III Results

III. Studies on the antennal flagellum of the cotton leafworm, Spodoptera littoralis (Boisd.):

III.I. A SCANNING ELECTRON MICROSCOPE STUDY

The present study on the morphology of the antennae of <u>Spodoptera littoralis</u> (Boisd.) was carried out by the aid of the scanning electron microscope as a trial for better understanding of this insect's chemical communication system. Thirty male and thirty female moths were captured from a laboratory culture of the cotton leafworm. Antennae of adults were prepared for examination by the aid of a light binoculer microscope. Several specimens were also prepared for examination by scanning electron microscope. Measurements of length, tip and base diameters of the determined sensilla were made.

III.I.I. <u>Identification and distribution of sensilla</u> on insect antennae:

As shown in Table (III.I.I), the antennal flagellum of female <u>S.littoralis</u> is longer than that of male being 8.91 ± 0.04 mm in the former case opposed to 7.72 ± 0.08 mm in case of male . Also, the mean number of flagellar segments was 64.4 ± 0.16 in

female, and 62.98 ± 0.13 in male. As shown in Fig. (III.I.I) the first 5 proximal segments of the flagellum have scales present on nearly all surfaces, while on the higher segments, scales are restricted on the dorsal surface, Fig. (III.I.2). The ventral and lateral surfaces are covered with a network of micro-ridges among which most antennal sensilla are located. The only evident sensilla present under the scales were sensilla squamiformi, Fig. (III.I.I). Accordingly, all the subsequent presentation will be confined to sensilla present on the ventral and lateral surfaces which are nearly bare of scales. Eight types of sensilla are localized on ventral sensillar fields on the antenna. These are: three types of sensilla trichodea I, II, & III, small chemoreceptor pegs, sensilla chaetica, (two types I & II), sensilla squamiformia, sensilla auricillica, sensilla styloconica, sensilla basiconica and sensilla coeloconica (two types I & II). In the following paragraphs, the scanning electron micrographs of the different sensilla, will be described. Comparison between the sensilla of males and females, regarding their lengths, tips and base diameters, will be made in order to distinguish between them and predict a relationship between their shapes and functions.

Table: III.I.I. Length (mm) and number of segments of the antennal flagellum in male and female

S. littoralis (records from 30 replicates).

Sex	Length of flagellum $\bar{x} \stackrel{+}{-} \text{S.E}$	Number of segments $\bar{x} \stackrel{+}{=} S.E$
Male	7.72 ± 0.08	62.98 ± 0.13
Female	8.91 + 0.04	64.4 ± 0.16
		<u>-</u>

I. Sensilla trichodea:

antennal sensilla on <u>S.littoralis</u> is sensilla trichodea. Three morphological types of these sensilla can be recognized in male and female antennae as illustrated in Figs.(III.I.3,5&6). These sensilla can be easily distinguished by their dimensions and curvature. Dimensions of trichoid sensilla on the male antennae of <u>S.littoralis</u> differed from those recorded in case of female (Table.III.I.2), (Figs.III.I.7,8). Generally the hair shafts of trichodea were shorter on female than those on male antennae (Figs.III.I.2,9&IO)..Regarding the shape and dimensions of the three types of trichodea, one can notice that the trichodeum I is the most

numerous and longest, measuring 43.26 ± 0.12 and $32.2 \stackrel{+}{-} 0.22$ um in male and female, respectively . It is relativelly straight and hooked at its tip . Type II sensillum can be differentiated from type I as it is shorter and more curved . In male moth, it measures 18.35 + 0.18 in length, while in case of female it is I6.7 - 0.14 long . However, type III trichodeum is the shortest sensllum, where the hair shafts were 6.4 ± 0.15 long in male and 4.9 ± 0.15 long in female . The sensilla trichodea have blunt tips, where the tip diameter of hair shafts of female is smaller than that of male (Table.III.I.2). The longest tip diameter was found in sensillum trichodeum type I, while the least tip diameter was recorded from sensillum trichodeum III. The base diameter of trichodeum I (Fig.III.I.4) averaged 2.7 ± 0.07 and 2.53 \pm 0.05 in male and female antennae, respectively. Other while the base diameter of trichodeum II (Figs.III.1.7,8) averaged 2.57 \pm 0.06 and 2.67 \pm 0.04 in male and female antennae, respectively . The smallest base diameter was recorded from trichodeum III (2.18 \pm 0.12 and 2 \pm 0.12 in male and female antennae, respectively) (Fig. III. I.7) . Mahmoud (1985), examined the surface structure of the three types of sensilla

Table.III.1.2. Dimensions (um) of trichoid sensille on the entennae of the cotton leafworm moth . (records from IO replicates)

Measurements	Type I	Н	¢T,	Type II	III adan	III
	0,,	*0	0,	₹0	2	5 0
Length	43.26 ± 0.I2	32.2 ±0.22	I8.35±C.I8	16.7 ±0.14	6.4 ±0.15 4.9 ±0.15	4.9 ±0.15
Tip diameter	I.08 + 0.05	0.91±0.06	C.65±C.05	0.35±0.05	0.35±0.04	0.33 [±] 0.04
Base diameter	2.7 ± C.07	2.53±0.05	2.57±c.06	2.67±0.06	2.18 [±] 0.12 2	2 ±0.12

sensilla trichodea. He found that the surface of type I is covered with annular ridges, while that of type II has spiral ridges, and that of type III, (Fig. III.I.6) has a median longitudinal ridge or ridges with diagonalridges on each side. In this work, it was observed that sensilla trichodea found near the midventral line of the sensillar field are longer than those found at the lateral edges, (Fig. III.I.8, 9, & IO).

2. Small chemoreceptor pegs:

Sensilla of this type are characterized by the cone-shaped sensory structures. As shown in table (III.I.3) these sensilla were of nearly the same measurements on antennae of both sexes of S. littoralis. The peg, (Fig. III.I.II) is slightly curved but not tapered, and has a tip diameter of 0.52 \pm 0.05 um. This sensillum appeared identical in shape to the conical extremity of the styloconica sense organ, (Fig. III.I.25). The length of the small chemoreceptor peg is I.55 + 0.04um long and I.33 \pm 0.03 um at its base diameter. The terminal segment of female antenna bears three pegs on the narrowed tip, (Fig. III.I.I4), while that of male antenna bears more than five pegs.

This sensillum was found in fewer number on the most

Table. III. I.3. Dimensions (um) for different sensilla on the antennal flagellum S.littorelis moth (records from IO replicates) .

Sensillym	Ler	Length	Tip .	Tip diameter	Base dia	diameter
types	0,	₹0	ON	*0	a,	50
	I.55±0.C4	I.55 [±] 0.04	0.52±0.05	0.52±0.05	I.33±0.03	1.33 [±] 0.03
receptor peg		+	I	1	o N	ນ ກ
Chaetica I	8I.I ±0.47	68.8 ±0.28	I.5	H. 5	(()	• 0
II	50.7 ±0.44 43.4 ±0.44	43.4 ±0.44	I.5	H.5	ω •5	3.5
Squemiformia	20	20	ω •ω	ω w	ı	ŀ
Auricillics	9.2 ±0.26	7.5 ±0.15	1	l	4.25±0.4	4.85±0.37
Styloconic	23.I5±0.25	20.3 ±0.47	I.09 [±] 0.03	0.72±0.02	6.6 ±c.15	5.08±0.24
Basiconica	IO.9 ±0.25	8.8 ±0.16	0.45±0.02	0.45±0.02	I.6 ±c.02	I.6 ±0.02
Coeloconica	5.25-0.I4	5.25-0.14	0.87-0.03	0.67-0.03	2.5 -0.03	2.5 -0.03

Temale . Each sensillum has a blunt end, where the tip diameter measured I.5 um and have a pore (I um in diameter) which is located centrally (Fig.III.I.16, 23). Also, the typical sensillum chaeticum is straight, and has a base diameter of 3.5 um . A high magnification scanning micrograph (Fig.III.I.18) shows that the surface of the sensillum is coverd with radial ridges. The main feature of these sensilla is the high collar-like socket in which the hair is set (Fig.III.I.1).

4. Sensilla squamiformia:

These sensilla (Fig.III.I.I) are the only evident sensilla present under the scales. They are found on the scape, pedicel, and the first 3 - 5 segments on the antennal flagellum of S.littoralis. Sensilla squamiformia are more slender than the normal scales, They are flattened, sword-shaped and have a serrated rim. These sensilla have a tip diameter 3.3 um and the length of the apparent part of sensillum without removal of the scales is 20 um. They are bedded in a socket such as sensillum chaeticum.

5. Sensilla auricillica:

A sensillum auricillicum was noticed on each segment of the antennal flagellum of S.littoralis moths except on the first two segments on which no sensillum of this type could be detected . These sensilla are usually found on the distal end of the segment at the edges of the sensory area, close to the dorsal row of scales (Fig.III.I.2I) . Some sensilla auricillica are also found located away from the lateral margin of the segment (Fig.III.I.20) . Sensilla auricillica are concave small and thin-walled . It is ear-shaped in outline (Fig.III.I.I9). These sensilla occur on male and female antennae of S.littoralis and have a mean length of 7.5 \pm 0.15 and 9.2 \pm 0.28 um in female and male, respectively. The respective measurements of the type of sensilla at its broadest width for female and male was 4.85 ± 0.37 and 4.25 ± 0.11 um (Table III.I.3) .

6.Sensilla styloconica:

On <u>S.littoralis</u> antenna, sensillum styloconicum (Fig.III.I.22) could be recognized by its reticulated base, a relatively smooth stalk, and conical extremity. Sensilla of this type are present in both sexes on the

middle of the distal edge of all flagellar segments (Fig.III.I.5, I0, &22) except the apical segment of the female antennae on which no sensillum styloconicum has detected (Fig.III.I.I4) . In case of male, the apical flagellar segment bears two sensilla styloconica (Fig. III.I.25) . The pegs rise from the distal edge of the segments in the center of the sensory area . The conical tip of the projections exhibit a variety of appearance indicating possible retractability and a pore in the tip (Fig.III.I.23) . Occasionally a segment may lack a styloconicum, and in some other times a peg may have two styloconica. These sensilla have mean length of 23.18 \pm 0.25 and 20.3 \pm 0.47 in male and female, respectively. The tip diameter of conical extremity were I.09 \pm 0.03 and 0.72 \pm 0.02 um, while the base diameter were 6.6 \pm 0.15 and 5.08 \pm 0.24 in male and female, respectively .

