternary blend HP7 was as active as virgin females and more active than the one-ovipositor extract (1FE).

Field Tests Most of the field tests were performed during 1987 at two locations on two different crops at the beginning of the season, and later on cotton alone (Table IIA 4). Initial results (test 1) indicated that the mixture

of Z11-16:Ald + 2.5% Z9-16:Ald (HA), the two major sex pheromone components of H. armigera (Kehat et al., 1980), did not attract H. peltigera males. The addition of 5% Z9-14:Ald to this mixture (HP8) resulted in trapping of H. peltigera on the one hand, and in a significant decline in captures of H. armigera on the other hand. Increasing the amount of Z9-14:Ald up to 20% (HP9) significantly increased the captures of H. peltigera and further reduced the number of trapped H. armigera males (HP8 vs HP9). Results presented in test 2 indicated that increasing the amount of Z9-14:Ald up to 50% (HP10) did not enhance captures of H. peltigera as compared with the addition of only 20% Z9-14:Ald (HP9), but drastically reduced captures of H. armigera. The minor component in HA, Z9-16:Ald, did not affect the captures of H. peltigera, as shown by the comparison of HP10 with HP5. Therefore, this compound was eliminated from additional field tests. In test 3, two binary blends of Z11-16:Ald and Z9-14:Ald (HP11 and HP5) were compared with a ternary blend of these components and Z11-16:OH (HP7). The results showed that all three lures captured the same numbers of H. peltigera males, but larger amounts of Z9-14:Ald (HP5 vs HP11) resulted in a smaller trap catch of H. armigera. Addition of 30% of Z11-16:OH, which is a strong inhibitor to H. armigera (Kehat et al., 1980), resulted in complete elimination of males of H. armigera from the traps (HP5 vs HP7).

Two field tests were performed in the spring of 1988, when the population density of H. peltigera was higher (Table IIA 5). The first test again compared the efficiency of the best binary blend (HP5) with the ternary blend (HP7) and results indicated that they have the same attractancy in the field. The second test compared blend HP5 with two virgin females, and the synthetic lure attracted significantly more males than did the females.

DISCUSSION

The sex pheromones of several Heliothis species - H. virescens (Klun et al., 1980a), H. zea (Klun et al., 1980b), H. armigera (Nesbitt et al., 1979, 1980, Dunkelblum et al., 1980), H. subflexa (Teal et al., 1981, Klun et al., 1982), H. puntiger (Rothschild et al., 1982) and H. phloxiphaga (Raina et al., 1986) have been investigated in recent years. For most Heliothis species gland extracts were analysed and multicomponent pheromones were found. Only in H. virescens was airborne pheromone from live females (Teal et al., 1986) and from gland volatiles (Pope et al., 1982) collected and analysed. Gland volatiles were also analysed from H. zea (Pope et al., 1984). In all the Heliothis species the main pheromone component was Z11-16:Ald, accompanied by varying amounts of other C16 aldehydes, acetates and alcohols. Only in H. virescens was Z9-14:Ald found to be an essential component in addition to the C16 components (Vetter and Baker, 1983, Teal et al., 1986).

Comparison of the content of the gland extract from H. peltigera (Table 1) with the pheromone components of the other Heliothis species showed that H. peltigera contains most of the compounds found previously in the other species. The composition of the gland extracts from H. virescens (Teal et al., 1986) is similar to that of H. peltigera, although the latter contains also the two acetates, Z9-14:Ac and Z11-16:Ac, not present in H. virescens. There is also some similarity to the composition of gland extracts of H. subflexa (Teal et al., 1981, Klun et al., 1982). However, the latter contains additional alcohols and acetates. Most important, H. subflexa contains relatively large amounts of Z9-16:Ald and only trace amounts of Z9-14:Ald, whereas in H. peltigera the opposite was found.

