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NERVOUS AND HORMANAL FACTORS AFFECTING THE REPRODUCTIVE EFFICIENCY OF *Spodoptera littoralis* FEMALE MOTHS

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ABSTRACT

Mating process in *Spodoptera littoralis* female moths started 2 – 3 hrs after light off and the peak of mating was noticed 6.30 – 7.30 hrs after starting the scotophase period. Most of allatectomized females were observed to mate successfully with normal males. In virgin moths, 3 day old normal female laid an average of 371.84 eggs and found a mean of 11.76 eggs in its ovarioles, with a total number of 383.60 eggs/ female as fecundity. The relatively same numbers were found in case of sham operated females. . The reduction in total number of eggs in sham operated virgin females was only 3.97%. The opposite was, however, true in case of allatectomized females. Few number of eggs was produced by allatectomized females as compared with those of non-operated females. Mean of 12.26 eggs were laid and 15.56 eggs were found in the ovarioles with a total number of 27.82 eggs per allatectomized female, representing a reduction rate of 92.75% as compared with the fecundity of non-operated females. Transection of the ventral nerve cord (VNC) of females did not affect mating success. Transected VNC of females at emergence and mated 24 hrs later exhibited mating-induced stimulation of egg production. The mating signal, in this case, may not be transmitted through the VNC, but it may be due to the release of chemical factor into the haemolymph that activates the brain to release an allatotrophic hormone which affects corpora allata to secrete the juvenile hormone. Such hormone. Such hormone causes the physiological changes of mated females.

Keywords: Neural, Hormonal Physiology, Reproduction, Cotton leafworm, *Spodoptera littoralis,* Allatectomy, Sham operation.

INTRODUCTION

Lepidopterous insects release sex pheromones to attract the other sex of the same species for copulation. Pheromone production, emission and response to the other pheromones are controlled by an endogenous circadian rhythm. The species specific pheromone for mating in moths is released during calling, which is controlled by the brain or other nerve centers (Itagaki and Conner,1987 and Tang *et al*.,1987).

In earlier studies provided for both neural and hormonal influences on calling and oviposition behaviour of various insect species (Hollander and Yin, 1982, 1985; Sasaki and Riddiford, 1984; Webester and Cardé, 1984). However, Sasaki *et al.* (1983) reported that corpora cardiaca-corpora allata complex had no effect on calling and oviposition behaviour of *Hyalophora cecropia (L.)* moths. According to Webester and Cardé, (1984), juvenile hormone releases from corpora allata in adult females may be involved in the switch from virgin to mated behaviour in *Platynota stultana* (Walker).

The aim of the present study is to determine the neural and hormonal regulators of mating process, egg development and oviposition in the *Spodoptera littoralis* female moths.

MATERIALS AND METHODS

Experimental insect and rearing technique:

Egg masses of the cotton leafworm*, Spodoptera_littoralis* (Boisd.), collected from fields located in Kaluobia Governorate were transferred to the laboratory and placed in small glass jars covered with clean muslin, held with a rubber band. The rearing technique adopted by Gomaa (2001) was followed. Newly hatched larvae were transferred to rearing jars (3 liters in capacity) and supplied with fresh clean leaves of castor oil bean, *Ricinus communis* L. Saw dust was placed at the base of each jar to absorb any excess moisture. Daily, larvae were provided with sufficient amounts of fresh and clean castor bean leaves, feces and dried leaves were removed and replaced by fresh leaves. Towards the end of the last (sixth) instar larvae, moist saw dust was placed at the base of the rearing jars to provide a pupation site. Newly formed pupae were carefully collected daily, sexed and placed in clean jars until adult emergence to be used in the following experiments.

Dissection technique of ventral nerve cord of female moths:

To study the role of ventral nerve cord of female moths in mating and oviposition capacity, the females were carefully collected soon after emergence and left about 3 hrs for wing expansion and divided into two main groups. In the first group, females were divided into two subgroups. The first was operated and introduced to normal males for mating, and the second subgroup was left without operation and mated with normal males. The second main group of females was reared without mating (virgin) and divided to two subgroups. The first was operated and the second was left without operation as control. Observation was made every 30 minutes for mating occurrence. The following operation was made to females using the technique adopted by Park and Ramaswamy (1998) as follows: Females were anesthetized with ether for 30 seconds and immobilized using a piece of a modeling clay strips, which were flattened on their thorax and the last abdominal segments to immobilize the abdomen. The body was situated ventral side up and the ventral nerve cord (VNC) was transected anterior to the terminal abdominal ganglion under a dissecting microscope. The wounds were closed by nail polish. Females of each subgroup were placed separately in one liter glass containers and observed for daily egg deposition. The daily number of eggs laid either by mated or virgin female moths was recorded every day till the end of the oviposition period. Each subgroup was replicated 8 times.