7. Sensilla basiconica:

Sensilla basiconica could be determined on the antennal flagellum of <u>S.littoralis</u> males and females. They appear identical to the type III sensilla trichodea of Jefferson <u>et al.</u>,(I970). Sensilla basiconica were observed on the ventral surface of each flagellar

segment (Figs.III.I.2,7,&IO) and appeared to be distributed at random (Fig.III.I.2,24). Sensillum basiconicum has the appearence of a short peg measuring IO.9 ± 0.25 and 8.8 ± 0.I6 um in male and female, respectively, thus indicating longer sensillum in the former case. The sensillum is also characterized by a blunt tip that measured the same diameter in both sexes 0.45 ± 0.02 um Table (III.I.3). Also, the typical sensillum basiconicum is found situated in a pit (Fig.III.I.25) and has a base diameter of I.6 ± 0.02 um. As stated by Lin and Chow (I972), the wall of the sensillum is thin and transparent, also the thin-walled pegs are usually stout and rounded.

8. Sensilla coeloconica:

Sensilla coeloconica are commonly called pit pegs. There are 2 morphological types of these sensilla (pig like sensillar apparatus recessed in a cuticular pit). Also sensilla coeloconica are situated in deep pits have mean diameter ranged from 6-8 um with an average of 7 um. The coeloconica are present on all segments of both sexes, generally located in the median portion of the segment. The commonest type has peripheral fringes of II.8 $^{\pm}$ 0.44 (IO - I4) average that tapered

distally, and a longitudinally striated pegs around a shallow cuticular depression (Figs.III.I.26,27). The tips of the fringe pegs curve over the stouter central peg of the sensillum. The sensory pegs arising from the bases of the pits, in S.littoralis measured 5.25 ± 0.12 um long, a tip diameter of 0.87 ± 0.03 and a base diameter of 2.5 ± 0.03 um. Also, the mean length of fringe pegs is II.3 ± 0.29 um. The second morphological form occurs regularly in deep pits, there are no teeth-like projections (Fig.III.I.25) surrounding the pit as with sensilla coeloconica.



Fig.III.I. SEM showing the scape, pedicel and the basel proximal segments of female <u>S.littoralis</u> flagellum, showing scales present on nearly all surfaces and sensilla squamiformia (S.sq) (x 200).



Fig.III.I.2. Scanning electron micrograh, showing the lateroventral view of the 26-28 female

S.littoralis flagellar segments, showing scales on the dorsal surface and different types of sensilla chaetica (S.ch I,II);

(S.a) sensillum auricillium; sensilla trichodea

(S.T.I,II,III) and sensilla basiconica (S.B)

(x 400).



Fig.III.I.3. SELI on the ventral side of the 8th antennal segment of S.littoralis male, showing sensillum trichodeum type I (S.TI). (x2000).



Fig. III.1.4. Scanning electron microscope of lower portion of type I trichodeum in <u>S.littoralis</u> male (x 3000).



Fig.III.I.5. SEM of third antennal segment of S.littoralis male showing sensillum trichodeum type II

(S.T₂) found on the midventral line of the segment distal end sensillum styloconica (S.st)

(x 5000).



Fig.III.I.6. SEM showing one sensillum trichodeum type III (x 9000).

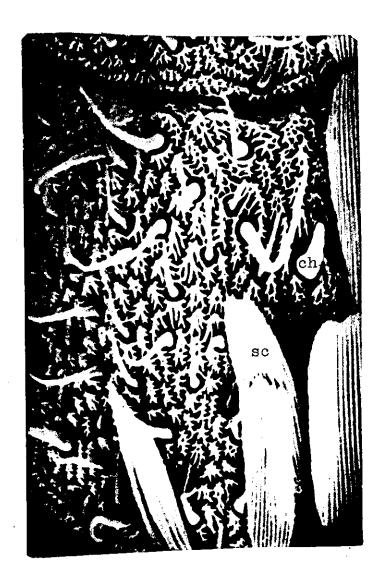


Fig.III.I.7. Scanning electron microscope of the tenth flagellar segment of male <u>S.littoralis</u> antennae showing sensilla trichodea II&III, sensillum basiconicum, and sensillum chaeticum type II (x ICOO).

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Fig.III.I.9. SEM showing sensilla chaetica type I

(S.ch_I) sensilla trichodea type I,II (S.T_{I,2})
and type I sensilla coeloconica (S.co_I) on
the dorsal surface of male apical segment
(x I500).

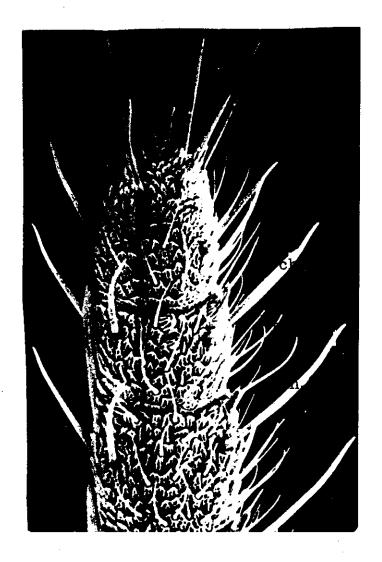


Fig.III.I.O. General view of the distal three segments of the male <u>S.littoralis</u> antennae, showing the sensilla styloconica (S.st) in the apical segment, two types of sensilla chaetica (S.ch), three types of sensillum trichodeum (S.T) sensillum basiconicum (S.B) and the coeloconica type I (S.co) on the narrowed portion (x 400)

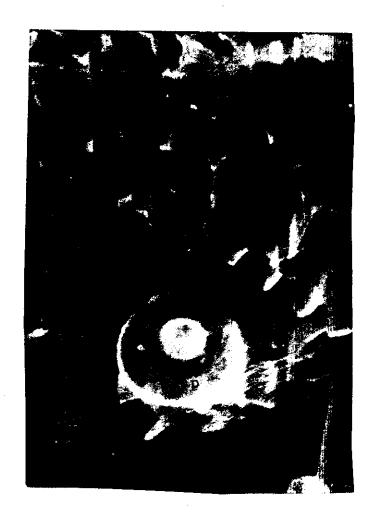


Fig.III.I.2. Scanning electron microscope of a small chemoreceptor peg arising in the center of the sensoty area (x 8000).



Fig.III.I.13. Scanning electron micrograph of sensillum chaeticum I on antenna of male $\underline{S.littoralis}$ moth (x 2000).



Fig.III.I.14. SEM of the terminal female <u>S.littoralis</u> antennal segment showing three small chemoreceptor pegs (S.scp) at the narrow portion and IO - I2 sensilla chaetica type I (S.ch_I) (x IOCC).



Fig.III.I.15. SEM of the ventral view of the I7 & I8 th male S.littoralis flagellar segments, showing the sensilla chaetica type I (S.ch_I) (x 400)



Fig.III.I.16. SEM of male <u>S.littoralis</u> antenna, showing the tip of a sensillum chaeticum type II (S.ch_I). Notice the pore (p) at the tip of sensillum (x 300).



Fig.III.I.7. High magnification of the basal part of sensillum chaeticum type I, showing the collar like socket through which the sensillum is fixed (x 10000).



Fig.III.I.8. The basal portion of sensillum chaeticum type I as figured by the MBM. Notice the collar-like socket of sensillum (x 4500).



Fig.III.I.19. Sensilium auricillum (S.a) and small chemoreceptor peg sensillum (scp) figured by SEM from the 32 nd flagellar segment of female S.littoralis antenna (x 9000).



Fig.III.I.22. Sensillum styloconicum (S.st) as magnified by the SEII, appearing with its smooth wall and arising directly from the antennal surface without a difinite socket (x SCOO).



Fig.III.I.23. General view of the apical portion of the terminal segment of <u>S.littoralis</u> male sensilla that can be determined include: three sensilla styloconica (S.st), sensilla chaetica type I (S.ch_I) showing the pore of sensillum on its tip, and sensilla coeloconica type I (S.co_I) with I4 teeth (x 300).

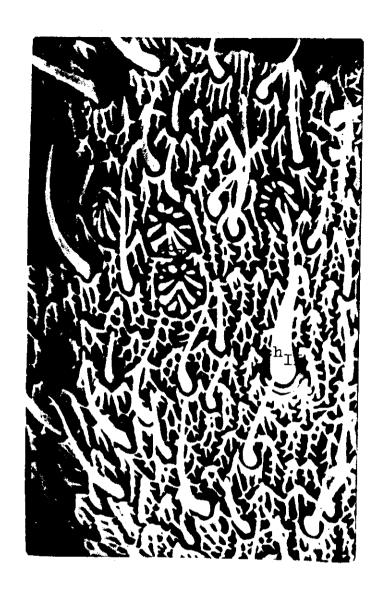


Fig.III.I.24. Ventral view of the I2 th flagellar segment of S.littoralis female figured by SEM, showing different types of sensilla: (sensilla trichodea I,II,& III, sensillum chaeticum II, coeloconica I and sensilla basiconica) (x 1000).

III.2. Electroantennogram recording:

Various types of sense organs are present on the antennae of <u>Spodoptera littoralis</u> (Boisd.), and different types of sensilla have been recognized on it. The electroantennogram (EAG) is one of the most important electrophysiological record performed on insect's moth antennae in order to reflect light on its function.