The gland extracts of H. peltigera contain a large number of pheromonal compounds, but probably only some of these compounds are emitted by the female,

the rest of the components may be biosynthetic precursors and intermediates (Morse and Meighen, 1987). The different functional groups of aldehyde, alcohol and acetate can be biosynthetically interconverted, as shown in Choristoneura fumiferana (Morse and Meighen, 1987) and in H. virescens and H. subflexa (Teal and Tumlinson, 1987). The presence of the three functional classes in H. peltigera may indicate the biosynthetic pathway of the formation of the pheromone components in the glands of this moth.

The principal goal of the present study was to identify the main pheromone components of H. peltigera and to develop a species-specific lure for monitoring H. peltigera in the presence of the sympatric species, H. armigera. Therefore, the major effort was directed at the identification of the compounds in the pheromone gland which are attractive to males in both the flight tunnel and field. The flight tests were planned to find the main components which evoke maximal response from the males and eventually would be useful in the field. The tested lures were evaluated by comparing their activity with that of calling females. A relatively high load per septum, based on 2 mg of Z11-16:Ald, was used because this is the standard load of pheromones in use for monitoring other Lepidopteran pests in Israel. In view of the information on the pheromone blends of other Heliothis species, the obvious choices to start these behavioural tests were the two most abundant aldehydic compounds, Z11-16:Ald and Z9-14:Ald. The tests showed that these compounds initiated individually only upwind flight, but a mixture of the two evoked a strong response from the males, comparable to that of calling females. The fact that the binary blends of Z11-16:Ald + Z9-14:Ald evoked the whole courting sequence from the males was very encouraging. The alcohol Z11-16:OH, which was one of the more abundant compounds in the gland extracts of H. peltigera, is an inhibitor to the sympatric H. armigera males (Kehat et al., 1980). Therefore, it was important to

evaluate the response of the H. peltigera males to this compound. Addition of 30% Z11-16:OH to the binary blend of aldehydes indicated that this alcohol has no inhibitory effect on H. peltigera males. Subsequent field tests showed that a 2:1 mixture of Z11-16:Ald and Z9-14:Ald is an efficient attractant for H. peltigera, better than two virgin females. The addition of Z11-16:OH did not enhance the trap catch, but produced a more species-specific lure for H. peltigera by reducing completely the capture of the sympatric H. armigera males. The minor component Z9-16:Ald, which is present in both H. armigera and H. peltigera, is an essential component in the pheromone blend of H. armigera (Kehat et al., 1980) but seems to have no effect on the trap catch of H. peltigera. Despite that fact that the present results are based on the composition of gland extracts, the data from both the flight tunnel and field tests indicated strongly that Z11-16:Ald and Z9-14:Ald are the essential pheromone components of H. peltigera. The alcohol Z11-16:OH might be a pheromonal component responsible for enhancement of the pheromone specificity. Therefore, the ternary blend of Z11-16:Ald + Z9-14:Ald + Z11-16:OH is recommended for monitoring H. peltigera.

Careful airborne collection from live females is necessary in order to determine which components, from the large pool of compounds in the gland, are emitted as pheromone components. Based on these findings, additional behavioural tests with synthetic lures emitting the same blend should be performed. At the present time, the major pheromone components have been identified and evaluated by flight and field tests. A lure has been developed which efficiently and specifically attracts H. peltigera, thus enabling monitoring of the pest and possibly leading to improved procedures for its control.

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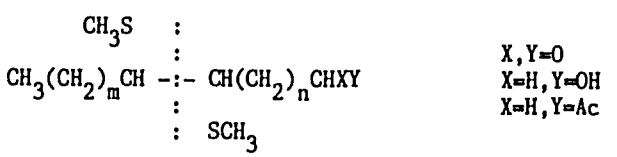
Table IIA 1. Relative proportion of compounds identified from sex pheromone ovipositors of Heliothis peltigera[a]

Compound	Average (%)	Range (%)
14:Ald	0.8	0.5-1.0
Z9-14:Ald	14.6	13.0-17.5
Z9-14:OH	6.5	4.4-7.6
Z9-14:Ac	2.0	1.6-2.5
16:Ald	3.7	3.3-4.0
Z7-16:Ald	1.1	0.5-1.5
Z9-16:Ald	1.5	1.2-2.0
Z11-16:Ald	100	
Z11-16:OH	24.3	21.7-26.1
Z11-16:Ac	4.8	3.3-8.0

[a]Relative proportions were calculated with respect to the main component Z11-16:Ald.