Allatectomy and sham operation in female moths:

Another surgical treatments were made to the newly emerged females by dividing them to three main groups. In the first, sham operation was made and in the second , corpora allata was removed to obtain allatectomized

females, while the third group was left without operations as control. Each main group was divided to two subgroups; the first was mated to normal males and the second was left without mating as virgin females. Allatectomy and sham operation were made according to the method adopted by Gaddene (1993) and modified by Park and Ramaswamy (1998) as follows. Newly emerged adult females were anesthetized with ether for 30 seconds. Anesthetized females were placed on a modeling clay bed and clay strips were flattened on their thorax and head to immobilize the head. The neck membrane in the posterior region of the head capsule was cut open to expose the corpora cardiaca-corpora allata complex. Because the corpus cardiacum and corpus allatum are closely appressed, the complex was excised in its entirety and the integument cover pushed back in place. When corpora allata of the female moth were removed at the require age, care was taken to ensure the presence of corpora cardiaca. Sham operated males were treated similarly by cutting the nerves between each corpus cardiacum and corpus allatum, but not removing the later.

For mating females, couples of one day old moths, either operated or not, were transferred to mating cages measuring about 30 cm in length, 30 cm in width and 40 cm in height. A piece of cotton wool soaked in a 20% sugar solution was immersed as a wick in a small glass tube to provide a source of nutrients for emerged moths. The sugar soaked cotton wool was renewed daily to avoid the growth of microorganisms and fermentation. Furthermore, a small fresh branch of Tefla. *Nerium oleander* (L.) was introduced into each breeding cage to provide an oviposition site. Any deposited egg masses were collected daily, counted and transferred to clean rearing jars as previously described. The laboratory conditions during the experimental work were 24± 3 ^{o}C, 65 ± 5% R.H. and 16: 8, L: D photoperiod.

RESULTS AND DISCUSSION

In the available literature, mating in insects appears to provide a chemical or/and neural stimulus that promotes egg maturation in females and triggers oviposition. Substances stimulating oviposition are produced in accessory glands of the male reproductive system and are transferred to females during copulation (Friedel and Gillott, 1977; Gomaa, 2001). These male secretions are called male factor or sex peptides (Gomaa, 2001).

Several mechanisms may regulate egg maturation in mated female insects. Mating signal may act on the brain via the ventral nerve cord or the haemolymph. The brain in turn may affect the female reproductive system through the ventral nerve cord triggering enhancement egg production and deposition. Therefore, the role of neural and hormonal factors in mating, egg maturation and oviposition are considered.

The role of ventral nerve cord in mating process and oviposition behaviour of *S. littoralis* female moths:

In the present work, mating process started 2 – 3 hrs after light off and the peak of mating was noticed 6.30 – 7.30 hrs after starting the scotophase period.

Virgin and mated females laid a total of 349.23 and 1778.48 eggs/ female during the oviposition period of 8 and 10 days, respectively (Table 1).

Transection of the ventral nerve cord (VNC) of *S. littoralis* female moth did not affect mating success adversely, as the rate of successful mating of transected female (98.25%) was relatively similar to that of the normal females mated to normal males (97.50%).

Table (1): Number og eggs laid during the oviposition period by virgin and mated normal (N) and transected ventral nerve cord (TVNC) *S. littoralis* females. (Means of 8 individuals ± S.E.).