In Egypt, the EAG studies are very rare. This is may be due to the difficulties of recording and lack of available resources necessary for this study. This work was stressed on the EAG studies in order to determine the functions of sensilla of suitable lengths which are expected to have an important role in methods of communication, either between males and females or between the insect and its host plant. Two types of sensilla were chosen; the sensillum trichodeum, and the small chemoreceptor peg, in order to define the speifity of each sensilla to certain agents such as sex pheromone or other oders. The EAG was recorded by using a glass microelectrode of tip diameter (50-I00) micron and resistance (5-I0) X IO⁴ \mathbf{\Omega}, filled with a conducting electrolytic solutions such as NaCl, Kcl

or CaCl₂. The tip of the sensillum was introduced inside the tip of the glass microelectrode under microscopic control of high magnification. The conductance between the microelectrode and the recording system (400 MD 2C) was made through an Ag-AgCl electrode immerssed in the conducting solution filling the glass microelectrode. Preliminary experiments were made on different concentrations of each of those solutions (NaCl, KCl and CaCl₂) in order to chose the most suitable type of electrolyte and so its concentration which can be used in the recording glass microelectrode. Therafter, the effect of sex pheromone or other odors can be investigated.

To achieve the previous aim the results of the experiments in this part of work were divided into

experiments in this part of work were divided into four parts:

I. The effect of NaCl on the recorded electroantennogram:

A set of glass microelectrodes of tip diameter (50 - I00 u), filled with different concentrations of NaCl (0.0I, 0.05, 0.I, 0.25, 0.5, I.0, I.5, 2.0 and 3.0 M) were used for recording the EAG of sensilla trichodea.

2. The effect of KCl on the EAG recorded:

A set of glass microelectrodes of the same tip diameter, but filled with different concentrations of KCl of (0.025, 0.05, 0.1, 0.25, 0.5, I.0, I.5, and 3.0 M) were used for recording the EAG of the sensilla trichodea and small chemoreceptor pegs.

3. The effect of CaCl, on the EAG recorded:

A set of glass microelectrodes of the same tip diameter, filled with different concentrations of CaCl₂ of (0.05, 0.I, 0.25, 0.5, I.O, I.5, and 3.0 M) were used for recording the EAG of the sensilla trichodea and small chemoreceptor pegs .

4. Comparison between the effects of using NaCl, KCl and CaCl₂ microelectrodes, on the EAG and the choice of the most suitable one which can be used in studying the effect of the sex pheromone and other odors on the two sensilla.

III.2.I. Effect of NaCl on the recorded electroantennogram (EAG):

In this part of work, the EAG was recorded from the sensillum trichodeum by using glass microelectrodes filled with different concentrations of NaCl from 0.0I up to 3.0 M. Figure (III.2.I) represents typical records of the EAG which showed a great variations in its form, frequency and amplitude, as the NaCl concentration was changed. The relation between the characteristics; frequency and amplitude, of the EAG spikes and the NaCl concentrations are tabulated in Table (III.2.I) and represented in Figs.(III.2.2A,B) respectively.

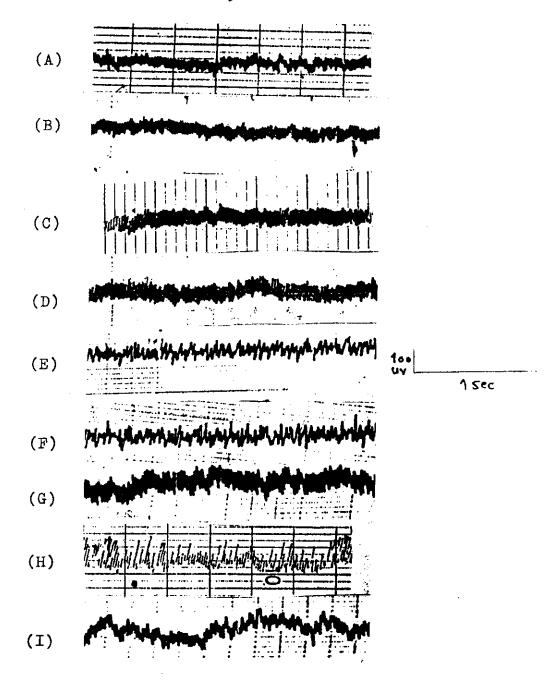


Fig.III.2.I. Typical records of the EAG registered from the sensillum trichodeum tip by using glass microelectrodes filled with different concentrations of the NaCl solutions.

(A) O.OI M) 0.0I	M
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(B) 0.05 M

(C) O.I M

(D) 0.25 M

(E) 0.5 M

(F) I.O M

(G) I.5 M

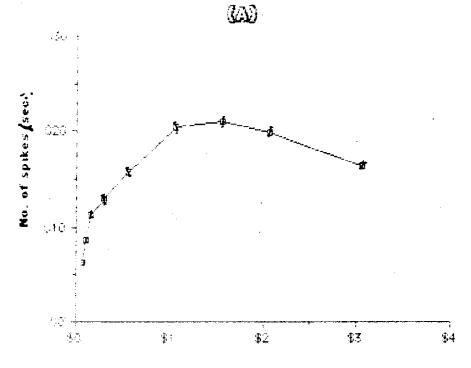
(H) 2.0 M

(I) 3.0 M

Table.III.2.I. Relation between the NaCl concentrations and the frequency (F) and amplitude (A) of the EAG spikes recorded from sensillum trichodeum.

Concentration/ Mol. NaCl	(F)	(A)		
0.01	5.7 ± 0.12	46 ± 1.4		
0.05	8.2 ± 0.II	61.6 ± .87		
0.1	10.6 ± 0.13	76.6 ± 1.49		
0.25	12.26 ± 0.18	79.3 ± 0.36		
0.5	I5.2 ± 0.14	85.7 ± 1.39		
I.O	19.7 ± 0.12	103.3 ± 2.68		
1.5	20.26 ± 0.18	II7.5 ± I.29		
2.0	19.2 ± 0.10	IIO ± 3.66		
3.0	15.65 ± 0.19	90 ± 1.65		

Mean \pm SE $_{\overline{X}}$ from I5 replicates .



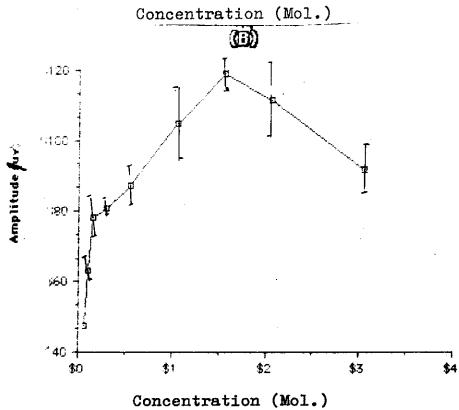


Fig.III.2.2A,B. Relation between the NaCl concentrations and the frequency (upper curve A) and amplitude (lower curve B) of the EAG spikes recorded from sensillum trichodeum.

They indicated that both the frequency and amplitude of the EAG spikes showed a gradual increase as the NaCl concentration was increased up to I.5 M and then they slightly decreased.

Generally, the obtained data clearly showed a . possitive relationship between the recorded frequency and the mean amplitude on one side and the NaCl concentration up to I.5 M on the other side. By increasing the concentration than I.5 NaCl, a successive decrease occured in both the frequency of the EAG & amplitude. This led the conclusion that the frequency & spike amplitude of the EAG changed in association with each other in insect's behaviour. Moreover, from the previous mentioned results it could be concluded that the S. littoralis moth's activity is low in both NaCl concentrations of 0.5 & 3.0 M. These concentrations may be used if ane wants to study the effect of other chemical agents on the EAG of sensillum . In this work, the 3.0 M NaCl electrode was chosen since it is a good conductor and a suitable stimulator for initiating EAG of activity low enough for investigating any other external effect on sensilla .

This finding is in agreement with that demonstrated by Mayer (1973), who used the 3.0 M NaCl electrodes for electrophysiological studies correlated to attraction in <u>Trichoplusia</u> ni.

III.2.2: Effect of KCl on the recorded electroantennogram (EAG):

The typical responses of the EAG of the sensillum trichodeum and the small chemoreceptor peg are shown in Fig. (III.2.3). These records were obtained by using glass microelectrodes filled with different concentrations of KCl from 0.025 M up to 3.0 M. The data were tabulated in Table (III.2.2) and represented in Figs. (III.2.4,5) . They showed the variations of both frequency and amplitude of the EAG of the two sensilla with the variation of the KCl concentrations . These data indicated similar general trend of response to those obtained by using NaCl electrodes . The frequency of impulses of the two sensilla increased gradually with the increase of KCl concentration up to I.O M with peak values of I6.33 - 0.13 cycle/second and I3.85 $\stackrel{+}{=}$ 0.I2 cycle/second for the sensillum trichodeum and small chemoreceptor peg respectively. By increasing the concentration up to 3.0 M the frequency decreased gradually to be 9.4 - 0.14 C/sec. and 9.3 ± 0.13 C/sec. for the sensillum trichodeum and the small chemoreceptor peg respectively .

Regarding the amplitudes of the EAG recorded from the two sensilla one can notice that they have

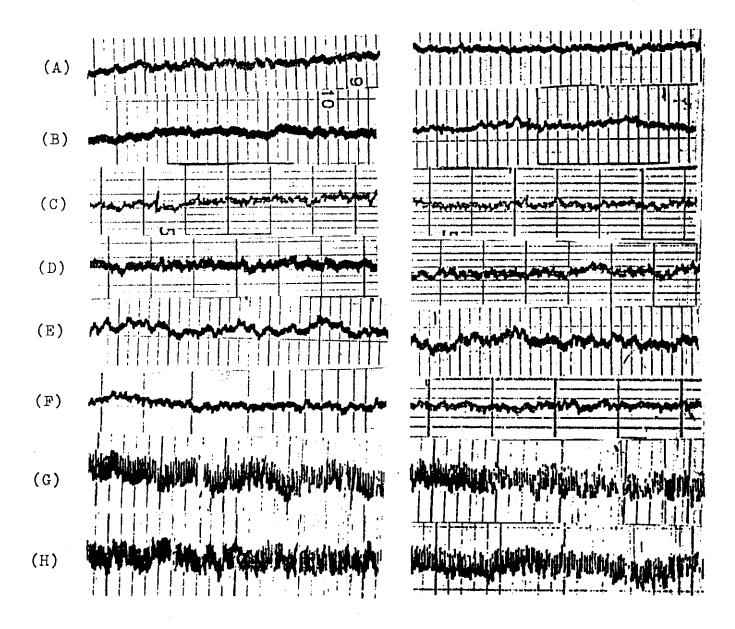


Fig.III.2.3. Typical records of the EAG registered from two sensilla (trichodeum (I) and small chemo. peg (2) by using electrodes filled with different concentrations of the KCl solutions.