Table IIA 2. Mass spectrometric data of DMDS derivatives of unsaturated compounds from pheromone blends of *Heliothis peltigera*



DMDS adduct[a]	A^+	B^+	B^+-60	M^+
Z9-14:Ald	117	187		304
Z9-14:OH	117	189		306
Z9-14:Ac	117	231	171	348
Z7-16:Ald	173	159		332
Z9-16:Ald	145	187		332
Z11-16:Ald	117	215		332
Z11-16:OH	117	217		334
Z11-16:Ac	117	259	199	376

[a]Presented in the order of elution on the SE 54 column.

Table IIA 3. Response (%) of male Heliothis peltigera in a wind tunnel to pheromonal blends, gland extracts and live females[a]

Bait[b]	Components (ug)			Number	Upwind	Close to	Source	Copulation
	Z11-16:Ald	Z9-14:Ald	Z11-16:OH	tested	flight	source	contact	attempts
HP1	2000	-	-	26	100a	8d	0c	0c
HP2	-	1000	-	33	100a	18cd	0c	0c
HP3	2000	20	-	24	100a	29c	8c	8c
HP4	2000	100	-	41	98a	73b	66ab	59ab
HP5	2000	1000	-	71	100a	92a	73ab	61ab
HP6	2000	-	600	32	100a	12cd	0c	0c
HP7	2000	1000	600	61	95a	90a	77a	74a
1FE				26	92a	62b	46b	46b
♀♀[c] ♂♂				28	100a	57b	54b	54ab

[a]Percentages followed by the same letter are not significantly different according to X^2 test of independence.

[b]All baits were rubber sepata, impregnated with the tested compounds, except for 1 FE, which was a one gland extract on a 1 cm^2 filter paper.

[c]Four or five females confined in a 30 x 30 x 30 cm screen cage.

Table IIA 4. Captures of Heliothis peltigera and Heliothis armigera (mean number of males/trap/night)[a] in traps with different pheromonal blends

Test[b]	Bait	Components (ug)				Location/Crop			
		Z11-16:Ald	Z9-16:Ald	Z9-14:Ald	Z11-16:OH	H. arm.	H. pelt.	H. arm.	H. pelt
						Mai Ami/Cotton		Mai Ami/Chickpea	
1	HA	2000	50	-		22.7a	0c	57.5a	0c
	HP8	2000	50	100		0.5b	0.1b	2.7b	0.6b
	HP9	2000	50	400		0.1c	0.9a	0.1c	1.5a
						Mai Ami/Cotton		Bet Shean/Cotton	
2	HA	2000	50	--	--	-	0	21.0a	0
	HP9	2000	50	400	--	-	1.10a	0.30b	0.60a
	HP10	2000	50	1000	--	-	1.50a	0.01c	0.60a
	HP 5	2000	--	1000	--	-	1.70a	0.05c	0.80a
3	HA	2000	50	--	--	7.20a	0b	6.3a	0b
	HP11	2000	--	400	--	1.10b	0.20a	0.14b	0.10a
	HP 5	2000	--	1000	--	0.10c	0.20a	0.02c	0.11a
	HP 7	2000	--	1000	600	0.01d	0.45a	0c	0.28a

[a]Means followed by the same letter are not significantly different at $P<0.05$ according to Duncan's New Multiple Range Test.

[b] Test 1, 27 April - 15 May, 1987, Test 2, 6 June - 24 June 1987, catches of H. armigera were not recorded at Mai Ami; Test 3, 26 June - 14 July 1987.