Age of female moth (days)	Number of eggs laid by virgin female			Number of eggs laid by mated female		
	N	TVNC	Rate of decrement to normal (%)	N	TVNC	Rate of decrement to normal (%)
3	24.12± 1.98 d	20.43± 1.36 d	15.30	159.75 ± 5.44 d	145.41 ± 4.78 c	8.98
4	38.87± 1.84 c	30.36± 1.48 c	21.89	287.42± 11.16 b	270.92± 10.96 a	5.74
5	48.42± 2.15 c	36.92± 2.02 c	23.75	308.26± 16.23 a	239.45± 9.18 b	22.32
6	62.63± 3.82 b	46.41± 2.36 b	25.90	279.73± 11.85 b	223.32± 8.91 b	20.17
7	68.85± 4.01 b	49.22± 2.44 b	28.51	285.56± 10.94 b	210.73± 8.84 b	26.20
8	81.16± 4.66 a	60.66± 3.95 a	25.26	191.84± 8.18 c	156.55± 6.62 c	18.40
9	23.73± 1.52 d	18.36± 1.20 d	22.63	149.61± 6.02 d	129.69± 4.16 c	13.31
10	8.45± 0.75 e	7.08± 0.81 e	16.21	87.87± 4.76 e	78.41± 3.22 d	10.77
11	0.00	0.00	0.00	23.36± 1.54 f	21.18± 1.96 e	9.33
12				5.08± 0.42 f	4.54± 0.36 e	10.63
13				0.00	0.00	0.00
Total	349.23± 15.96	263.04± 11.72		1778.48± 22.19	1480.20± 20.25	
Mean			22.43			14.59
F between ages	29.44**	32.25**		28.92**	19.48**	
L.S.D. at 0.05	10.01	9.11		22.78	29.04	

Similar results were given by Park and Ramaswamy (1998), who reported that tobacco budworm females, *Heliothis virescens* (F.) with or without an intact VNC is not necessary for pheromone emission or mating in *H. virescens*. The same findings are also given by Rafaeli *et al*. (1990), Jurenka *et al*. (1991), Rafaeli (1994) and Ramaswamy *et al.* (1995 and 1996) for pheromone production in moths belonging to genus *Heliothis*.

The data given in Table (1) clearly show that the transected VNC of virgin and normal *S. littoralis* female moths produced eggs, whose numbers increased gradually until the 8th day after emergence, and decreased thereafter. This was common for normal and transected females.

Normal *S. littoralis* females mated with normal males produced significantly more mature eggs until the 9th day, especially peaking at the 4th or 5th day, regardless of treatment. After the 9th day, the mated females laid markedly fewer eggs till the end of the oviposition period. These data suggest that mating can prolong activation of reproductive system in females for 2 days.

Females that underwent VNC transection at emergence and mated 24 hrs later exhibited significant mating-induced stimulation of egg production. This suggests that the mating signal might not be transmitted through the VNC in the experimental insect, but it may be due to the release of a chemical factor into the haemolymph that activates the brain to release an allatotrophic hormone which affects corpora allata to secrete the juvenile hormone. Such hormone causes the subsequent physiological changes of mated females.

Effect of *S. littoralis* female neck legation on number of egg deposition:

It is well known that in most insects, cephalic structures (the brain, suboesophageal ganglion, corpora cardiaca, corpora allata complex) are the major regulators of endogenous activities.

As shown in Table 2, normal virgin female moth laid an average of 56.14, 101.62 and 153.37 eggs/ female 3,4 and 5 days after emergence, respectively. When neck legation was made 24 hrs after eclusion, the mean number of egg deposition significantly decreased, being 16.38, 27. 65 and 38.92 eggs/ female 3, 4, and 5 days after emergence. The rate of decrement in the number of egg deposited by neck legated females compared to those of normal females ranged between 70.82 and 74.61% with no significant difference between both (Table 2).

Normal mated females laid a mean of 423.54, 821.06 and 1015.84 eggs/ female 3, 4 and 5 days after emergence when paired with normal males. When legation occurred 24 hrs after emergence and before mating, the mean number of deposited eggs highly significantly decreased as 87.73, 152.24 and 215.18 eggs/ female were laid 3, 4 and 5 days after eclusion. The rate of decrement ranged between 78.82 and 81.46%, with no significant difference between both.