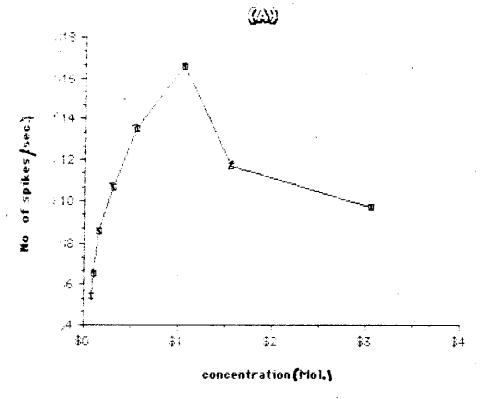
(A) 0.025 M (B) 0.05 M (C) 0.1 M (D) 0.25 M

(E) 0.5 M (F) I.O M (G) I.5 M (H) 3.0 M

Table.III.2.2. Relation between the concentrations of
KCl and the frequency (F) and amplitude
(A) of the EAG spikes recorded from two
sensilla (trichodeum & small
chemoreceptor peg).

Concen./ Mol. KCl	Sensillı (F)	um trichodeum (A)	Small chemo	receptor peg
0.025	5.2 ± 0.II	29.6 ± 0.4I	4.6 ± 0.II	27.2 ± 0.59
0.05	6.25 [±] 0.13	34.4 ⁺ 0.4I	6.52 ± 0.10	29.6 ± I.6
0.1	8.3I [±] 0.I4	36.4 ± 0.76	8.45 ± 0.09	39.2 ± 1.18
0.25	10.4 ± 0.18	47.6 ± 0.67	10.38 ± 0.08	42 ± 1.08
0.5	13.25 [±] 0.12	45.2 ± 1.07	II.72 ± 0.08	45.6 [±] I.39
I.O	16.33 [±] 0.13	38.7 ± 0.6I	13.85 ± 0.12	36 ± 0.82
I.5	II.4 ± 0.18	I24.8 ± 0.98	II.I2 ± 0.I4	II8.5 ± 0.49
3.0	9.4 - 0.14	105.3 ± 0.88	9.3 ± 0.13	102 ± 0.41

Mean - $SE_{\overline{x}}$ from 15 replicates .



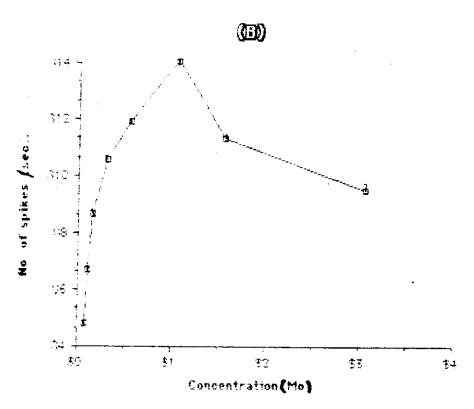
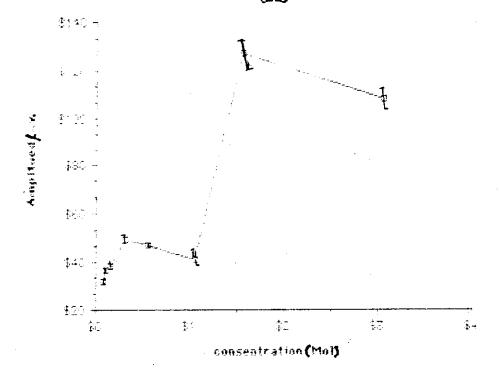


Fig.III.2.4A,B. Relation between the KCl concentrations and the frequency (F) of the EAG spikes recorded from two sensilla (trichodeum (A) and small chemoreceptor peg (B).





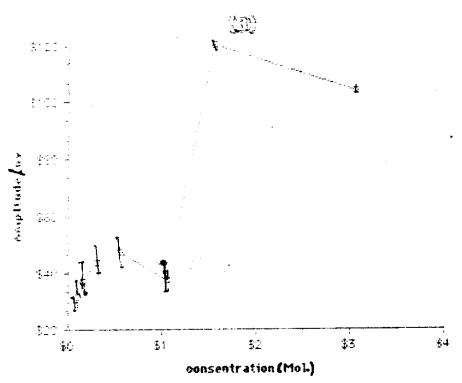


Fig.III.2.5A,B. Relation between the KCl concentrations and the amplitude (A) of the EAG spikes recorded from two sensilla (trichodeum (A) and small chemoreceptor peg (B).

the same form of change. In both cases the amplitudes showed a little increase as the concentration was increased from 0;025 M up to 0.25 M and then they slightly decreased until the KCl concentration reached I.O M. Therafter, a rapid high increase followed by a slight decrease was noticed as the KCl concentration was increased up to 3.0 M.

By comparing the results obtained from the two sensilla for both amplitude and frequency one can deduce that:

- I. The response of sensillum trichodeum by using KCl electrode is higher than that of the small chemoreceptor peg.
- 2. S.littoralis moth's activity is low in both KCl concentrations of 0.25 and 3.0 M. These concentrations may be used for filling the glass microelectrode used for studying the effect of chemical agents on the EAG of sensillum.

III.2.3: Effect of CaCl2 on the recorded electroantennogram (EAG):

The typical responses of the EAG of the sensillum trichodeum and the small chemoreceptor peg are shown in Fig. (III.2.6) . These records were obtained by using glass microelectrodes filled with different concentrations of CaCl, from 0.05 M up to 3.0 M . The data tabulated in Table (III.2.3) and represented in Figs. (III.2.7&8) showed the variations of both frequency and amplitude of the EAG of the two sensilla with the variation of the CaCl, concentrations . These data indicated that at concentration lower than 0.5 M, the obtained results were nearly similar to those obtained by treatment with sodium chloride at the same concentrations . However at concentrations 0.5 M and above the frequency was clearly lower by using CaCl2 than sodium chloride . The frequency of impulses of the two sensilla increased gradually with increasing the CaCl2 concentration from 0.05 M up to 0.5 M and then they decreased at higher concentrations up to 3.0 M CaCl, .

Regarding the amplitudes of the EAG recorded from the two sensilla one can notice that they have



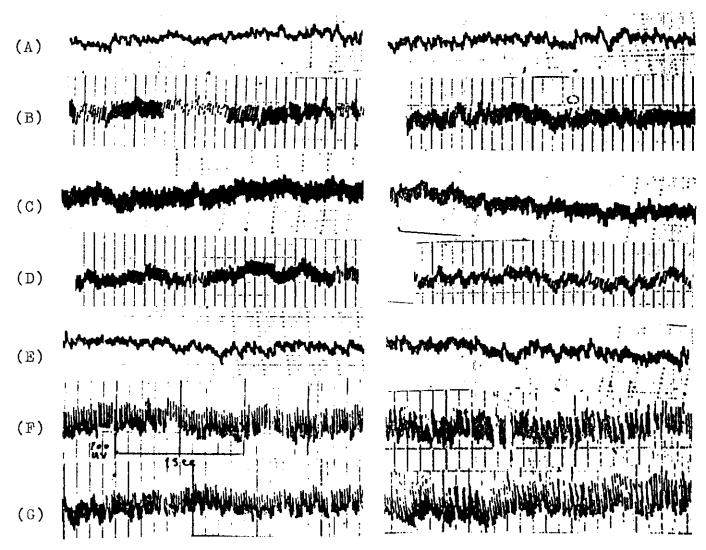


Fig.III.2.6. Typical records of the EAG registered from two sensilla (trichodeum (I) and small chemo. peg (2) by using different concentrations of CaCl₂ solutions .

(A) 0.05 M (B) 0.1 M (C) 0.25 M (D) 0.5 M

(E) I.O M (F) I.5 M (G) 3.0 M .

Table. III. 2.3. Relation between the CaCl concentrations and the chemoreceptor peg.) frequency (F) and amplitude (A) of the EAG spikes recorded from two sensilla (trichodeum & small

3.0	I.5	I.0	0.5	0.25	0.I	0.05	CaCl	Concentration/
8.04 ± 0.05	9.04 + 0.06	II.7 ± 0.06	12.5 ± 0.18	IO.4 ± 0.14	8.6 ± 0.I3	7.5 ± 0.14	(F)	
III.5 ± 0.85	132 ± 0.67	44.5 ± D.I4	65.2 ± 0.59	74.6 + 0.85	89.6 ± 0.38	38 ± 0.85	(A)	Sensillum trichodeum
7.76 ± 0.09	8.32± 0.II	9.75± 0.I2	II.2 ± 0.13	10.3 ± 0.14	8.3 ± 0.I3	7.3 ± 0.15	(F)	Small c
I08.2 ± 0.85	127.5 ± 0.53	44.7 ± 0.46	53.2 ± I.2	67.2 ± 0.59	85 + 0.95	43 t 0.54	(A),	Small chemoreceptor peg

Mean + SE from 15 replicates .