Table IIA 5. Captures of Heliothis peltigera (mean number of males/trap, night)[a] in traps with a blend of HP5, HP7 and virgin females in cotton fields

Test[b]	Bait	Components (ug)			H. peltigera
		Z11-16:Ald	Z9-16:Ald	Z11-16:OH	
1	HP5	2000	1000	---	7.7a
	HP7	2000	1000	600	7.5a
2	HP5	2000	1000	---	10.2a
	2♀	--	--	---	4.2b

[a]Means followed by the same letter are not significantly different at $P<0.05$ according to Duncan's New Multiple Range Test.

[b]Test 1, 24 March-31 March 1988 in Bet Shean, Test 2, 5 May-9 May 1988 at Bet Dagan.

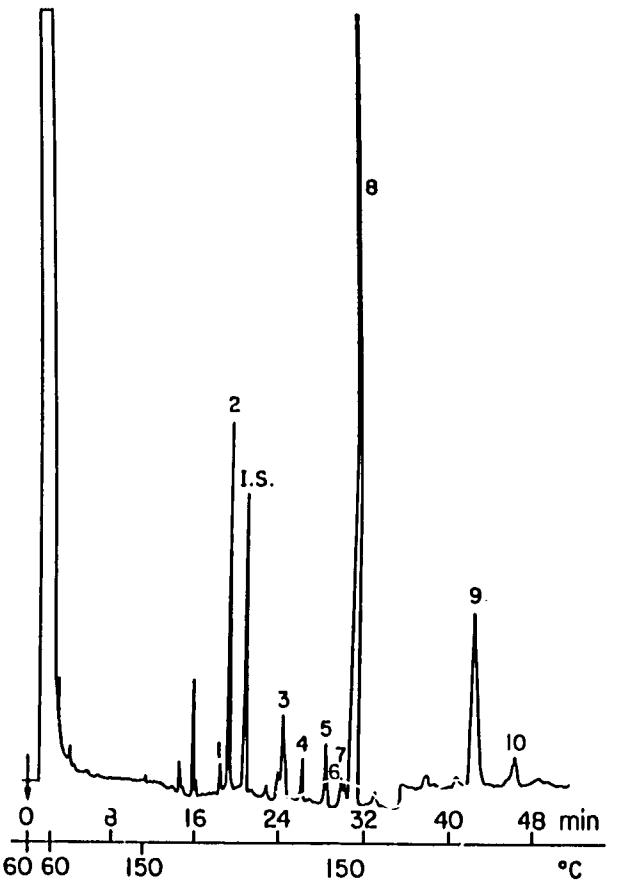


Fig. II 1 . Capillary GC trace of gland extracts of *Heliothis peltigera:* 1 = 14:Ald, 2 = Z9-14:Ald, 3 = Z9-14:OH, 4 = Z9-14:Ac, 5 = 16:Ald, 6 = Z7-16:Ald, 7 = Z9-16:Ald, 8 = Z11-16:Ald, 9 = Z11-16:OH, 10 = Z11-16:Ac, and I.S. = Z8-13:Ac.

II B. Sexual Behavior

(a) Effect of age and time on females calling

For behavioral tests with *H. peltigera* males, rubber septa were loaded with 1.5 mg Z11-16:Ald and 0.2 mg of Z9-14:Ald. This blend gave a release of approximately 60 ng/min of each component at a flow of 2 lit/min. The release rate from the dispensers was measured in a similar flow system used for collecting natural pheromone from the females.

Fifty females were kept in a dark room (14 l : 10 d) in 30 x 30 x 30 cm cages (10 females in a cage) and observed during the scotophase for calling. Observations were continued form 1 day old to 10 days old females. Number of calling females was recorded, and percent calling was calculated

Results indicate that most females called during the second half of the scotophase with a peak activity during the 7-9 hrs into scotophase. Five to seven days old females called more frequently than 1-day-old females (Fig. IIB 1). Peak time of female calling correspond with peak activity of males' flight (Fig. IIB 3).