The fore-mentioned results revealed that at any age, mated female neck legated 6 hrs after emergence produced about 5.5 times more eggs than neck legated virgin female. These results suggest that a factor transferred by *S. littoralis* male moth during copulation evokes stimulation of egg production and/or acts upon the female cephalic system to activate a release of endogenous juvenile hormone during mating. This was common in normal (non-legated) mated females. The same findings are recorded by Park and Ramaswamy (1998) on *H. virescens*. The accessory sex glands in *H. virescens* contain large amounts of juvenile hormones, the titers of which are reduced significantly during mating, concomitantly, the bursa copulatrices

of virgin females do not have any juvenile hormone, but those of mated females have juvenile hormone titers similar to those of the virgin male accessory sex glands (Park *et. al*., 1998a).

Table (2): Number of eggs laid by virgin and mated *S. littoralis* female moths after different days from neck legation* (Means of 8 individuals ± S.E.).

Female status	Age of legated female moth (days)	Number of deposited eggs/ single female		
		Normal female	Neck legated female	Rate of decrement to normal female(%)
Virgin	3	56.14±3.84 c	16.38±2.09 c	70.82
	4	101.62±4.11 b	27.65±2.86 b	72.79
	5	153.27±5.68 a	38.92±4.21 a	74,61
	F between ages	22.02**	18.55**	1.83 (Insig.)
	L.S.D. at 0.05	28.59	10.44	-
Mated	3	423.54±18.62	87.73±10.02	79.29
	4	821.06±30.06	152.24±17.14	81.46
	5	1015.84±38.15	215.18±28.30	78.82
	F between ages	47.52**	34.08**	4.26 (Insig.)
	L.S.D. at 0.05	172.86	53.15	-

*** Legation occurred 24 hrs after emergence**

Effect of female allatectomy and sham operation on mating and egg production:

In the present work, it is noticed that the allatectomized, sham operated and normal *S. littoralis* female moths always mated during the scotophase period. This means that males responded and mated with females 24 hrs after emergence whether or not females were allatectomized. Therefore, corpora allata do not regulate sexual behaviour such as sex pheromone production as occurs in other noctuid moths such as *Pseudaletia unipuncta, Agrotis ipsilon (Hufn.) and H. veriscens* (Cusson and McNeil, 1989; Duportets *et al*., 1996: Park and Ramaswamy, 1998).

From the observation of the present work, although the rate of mating was found to be less in allatectomized females (90.55%), most of females were observed to mate successfully with normal males.

In virgin moths, females 3 day old normal female laid an average of 371.84 eggs and found a mean of 11.76 eggs in its ovarioles, with a total number of 383.60 eggs/ female as fecundity. The relatively same numbers were found in case of sham operated females; means of 63.14 and 305.22 eggs/ female were laid and found in the ovarioles, respectively, with a total number of 368.36 eggs/ female. The reduction in total number of eggs in sham operated virgin females was only 3.97%, represented insignificant difference between fecundity of normal and sham operated females. The opposite was, however, true in case of allatectomized females. Few number of eggs was produced by allatectomized females as compared with those of non-operated females. Mean of 12.26 eggs were laid and 15.56 eggs were found in the ovarioles with a total number of 27.82 eggs per allatectomized

female, representing a reduction rate of 92.75% as compared with the fecundity of non-operated females. The difference between the fecundity of normal and allatectomized female moths proved to be statistically highly significant. According to Nijhout and Riddiford (1974), corpora allata were required for normal reproduction in *Manduca sexta (L.)*; however, egg production and oviposition decreased if females remained virgin. The authors speculated that this was because corpora allata stopped release juvenile hormones. Similar suggestions were made by Herman and Barker (1977) and Ramaswamy *et al.* (1997), who reported that the rate of egg maturation in virgin females decreased because of decreasing juvenile hormone titers.

Table (3): Fecundity of 3 day old virgin and mated *S. littoralis* female moths after two types of surgical operations* (Means of 8 individuals ± S.E.).

Female status	Type of female operation	No. of laid eggs/ female	No. of eggs in ovarioles/ female	Total No. of produced eggs**
Virgin	Allatectomy	12.26±1.02 c	15.56±1.66 b	27.82±1.98 b (92.75)
	CA sham operation	63.14±0.96b	305.22±15.98a	368.36±16.06 a (3.97)
	Normal (control)	371.84±14.48 a	11.76±0.46 a	383.60±17.14 a
	F between type of operation	5.56*	4.82*	5.79*
	L.S.D. at 0.05	9.01	21.15	70.46
Mated	Allatectomy	5.18±0.24 b	56.65±3.36a	61.83±3.90b (92.58)
	CA sham operation	763.74±10.18a	20.16±9.92b	783.90±19.28a (5.89)
	Normal (control)	791.42±21.30a	41.53±2.26a	832.95±23.12a
	F between type of operation	4.90*	5.15*	7.07*
	L.S.D. at 0.05	61.66	18.81	53.79