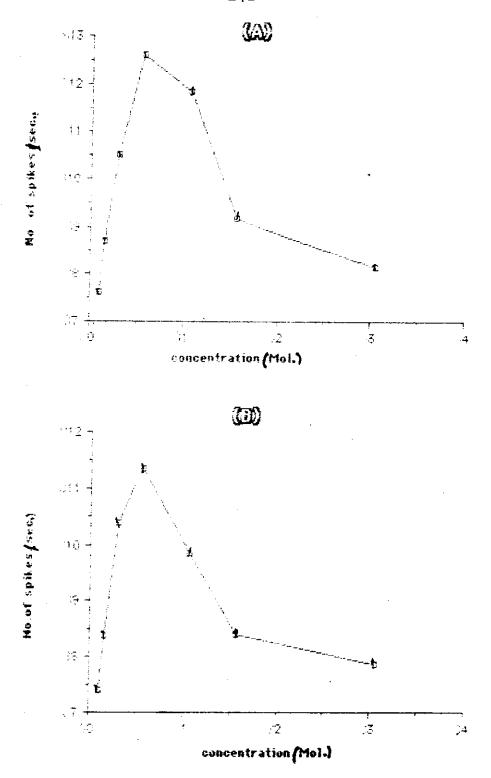
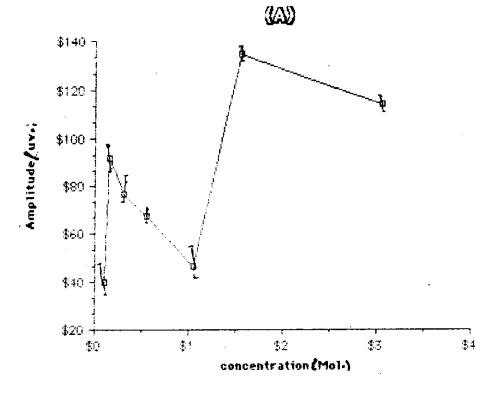


Fig.III.2.7A,B. Relation between the CaCl₂ concentrations and the frequency (F) of the EAG spikes recorded from two sensilla (trichodeum (A) and small chemoreceptor peg (B).



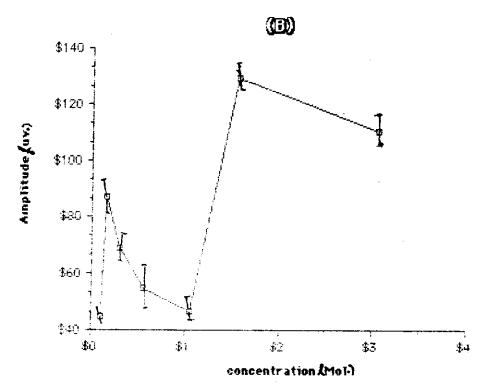


Fig.III.2.8A,B. Relation between the CaCl₂ concentrations and the amplitude (A) of the EAG spikes recorded from two sensilla (trichodeum (A) and small chemoreceptor peg (B).

the same form of change. The amplitudes showed two maxima at CaCl_2 concentrations of 0.I & I.5 M and two minima at 0.05 & 3.0 M CaCl_2 for both sensilla. Generally, one can noticed that the trichodium is more sensitive to CaCl_2 stimulation than the small chemoreceptor peg. Moreover, the sensitivity of the two sensilla to CaCl_2 is lower than that for both NaCl_2 and KCl_2 .

III.2.4. Comparison between the effect of NaCl, KCl and CaCl₂ on the EAG of antennal sensilla of S. littoralis:

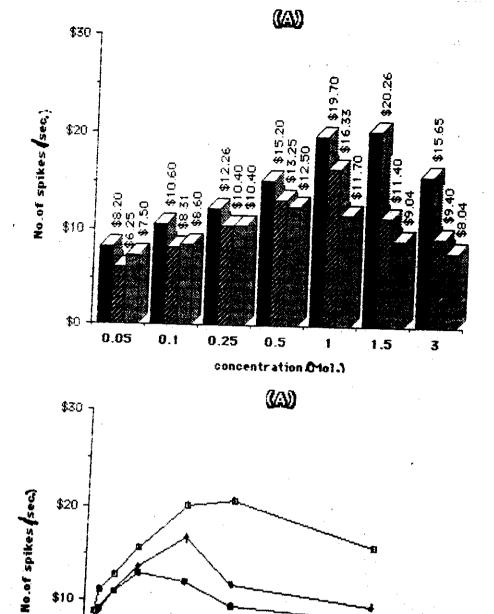
Results of the EAG responses of sensilla trichodea to different concentrations of NaCl, KCl and CaCl₂ are collected in Fig.(III.2.9), table (III.2.4) and diagramatically represented in Figures (III.2.10 A,B) These results indicated that:

- I. The minimum frequencies and amplitudes of the EAG were found at the lowest used concentration (0.05 M) for all of the three salt solutions.
- 2. The peak values of the frequencies were found at concentrations of I.5, I.O and O.5 M for NaCl, KCl and CaCl₂ solutions respectively.
- 3. The peak value of the amplitude, in case of NaCl was found at I.5 M, while in case of KCl and CaCl₂ solutions there were two peaks:
 - (I) small peaks at 0.5 M and 0.I M respectively, and
 - (2) big peaks at the same concentration of I.5 M of the two salts .
- 4. The peak values of the frequency and amplitude of the EAG of sensillum trichodeum were always higher in case of NaCl solutions than those in case of KCl and CaCl₂ solutions.

Table.III.2.4. Effect of seven concentrations of NaCl, KCl and $CaCl_2$ on the EAG frequency and amplitudes of sensilla trichodea on antennae of <u>S.littoralis</u>.

Concentrati	ion/	Frequency	
Mol.	NaCl	KCl	CaCl2
0.05	8.2 ± 0.II	6.25 ± 0.13	7.5 [±] 0.14
0.1	IO.6 ± 0.I3	8.3I ⁺ 0.I4	8.6 ± 0.13
0.25	12.3 ± 0.18	IO.4 ± 0.18	10.4 ± 0.14
0.5	15.2 ± 0.14	I3.25 ± 0.I2	12.5 ± 0.18
I.0	19.7 ± 0.12	16.33 ± 0.13	II.7 ± 0.09
I.5	20.3 ± 0.18	II.4 ± 0.18	9.04±-0.06
3.0	15.65 [±] 0.19	9.4 ± 0.14	8.04 [±] 0.05
Concen./Mol	•	Amplitude	
0.05	6I.6 ± 0.87	34.4 [±] 0.4I	38 ± 0.85
0.1	76.6 ± 1.49	36.4 ± 0.75	89.6 [±] 0.38
0.25	79.3 ± 0.36	47.6 ± 0.67	74.6 [±] 0.85
0.5	85.7 ± 1.59	45.2 ± I.07	65.2 [±] 0.59
I.0	103.3 ± 2.68	38.7 ± 0.6I	44.5 [±] I.I4
I.5	II7.5 ± 1.29	I24.8 ± 0.98	132 ± 0.67
3.0	90 ± 1.65	105.3 ± 0.88	III.5 ⁺ 0.85

Mean - $SE_{\overline{x}}$ from 15 replicates .



concentration (Mol.) Fig. III.2. IOA. Comparison between the effects of different concentrations of NaCl, KCl, and CaCl2 on the frequencies of the EAG recorded from sensilla trichodea .

\$2

\$3

\$1

\$10

\$0 \$0

NaCl Q.... KCL CaCl₂ From the last observations, it can be concluded that the least activity of the sensillum trichodeum was recorded by using Calcium chloride electrode, while the highest activity occured with sodium chloride one.

Other-while, at higher concentration of KCl and CaCl₂ the amplitudes of the EAG became higher than those obtained by using the same concentrations of NaCl.

The previous discussion results that:

The selection of the conductor solution for filling the micro-electrode used in EAG recording is based upon being a good conductor for initiating EAG of less activity, suitable for investigating the effects of any external chemical agents. For these reasons the 3.0 or 0.I M NaCl and I.O M CaCl₂ electrodes could be chosen and used in experiments especialised in sex pheromone or for using the chemical oder substances.

III.2.5 Effect of the attractant female sex pheromone on Spodoptera littoralis male moth.

Studies on pheromone reception by the insect's antennae was rapidly advanced in recent years. But the electrophysiological studies, in this field, on the S. littoralis are still obscure in literature. So, it is of great importance to pay attention to this study in order to know many new informations about this insect.

In this part of work the EAG of the sensillum trichodeum and the small chemoreceptor peg was recorded and exploited for the following studies:

- a- Determination of the site receptor antennae of <u>S</u>.

 littoralis male moth to sex pheromone.
- b- Effect of using different conductor solution with sex pheromone on response of sensillum trichodeum of S. littoralis male moth .
- c- Effect of higher pheromone concentrations on the adult response.

The insects were obtained from a laboratory reared culture of <u>S. littoralis</u>. Freshly emerged males and females which were previously seperated in pupul stage (to prevent mating), were fed on 5% aqueous sucrose solution for few days before being used in the experiments. The average length of the male antennal flagellum is

about 7.72 ± 0.08 mm long, and consists of approximately 63 segments. Each segment is covered with scales except for the rostroventral side of its proximal end where the scales are missing, and each segment bears many curved sensilla trichodea.

The antennae was fixed using the same technique as mentioned later. The airflow system and experimental method were previously in Fig.(II.4). The attractant (test materials) Cis 9 trans II tetradecadien I YL acetate was placed in a glass vessel, prepared for directing it over the antennae. Glass microelectrodes of tip diameter about 50 u filled with 3.0, 0.I or I.5 M NaCl and I.0 M CaCl₂ solutions were used. The tip of microelectrode was placed on the proximal part of the trichodeum type I of sensory hairs on the antennae under microscopic control. The electrode was led to an oscillograph (400 MD 2C) via an amplifier.

Airflow was continually directed over the antennal prepartion. The successive impulse responses were then recorded.

a- Site receptor on antennae of S. littoralis male moths to sex pheromone:

To investigate how males of <u>S. littoralis</u> are able to perceive female attracting substances, records of the EAG were made from the sensilla trichodea and small chemoreceptor pegs on antennae of both sexes during stimulation with 3.0 NaCl alone and after the application of the attractant Cis 9 trans II tetradecadien IYL acetate.

Typical records of the EAG of both sexes are shown in Fig. (III.2.II.). The data of the records is represented in table (III.2.5) and Fig. (III.2.I2 A,B). They showed the variations of both the frequency and amplitude of the EAG responses of the olfactory cells in the antennal sensilla trichodea and small chemoreceptor peg for both sexes. Both the frequency and amplitude of the EAG responses showed the same form of change towards the pheromone stimulation. The highest EAG response was recorded in case of the sensillum trichodeum of the male antennae. In this case, the frequency increased from 15.65 ± 0.18 cycle/second by using NaCl electrode alone to 20.I ± 0.22 cycle/second after stimulation with the attractant sex pheromone, while the amplitude changed from 90 ± 1.65 uv up to 109.I ± 0.8 uv respectively.