(b) Collection of airborne pheromone from live *H. peltigera* females

Airborne pheromone from live *H. peltigera* females was collected in a push-pull system as described by Landolt and Heath, 1987. Individual, 4-5-day-old females between 7-9 h into scotophase were placed in the system. An air flow of 1 lit/min with 60% RH was maintained at a temperature of $25 \pm 2^{o}C$. The emitted pheromone was collected for 1 hour on a commercial 1.5 mg charcoal filter (Brechbuhler AG, 8952 Schlieren, Switzerland). The filters were eluted with small volumes (10-50 ul) of carbon disulfide and 25 ng of Z8-13:Ac was added as internal standard. Samples were concentrated and analyzed by capillary

GC as described in the identification of the pheromone components. The average amount of Z11-16:Ald was 65.3±19.3 ng/h, Z9-14:Ald 54.3±17.6 ng/h and trace amounts of 16:Ald, Z7-16:Ald, Z9-16:Ald and Z11-16:OH were also dedected. These measurements indicate that H. peltigera females emit about the same amount of Z9-14:Ald and Z11-16:Ald. The relative ratios of these components in the gland extracts was very different from those found in the airborne collection.

(c) Effect of age and time on the flight of males

Males of different ages were flown in a wind tunnel (during the 7-9 hrs into scotophase) towards a source containing the sex pheromone. Males were flown in groups of five moths and the % of response (source contact) was recorded. Similarly, 6-days-old males were tested in the wind tunnel at differnt hrs. during scotophase.

Results clearly indicate that the % of response of 1-2 days old males was very low. No differences, however, in flight activity between 3-8 day old males were detected (Fig. IIB 2). Peak flight activity was during the 6-8 hrs into scotophase (Fig. IIB 3).

(d) Mating of H. peltigera

Since this species is prevalent, sometimes, in cotton field in Israel it was interesting to study its mating behavior as compared with that of H. armigera. Methods were the same as those descibed for H. armigera.

Results (Tab. IIB 1) indicate that (1) Most matings occurred between 5-8 hrs. into scotophase (80.8%). (2) % mating was relatively high (76.4% in 2-3 days old individuals) (3) Mean duration of mating (for 28 pairs was 97.1 mins (50-180) (4) Direct observations at the same nights, on mating of both H.

armigera and H. peltigera indicated a similar mating activity in these two species.

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Table IIB 1. Mating of laboratory bred Heliothis peltigera in outdoor screened cages (3,5) Aug, 11 Sep 1986, Bet Dagan)

No. of pair tested*	68	
Total no. of pairs mated	52	
No. of pairs mated at 0-3 hrs into scotophase*	1	(1.9)**
3-4	1	(1.9)
4-5	1	(1.9)
5-6	13	(25.0)
6-7	17	(32.7)
7-8	12	(23.1)
8-9	4	(7.7)
9-10	3	(5.8)
% mated (total)	76.4	
% mated within first 4 hrs of darkness	3.8	
% mated within last 6 hrs of darkness	96.2	