CA, Corpora allata

*** Operations were made 6 hrs. after emergence of female moths, then introduced to males 3 day old for mating.**

****Values between brackets represent the rate of decrement of produced eggs by operated females to to normal (control) female.**

Three day old normal mated female, previously introduced to normal male laid after separation an average of 791.42 and had a mean of 41.53 eggs in its ovarioles, with a total number of 832.95 eggs/ female as fecundity. Relatively similar numbers of eggs (763.74 eggs/ female) were laid by sham operated female and a mean number of 20.16 eggs/ female was calculated in the ovarioles, with a total number of 783.90 eggs/ female as fecundity. The reduction in total number of eggs produced by sham operated 3 day old mated female as compared with the normal mated female was only 5.89%.

The obtained data revealed that highly significant difference in egg production and deposition between normal females mated with normal males and allatectomized females mated to normal males. Contradicting results

were given by Park and Ramaswamy (1998), who stated that, although the allatectomized mated *H. veriscens* females produced numerically more eggs than did allatectomized virgin ones, there was no significaqnt difference between the two groups. Even though, Park *et al*. (1998 b) found no significant difference in egg deposition between normal *H. veriscens* females mated to normal males and normal females mated to allatectomized males.

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العوامل العصبية والهرمونية المؤثرة على الكفاءة التناسلية لإناث فراشات دودة ورق القطن

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تبدأ عملية تلقيح إناث فراشات دودة ورق القطن بعد ٢-٣ ساعة من حدوث الإظلام ، وقد لوحظ أن أقصى معدل لعملية التلقيح تحدث بعد ٦.٣٠-٧.٣٠ ساعة من بدء عملية الإظلام . وقد وجد أن قطع الحبل العصبى البطنى للإناث لا يؤثر على نجاح عملية التلقيح ، كما أن معظم الإناث التي اذيلت غدتيها الكرويتين بعد الخروج مباشرة قد لقحت بنجاح أيضا اذا ما ادخلت على ذكور عادية (بها الغدتين الكرويتين) .

في الفراشات غير الملقحة ، تضع الأنثى العادية التي عمرها ثلاثة أيام ٧١.٨٤ بيضة في المتوسط ، كما وجد في أنابيبها المبيضية ٣١١.٧٦ بيضه في المتوسط بمجموع قدره ٣٨٣.١٧ بيضة . وقد وجد نفس العدد الإجمالي تقريبا في حالة الإناث التي قطع الموصل بين الجسمين الكروى والقلبي ، حيث وصل الخفض في عدد البيض الناتج بواسطة الإناث الأخيرة إلى ٣.٩٧ % فقط . وقد لوحظ حدوث انخفاض حاد في عدد البيض الناتج من إناث اذيل غدتيها الكرويتين إذا ما قورن بعدد البيض الذي تضعه الأنثى العادية .

تنتج الفراشات العادية الملقحة ذات عمر ثلاثة أيام والتي سبق ادخالها على ذكور عادية عددا من البيض يساوى تقريبا ما تنتجه الأنثى التي قطع الموصل بين الجسمين الكروى والقلبي لها . وتوضح النتائج أن تحفيز عملية التلقيح لاينتقل من خلال الحبل العصبي البطنى ولكن من خلال افراز عامل كيميائي في الهيموليمف يقوم بتنشيط مخ الأنثى لافراز هرمون منبه للغدة الكروية ، وبالتالي يسبب تغيرات فسيولوجية في الإناث الملقحة . في أي عمر للفراشة ، تنتج الأنثى الملقحة والتي ربطت من رقبتها بعد ٦ ساعات من الخروج حوالي ٥.٥ مرة أكثر في عدد البيض من مثيلتها غير الملقحة . وقد يفسر ذلك بانتقال عامل من الذكر للأنثى أثناء التلقيح ينبه إنتاج البيض في الأخيرة و أو يعمل على النظام الدماغي للأنثى لينشيط افراز هرمون الشباب أثناء التلقيح

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