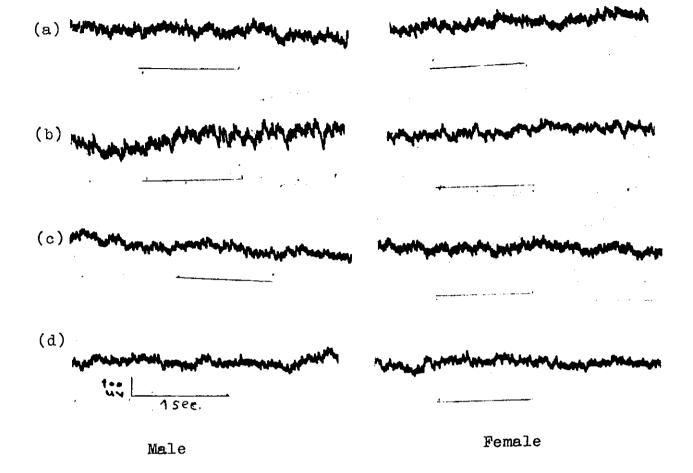


Fig. III.2.II. Typical records from the olfactory cells of sensilla trichodea and small chemoreceptor pegs where:

- (a) Response of sensilla trichodea recorded by NaCl electrode, (Male Female).
- (b) Response of sensilla trichodea recorded by NaCl electrode during exposure to sex pheromone (Male Female).
- (c) Response of small chemo. peg recorded by NaCl
- (d) Response of small chemo. peg recorded by NaCl during exposure to sex pheromone (Male-Female)

Table.III.2.5. Responses of the olfactory cells in antennal sensilla trichodea and small chemoreceptor pegs of <u>S.littoralis</u> (records from I5 rep.)

Type of S. Sensillum trichodeum Small chemoreceptor peg							
Sex	Treatm.	(F)	(A)	(F)	(A)		
M a	3.0 M NaCl	15.65±0.18	90 ± 1.65	I3.42 [±] 0.I2	64.8 [±] 0.8		
a l e	3.0 M NaCL + Sex phero.	20.I ±0.22	109.1±0.8	13.55±0.13	64 ±0.56		
F e m a l e	3.0 M NaCl	I4.6 ±0.I	65.8 [±] 0.77	I4.22 [±] 0.04	67.9±0.8 ⁷		
	3.0 M NaCl + Sex phero.	I4.3 ±0.I2	6I ±0.82	14.09 [±] 0.12	63.6±0.64		

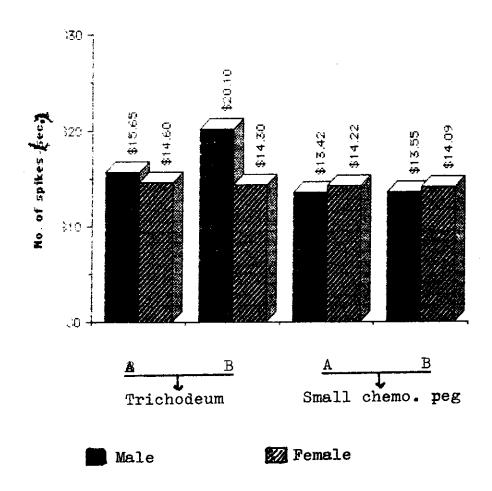


Fig. III.2.I2A. Responses frequencies of the EAG of the olfactory cells in antennal sensilla trichodea and small chemoreceptor pegs in both sexes of <u>S. littoralis</u>.

A-= 3.0 M NaCl

B = 3.0 M NaCl + sex pheromone.

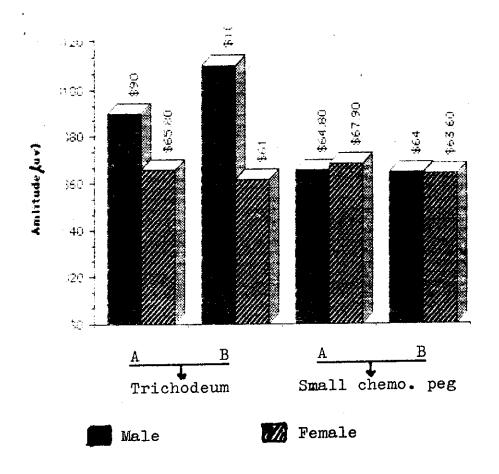


Fig.III.2.I2B. Mean spike amplitudes of the EAG of antennal sensillum trichodeum and small chemoreceptor pegs in both sexes of <u>S. littoralis</u>

A = 3.0 M NaCl

B = 3.0 M NaCl + sex pheromone.

On the other hand, no change in both frequency and amplitude was found when antennal sensillum trichodeum of female was stimulated with the sex pheromone. In this case the frequency was I4.6 ± 0.I cycle/second alone and showed a very little unnoticable decrease to I4.3 ± 0.13 cycle/second during stimulation with sex pheromone. The same finding is present in case of the small chemoreceptor pegs in both sexes exposed to sex pheromone. In this case no significant increase in impulses frequency or amplitude was observed, before and after the pheromone application. This is clearly demonstrated in that by using the 3.0 M NaCl solution alone. The recorded frequencies were I3.42 - 0.18 and 14.22 ± 0.04 in case of male and female respectively. Other while by exposing the small chemoreceptor pegs to stimulation with the sex pheromone, the recorded impulses from male and females became 13.55 ± 0.13 and I4.09 ± 0.12 cycle/second respectively. This indicates no significant change in this case.

The last results indicated that :

- I. Sensilla trichodea of female antennae didn't respond to sex pheromone, while in male, it was very sensitive to it.
- 2; The small chemoreceptor pegs were not stimulated by passing the sex pheromone oder neither in male nor in female antennae.

This study concluded that, if one wants to study the effect of sex pheromone on moth of \underline{S} . Littoralis the experiments must be carried out on the responses of sensillum trichodeum of male moth which is specialized in this function .

b- Effect of using different conductor solution with sex pheromone on response of sensillum trichodeum of S. littoralis male moth.

In this part of work, the effect of sex pheromone on the EAG of sensillum trichodeum was investigated when using glass microelectrodes filled with different conductor solutions (3.0, I.5, O.I of NaCl and I.O M CaCl₂). This was done in order to chose the best suitable electrode which can be used with sex pheromone experiments.

responses of sensilla trichodee on male <u>S. littoralis</u>.

The first set (A) represent the responses with one of the last conductors alone, while the second (B) represents the responses after using the sex pheromone with the different conductor solutions.

The data of the frequency and amplitude of the two sets are summerized in table (III.2.6) and diagrammatically represented in Fig. (III.2.I4). The obtained data indicated that:

I. By using NaCl electrode, the highest response of sensillum trichodeum to sex pheromone was induced at NaCl concentration of 3.0. In this case the mean frequency increased from I5.65 \pm 0.18 by using the conductor solution alone to 20.1 \pm 0.22 after sex pheromone exposure.

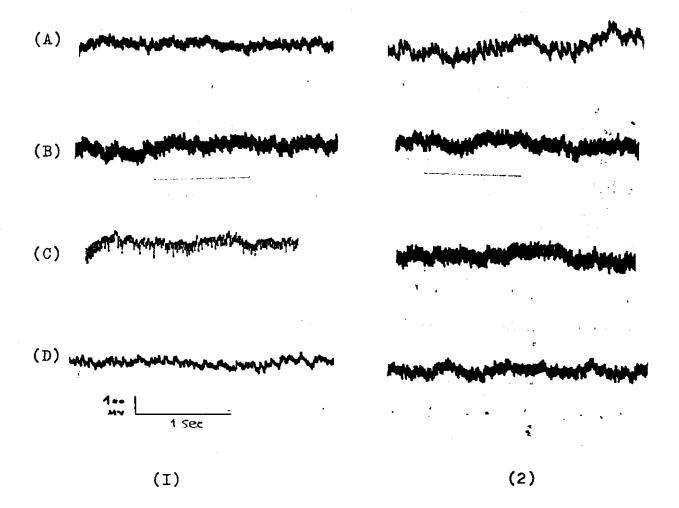


Fig.III.2.13. EAG responses recorded with microelectrode from olfactory receptor cells sensilla trichodea of the cotton leafworm male moth stimulated with:

- (A) 3.0 M NaCl (I) and 3.0 M NaCl + sex pheromone(2)
- (B) I.5 M NaCl (I) and I.5 M NaCl + sex phero. (2)
- (C) 0.I M NaCl (I) and 0.I M NaCl + sex phero. (2)
- (D) I.0 M $CaCl_2(I)$ & I.0 M $CaCl_2 + sex$ phero. (2)

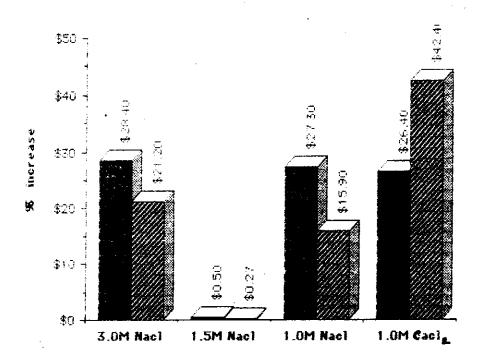
Table.III.2.6. Responses of the olfactory cells in antennal sensilla trichodea of <u>S.littoralis</u> male to different conductor solutions, (records from 15 replicates).

Treatment	Frequency	Amplitude	% inc	rease
iiea (meii	(F)	(A)	(F)	(A)
3.0 M Na0	15.65±0.19	90 ±1.65		
3.0 M Nac Sex phero	20 T IO 22	109.1 - 0.8	28.4	21.2
I.5 M NaC	20.26±0.18	117.5 [±] 1.29		
I.5 M Nac Sex phero	20.3 -0.06	117:8-0.67	0.5	0.27
O.I M NaC	1 10.6 ±0.13	76.5 [±] 1.49		
O.I M NaC Sex phero	13.5 -0.07	82.9 [±] 1.8	27.3	I5 . 9
I.O M CaC	1 ₂ II.7 ±0.09	44.5 [±] I.I4		
I.O M CaC Sex phero	- IA 8 10 IO	63.4 ± 0.9	26.4	42.4

This indicated an increase of about 28.4 % due to sex pheromone application. Also the mean amplitude of EAG impulses was increased by about 21.2 % after adding the sex pheromone from (90 ± 1.65 to 109.1 ± 0.8 uv for NaCl solution and sex pheromone + NaCl, respectively).