*2-3 days old **numbers in brackets indicate % mating of the total

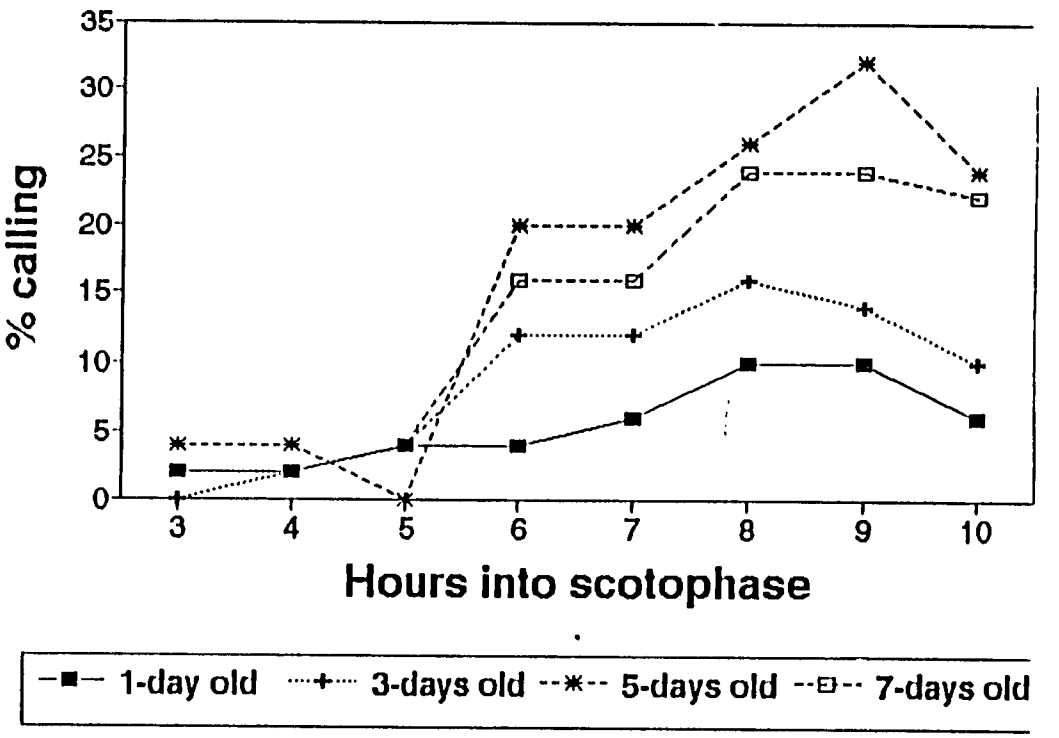

Fig. II B 1. Calling of K. peltigera females during scotophase.

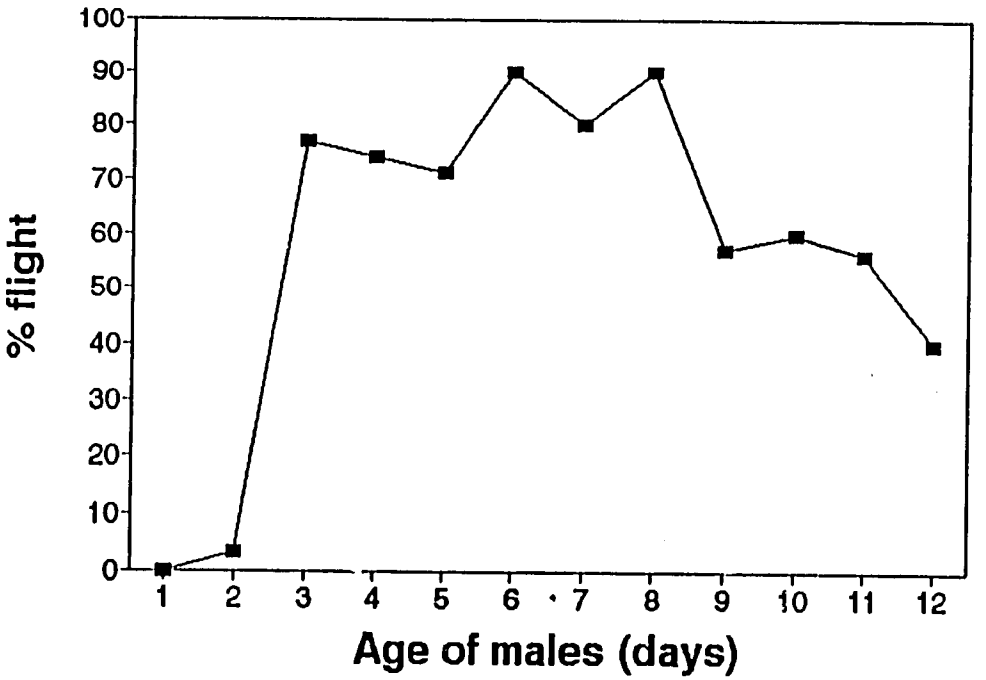

Fig. II B 2. Effect of age on H. peltigera male flight.

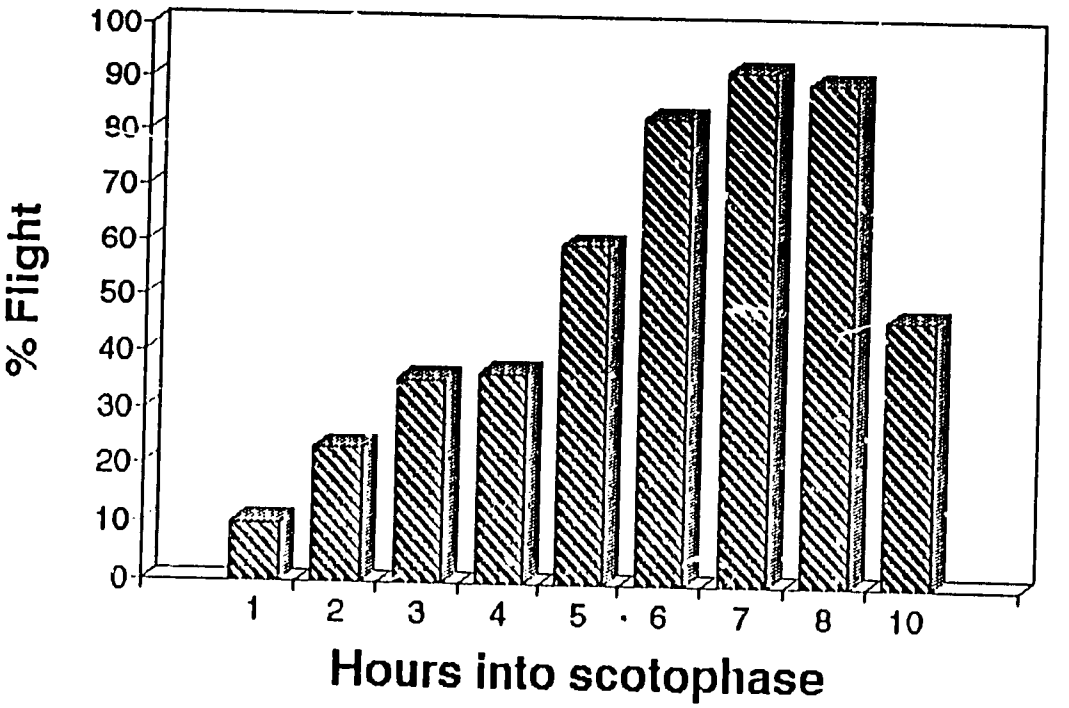

Fig. II B 3. Effect of time on H. peltigera male flight.

III *Heliothis assulta* (Guenne)

IIIA. Sex pheromone components of *Heliothis assulta*

Pheromone gland extracts of *H. assulta* usually containing 10 glands per sample in hexane, were sent from Thailand for chemical analysis. Many of the samples did not contain any expected pheromone components. However, in some, several aldehydes, typically found in other *Heliothis* species, could be detected. The separation was conducted on the same GC column as described for *H. peltigera* (III a). GC profile from one of these samples is presented in Fig. IIIA. The amount of pheromone in 1-3 day old females within the last hour of scotophase was 2.5-7 ng per gland. The main component was Z9-16:Ald, which is the minor component in *H. armigera*, and the two minor components were Z11-16:Ald and 16:Ald in the ratio of 8:3:2. The same components have been detected by Dr. Alan Cork from ODNRI in England in moths originating from South Korea.

Dispensers obtained from England and prepared in Israel were sent for field tests in tobacco fields in Thailand. Results indicate (Tab. IIIA 1) that baits, although not yet optimal, are now available for monitoring *H. assulta*.