2. In case of using 0.1 M NaCl electrode the recorded percentage of increase of mean frequency and mean , impulse amplitudes due to sex pheromone exposure were found to be 27.3 % & 15.9 %, respectively.

- 3. The percentage of increase in both the frequency and amplitude by using I.5 M NaCl electrode during the sex pheromone exposure, may be considered negligible as estimated only of about 0.5 % and 0.27 % respectively, table (III.2.6).
- 4. By using I.O M CaCl₂ electrode with sex pheromone application a considerable increase in the mean frequency was observed. It increased from II.7 ± 0.29 cycle / second by using the salt alone to I4.8 ± 0.I2 cycle/ second, after sex pheromone application. This indicated an increase of about 26.4 %. On the other hand, the amplitude showed a high percentage of increase which estimated of about 42.4 % after exposure to sex pheromone, table (III.2.6).



Different conductor solutions

Fig.III.2.14. Percentage of increase in EAG response (frequency and amplitude) recorded from sensilla trichodea on <u>S.littoralis</u> male antennae due to using the sex pheromone together with different conductor solutions.

/ increase in frequency in amplitude

C. Effect of high concentration of sex pheromone on the EAG responses of the male sensillum trichodeum:

The effect of different concentrations of sex pheromone was studied in this part of work.

I,2,3,4, and 5 mg, of sex pheromone were applied in capsules (each capsule contains one mg). Figure (III.2.I5) represents typical records of the EAG of the sensillum trichodeum. The data tabulated in Table (III.2.7) showed that no significant difference in response was detect among the tested pheromone concentrations. The precentage of increase in frequency and amplitude of the EAG due to the effect of different pheromone concentrations ranged from 25 % - 29 % and from 37.2 % - 48.3 % respectively.

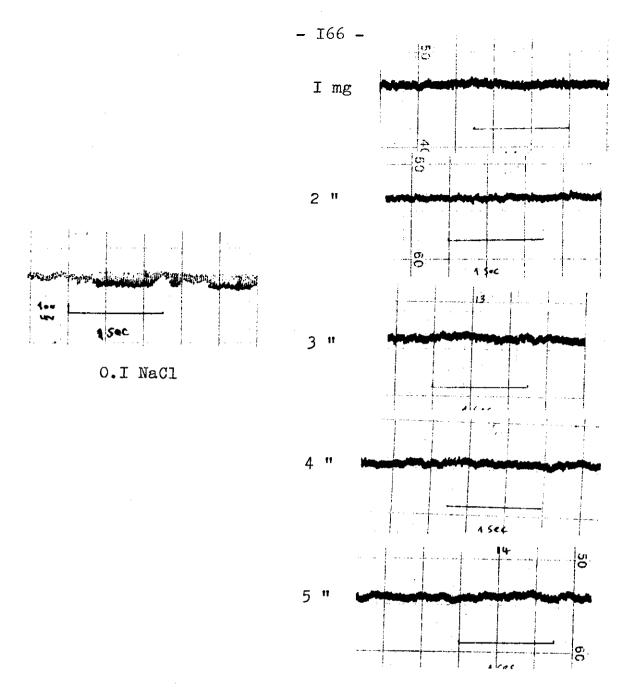


Fig.III.2.15. EAG recorded with microelectrode from sensillum trichodeum of the cotton leafworm male moth stimulated with different loads of sex pheromone (I,2,3,4,5 capsules).

III.2.6. Effect of the odor chemical substance on both sexes of <u>S.littoralis</u>:

Several studies on odor reception by antennae in different insect species have been done in recent years (Den Otter.et.al.,1979). But, due to the available knowlege, no work has been carried out on the odor reception by antennae in S.littoralis moths.

This part of work represents a trial to evaluate the possibility of using the EAG records to determine the important role of some sensilla as odor receptors in <u>S.littoralis</u> males and females.

The effect of two odor chemical compounds, amyl acetate ethyl butyrate on sensillum trichodeum and small chemoreceptor peg on the antenna of both sexes was investigated.

The EAG of the two sensilla was recorded from moth antennae, by using the previous mentioned recording technique described in chapter (III.2.5).

Female moths used in this experiment were obtained from a laboratory reared cultur after being left with males for a period of 2-4 days after emergence. This period was assumed as enough to insure mating and subsequently using them as gravid mated females. The average length of the female antennal flagellum measured

about 8.9I - 0.23 mm long, and consisted of approximately 65 segments, each segment bears many curved sensilla trichodea. The antennae was fixed, using the same technique as mentioned before. Also the airflow system and the experimental arrangement for the EAG recording are the same as previously shown schematically in Fig. (II.7) . A piece of filter paper, wet with the desired chemical substance, was placed in a glass vessel and directed to the antenna. Glass microelectrode of tip diameter 50 u filled with 3.0 M NaCl, was placed on the proximal part of the sensillum of sensory hairs on the antennae by the aid of micromanipolater together with a stereo-microscope. The electrode was led to the recording system via an amplifier. Continuous airflow was directed over the antennal preparation and the EAG responses were directly recorded on a chart paper .

III.2.6.I. Effect of amyl acetate as odor stimulus on sensilla trichodea and small chemoreceptor pegs on antennae of both sexes of S.littoralis:

Typical responses of the olfactory cells in antennal sensilla trichodea and small chemoreceptor pegs of S.littoralis males and females to amyl acetate are shown in Fig. (III.2.I6) . The data from this figure are tabulated in Table (III.2.8) and represented in Fig.(III.2.17) . The experimental results showed that: I- Both the frequencies and spike amplitudes of the EAG responses recorded from sensilla trichodea on male antennae were slightly varied when exposed to the amyl acetate odor. In this case the mean frequency was increased by only 0.82 % (I5.75 $^{\pm}$ 0.15 to I5.88 $^{\pm}$ 0.I cycle/second) and also the mean spike amplitude by only 0.54 % (from 73.6 \pm 0.67 to 74 \pm 0.47 uV • 2- In case of femals the sensilla trichodea showed high response to odor emanated from amyl acetate . This is clear from the increase in the frequency of the response by 44.7 %. Also, the EAG amplitude showed a very high percentage of increase of about II9.I % after using the amyl acetate stimulation . This is clearly shown in Table (III.2.8).

3- As for the stimulation of small chemoreceptor pegs with amyl acetate, an increase in impulses frequency and amplitude was observed in both sexes. In males, the frequency showed an increase of about 45.4 % while in females the increase in frequency indicated a percentage of about 41.8 % Table (III.2.8).

4-The amplitude of the responses of the small chemoreceptor pegs to 2 % amyl acetate in both sexes, showed a noticeable increase. In males, the spike amplitude was increased by I50 %, while in females the percentage of increase due to amyl acetate treatment estimated of about 66.6 %.

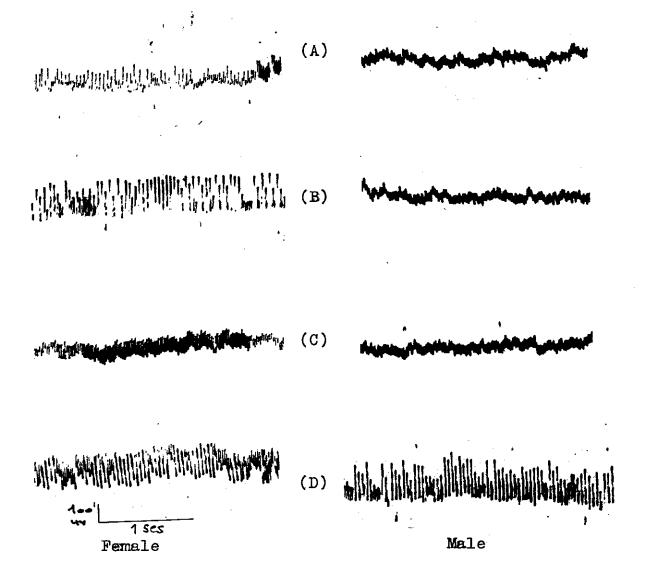


Fig.III.2.16. EAG records from olfactory receptor cells (sensilla trichodea & small chemoreceptor pegs) of the cotton leafworm moths before and after amyl acetate stimulation where .

- (A) Response of sensilla trichodea to NaCl (F-M)
- (B) Response of sensilla trichodea to NaCl + amyl acetate (Female Male)
- (C) Response of small chemoreceptor pegs to NaCl
- (D) Response of small chemoreceptor pegs to NaCl+ amyl acetate (Female Male).

Table.III.2.8. Responses of the olfactory cells in antennal sensilla (trichodea and small chemoreceptor pegs) of <u>S.littoralis</u> moths, (records from I5 replicates).

Type of S. Sensillum tricodeum Small chemoreceptor peg					
Sex	Treatm.	(F)	(A)	(F)	(A)
M a 1 e	3.0 M NaCl	15.75 [±] 0.16	73.6 [±] 0.67	14.7 [±] 0.11	71.1 [±] 0.42
	3.0 M NaCl	15.88±0.I	74. ±0.47	22.7 [±] 0.I4	177.8 [±] 3.6
	2 % Amyl ac				
Increase %		0.82	0.54	54.4	150
F e m a l e	3.0 M NaCl	15.2 [±] 0.I	85.8 [±] 0.06	I4.8±0.07	90 ±0.I
	3.0 M NaCl	22 ±0.16	188 ±0.13	2I ±0.I8	150 ±0.3
	2 / amyl acetate				
Increase %		44.7	119.1	41.8	66.6

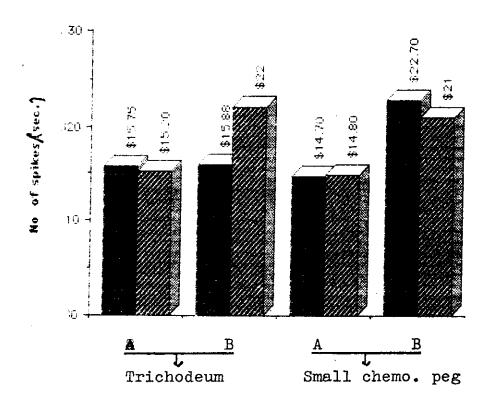


Fig.III.2.17A. Responses of the olfactory cells in antennal sensilla trichodea and small chemoreceptor pegs in both sexes of <u>S.littoralis</u>.