Table IIIA 1 Field test with different blends of Z9-16:Ald, Z11-16:Ald and 16:Ald in Thailand for *Heliothis assulta*

	Z9-16:Ald (ug)	Z11-16:Ald (ug)	16:Ald (ug)	Male/trap/night
		Test 1		
HS9	980	20	-	0 b*
HS10	960	40	-	0.3 b
HS11	900	100	-	0.6 b
HS12	870	130	-	1.9 a
HS13	800	200	-	0.8 b
HS14	500	500	-	0.03b
		Test 2		
HS11	900	100	-	2.9ab
HS12	870	130	-	3.2ab
HS13	800	200	-	3.0ab
HS15	4350	650	-	3.5ab
HS17	435	65	-	2.2bc
HS20	880	120	-	2.6abc
HS21	1000	300	-	1.3c
HS22	1000	300	500	3.3ab
HS23	2000	600	-	4.1a
HS24	2000	600	1000	2.5bc

HS-11-HS17 are English dispensers; HS20-HS24 are Israeli dispensers

*Numbers followed by the same letter are not significantly different ($P<0.05$)

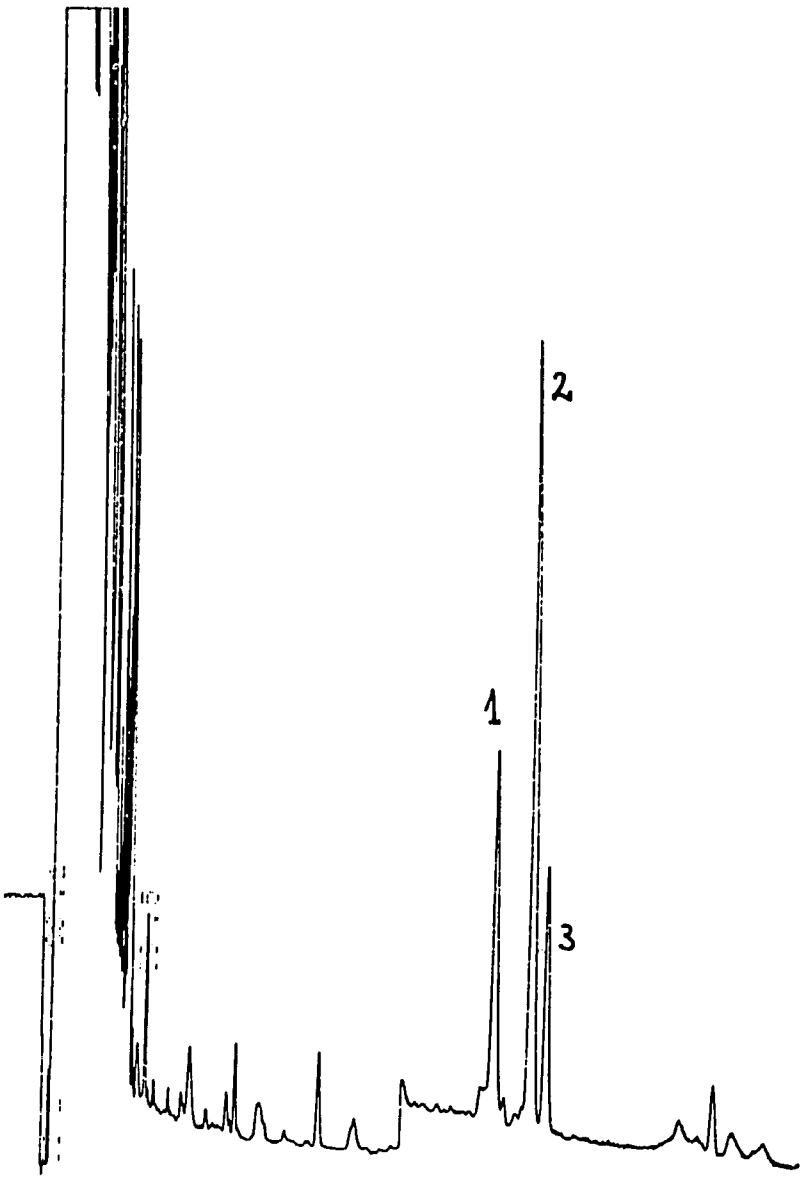

Fig. III A 1. Capillary GC trace of gland extracts of Heliothis assulata.

1 = 16:Ald, 2 = Z9-16:Ald, 3 = Z11-16:Ald.

Acknowledgments

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