Male Female

A= 3.0 M NaCl

B= 3.0 M NaCl + amyl acetate.

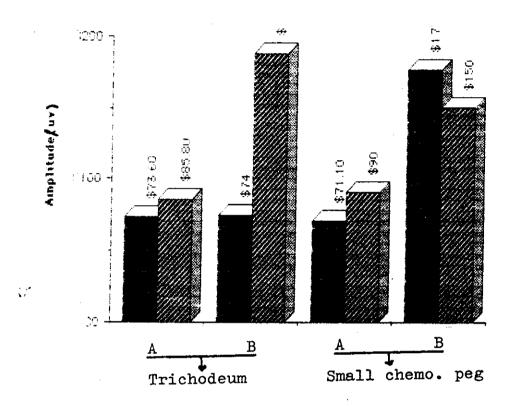


Fig.III.2.17B. Mean spike amplitudes in antennal sensillum trichodeum and small chemoreceptor peg in both sexes of <u>S.littoralis</u>.

Male Female

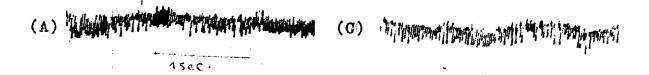
A= 3.0 M NaCl

B= 3.0 M NaCl + amyl acetate.

III.2.6.2. Effect of ethyl butyrate as odor stimulus on both sensilla (trichodea and small chemoreceptor pegs) of female moth S.littoralis.

To investigate were females of S. littoralis able to perceive ethyl butyrate, the EAG records Fig.(III.2.18) were made from two sensilla (trichodea and small chemoreceptor pegs) on antennae of females before and after the ethyl butyrate exposure. 3.0 M NaCl glass microelectrodes were used for these records. The obtained data were tabulated in Table (III.2.9) and represented in Fig.(III.2.19). The results indicated that the frequency of the EAG of sensillum trichodeum increased from I5.I $\stackrel{+}{=}$ 0.I4 to I9.6 $\stackrel{+}{=}$ 0.44 before and after ethyl butyrate exposure respectively indicating a percentage of increase of about 29.8 %. Other while when small chemoreceptor pegs were stimulated the spike frequencies showed a change from I4.05 - 0.26 cycle/sec. to I8.06 $\stackrel{+}{-}$ 0.19 C/s before and after exposure respectively. This represented a percentage of increase in response of 28.5 % (Table III.2.9) .

Stimulation of sensilla trichodea with 2 % ethyl butyrate resulted in an increase in the spike amplitude from 82.4 $^+$ 4.2 uV to I20.2 $^+$ 2.4 uV indicating



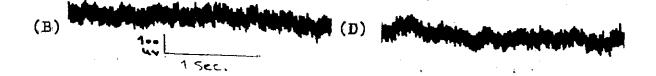


Fig. III.2.18. EAG records from olfactory receptor cells (sensilla trichodea & small chemoreceptor pegs) of the cotton leafworm female moth stimulated with. (A) 3.0 M NaCl

- (trichodeum)
- (B) 3.0 M NaCl + ethyl butyrate
- (C) 3.0 M NaCl (small chemoreceptor peg)
- (D) 3.0 M NaCl + ethyl butyrate (S.chemo. peg)

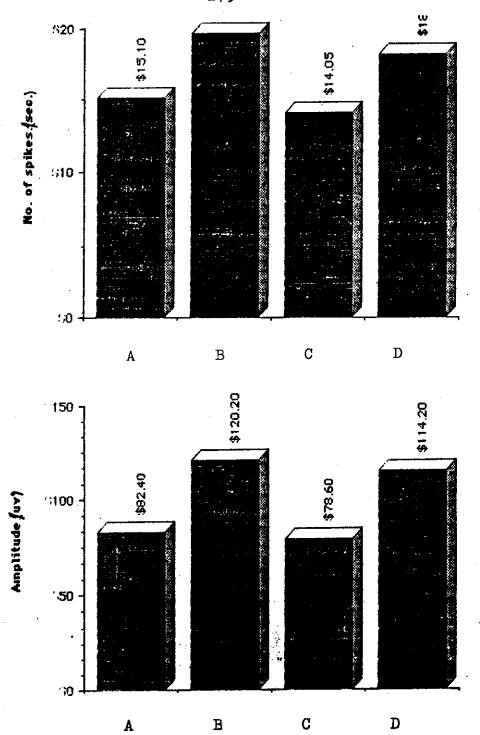


Fig.III.2.19. Responses of sensilla trichodea and small chemoreceptor pegs of <u>S.littoralis</u> female moths to ethyl butyrate .

(A), (B) = Sensilla trichodea

(C), (D) = Small chemoreceptor peg

(A), (C) = 3.0 M NaCl (B), (D) = 3.0 M NaCl + 2 % ethyl butyrate a percentage of increase of 45.8 % in response. Moreover, the small chemoreceptor pegs sensilla showed nearly the same response to odor emaneted from 2 % ethyl butyrate as those in case of sensilla trichodea, where the percentage of increase of amplitude was 45.2 %. This represented a change of amplitude from 78.6 ± 5.2 uV to II4.2 - 3.2 uV before and after treatment with ethyl butyrate respectively.

From the last results and discussion about the function of the sensillum trichodeum and small chemoreceptor peg as sensitive chemoreceptor. It is clear that the EAG recording is the most accurate method by which we can detect any change, even if it is very tinny in the sensilla's response towards any chemical stimuli.

Preliminary study on the role of sensilla chaetica as a mechanoreceptor:

Mechanoreception includes the perception of any mechanical distortion of the body. This may result from touching an object or from the impact of vibrations borne through the air, water or the substratum.

Mechanoreception includes the sense of haering.

In insects, the sensillum chaeticum on moth's antennae was thought to be a mechanoreceptor (Van der Pers and Den Otter, 1978),

The present study is a trial to detect this function of sensillum chaeticum by EAG recording .

Two types of sensilla chaetica are present on the antennae of <u>S.littoralis</u>. This sensillum has a blunt end, where the tip diameter measures I.5 um and contains a pore of I um in diameter (Fig.III.I.16). The mean length of type I is $8I.I \stackrel{+}{=} I.5$, $68.8 \stackrel{+}{=} 0.9$ in males and females respectively. The typical sensillum chaeticum is straight with a base diameter of 3.5 um.

For EAG recording, the sensillum chaeticum was fixed as previously mentioned in part 2.I chapter III. The recording glass microelectrode is filled with 0.I M NaCl as a conductor solution. This electrode was

placed on the tip of the sensillum chaeticum, and concected to the recording system. The sensillum was, then stimulated by different mechanical sounds from different sources. The EAG responses due to different types, rates and speeds of different sound were recorded.

Figures (III.2.20 & III.2.21) illustrate the sensillum responses of both sexes to mechanical sounds. This clear that, no differences occured between female and male. Also any change in the frequency of different sounds accompanied with the same changes in responses frequency. It could be observed that the number of spikes/second of the EAG responses was equal to the number of mechanical beats/sec.

The obtained results showed complete confirmation for the findings of Albert and Seabrook (1973), who thought that this type of sensillum could be concerned with mechanoreception.

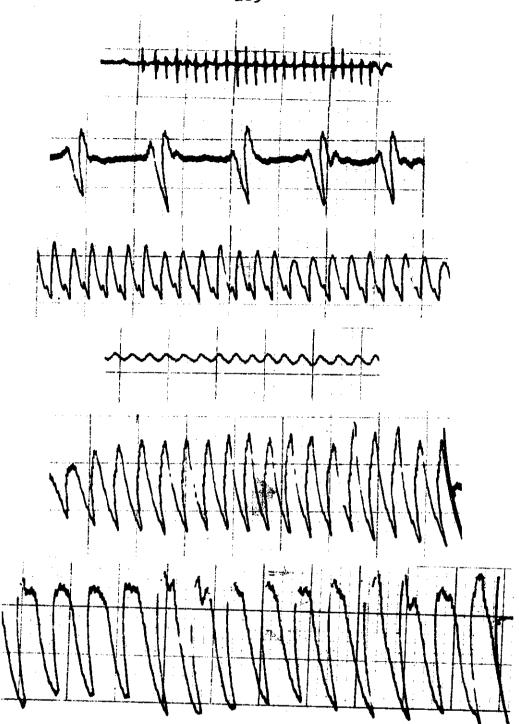


Fig. III.2.20. Responses recorded from the sensillum chaeticum tip on the female antennal flagellum of \underline{S} . littoralis by using different sounds from different sources .

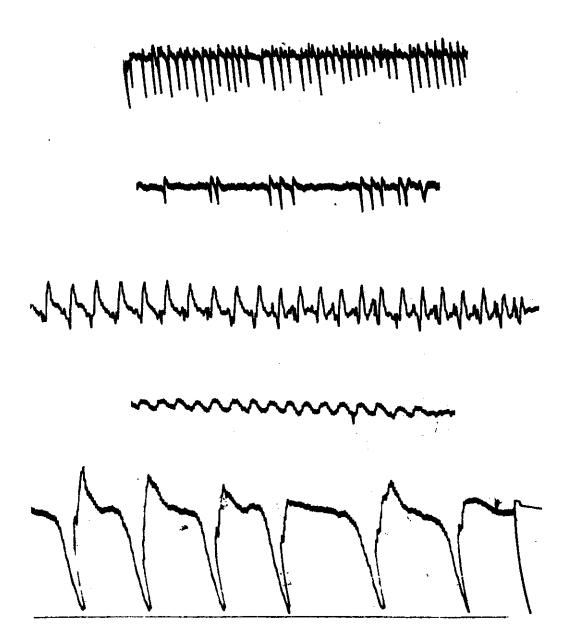


Fig.III.2.21. Responses recorded from the sensillum chaeticum tip on the male antennal flagellum of S.littoralis due to different sounds from different sources.