MALE ACCESSORY GLAND SUBSTANCE OF *SPODOPTERA LITTORALIS* (BOISD): A STIMULANT OF EGG-LAYING IN VIRGIN FEMALES IN RELATION TO THE MALE AGE

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ABSTRACT

Male substances were extracted from the whole male reproductive system (MRS), male accessory gland (MAG) and male reproductive systems free from accessory gland (AG free-MRS) of different aged *S. littoralis* adults. The amount of each male substance extract was increased with the increase in the age of the male. AG extracts were the most stimulatory ones in egg-laying induction since the ovipositing newly emerged (NE) virgin moths were responded 100 % to the injection of different concentrations. The higher the amount of injected MRS and AG free-MRS extracts the higher the percentage of the ovipositing virgin females. On the other hand, results showed that the younger the male, from which the extract was obtained, the higher the percentage of ovipositing females. The majority of male stimulatory factor(s) seems to be concentrated into the MAG. The stimulating activity was found to be concentration-dependent. All male substance extracts of NE males stimulated egg-laying, in NE virgin females, more than those of older males. The extracts obtained from younger males were more potent.

INTRODUCTION

Mating is known to increase fecundity in a number of insect species.The mechanism by which copulation enhances the rate of oviposition remain obscure. However, it is well known fact that in a number of Dipteran insects mating stimulates oviposition due to a male paragonial substance ejaculated into the female (Engelman, 1970; Leopold, 1976). As far as Lepidopteran insects are concerned, there is little information on the role of mating in ovipositional behaviour. Yamaoka and Hirao (1977) presented evidence that in Bombyx mori the injection of an extract of whole male reproductive system (MRS) in water or Ringer's solution into virgin females (VFs) resulted in stimulation of egg laying. Recently, Hashem and Megahed Metwalli (1985) revealed the occurrence of a similar male factor in the Egyptian cotton leafworm Spodoptera littoralis MRS extract which stimulates the oviposition when injected into the haemocoel of the virgin moth. Moreover, Hashem (1986) demonstrated that the mutual injection of the lipid-free extracts of the whole MRSs between B. mori and S. littoralis significantly stimulated the ovipositional activity (OA) in the VFs. He postulated that the male factors are not specific for either insect species.

No additional informations on such oviposition stimulatory factor in S. littoralis MRS have so far been available. However, such data might be important in understanding the mechanism of oviposition of such important insect pest and the similar ones. The present study was concerned to investigate the site of the male substance into the MRS and the role of the accessory gland (AG) of S. littoralis males in the oviposition induction.

MATERIALS AND METHODS

A stock culture of S. littoralis was kept in the laboratory at room temperature (25 ± 2°C) and 65 ± 5 % relative humidity. When needed, pupae were sexed, males and females were kept separately in isolated cages. Emerged adults of either sex were collected and used in the experiments at the desired ages.

Various numbers of male moths were carefully dissected at the desired age to obtain either intact MRSs, male accessory glands (MAGs) or accessory gland free-male reproductive systems (AG free-MRSs). In the experiments reported herein newly emerged(NE) males, males aged 48 and 72 hr were dissected. The tissues were carefully cleaned from fat body during dissection. Tissues were collected and preserved in deep freez (-25°C). The extraction of male substances from the abovementioned tissues and the removal of their lipid contents were carried out by homogenization, centrifugation and lyophilization essentially according to the method of Yamaoka and Hirao (1977) slightly modified by Hashem and Megahed Metwalli (1985). The powder extracts were obtained and the amounts in mgs of the MRS , MAG or AG free-MRS equivalent to one male (S mg) were calculated.

The injections of MRSs extracrs were carried out intraabdominally using 1 ml syringe and a hypodermic needle by means of a microapplicator. Dry powder extract suspended in the desired volume of glass distilled water (DW) and injected into the dorsal region of the sixth abdominal segment near the dorsal vessel. Females showed bleeding following injection were discarded. Each injected female received the desired amount of the powder extract suspended in 16.5 ul of DW.

Statistical analysis adopted in all experiments were 'F' test, and all probable comparison combinations were achieved using L. S. D. test at probability levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

Male substance extracts:

Fig. 1 clearly demonstrates that the "S" values, the amounts in mgs of the extracts equvalent to one male, are affected by the male age. The older the male, from which the tissue is obtained, the larger the amount of the resulted extract. The increase in "S" value of AG free-MRS is more pronounced. Haines (1981) demonstrated that the ductus ejaculterius simplex of NE males of S. littoralis was colourless or pale yellow, but after 2 days an orange pigmented secretion began to accumulate in 61.3 % of the examined males. By the day 4 this pigment had darkened to deep red in virgin males (VMs). However, present results, whi indicate the increase in the dry powder extract of the reproductive system of the VMs after emergence by time, suggest the accumulation of secretory material(s) from the AGs. The pronounced increase of S mg values of AG free- MRSs and the slight increase of the same value of AGs may indicate a transfer of this secretion(s) from AG into the ductus ejaculatorius simplex.

Percentage of ovipositing VFs:

Three main experimental groups of NE VF moths of the subject insect were established and used for the injection of male substances. VFs in each group were injected with 16.5 µl of DW containing 0.1 ,0.2 , 0.5 and 1.0 S mg of male substance extract. First group was used for the injection with extracts of whole MRSs obtained from NE, 48 hr old and 72 hr old males. The second group was used for the injection with AGs extracts obtained from males of the same ages. The third group was used for the injection with AG free-MRSs extracts obtained also from the same ages. Three types of control groups were also established: group M which contained females mated naturally with NE VMs; group V which contained untreated VFs; and group DW which contained VFs injected with 16.5 µl DW.

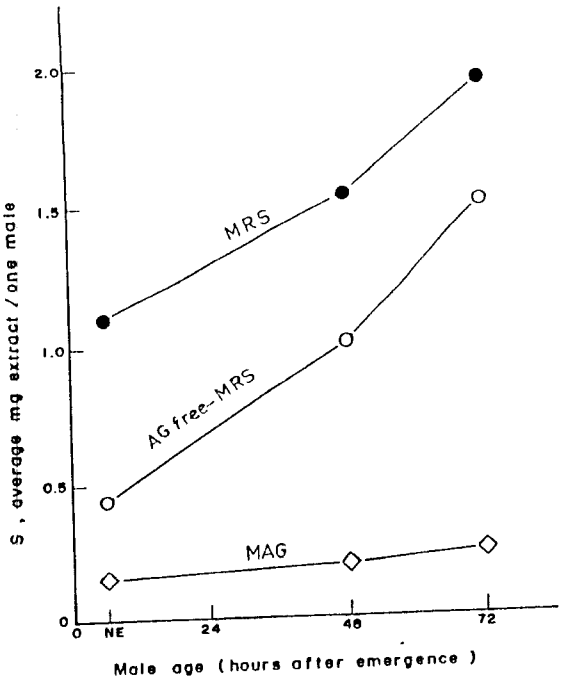

Fig. 1.: Average amounts, in mg, of male reproductive system (MRS), male accessory gland (MAG), and accessory gland free-MRS (AG free-MRS) extracts in relation to ages of S. littoralis males from which extracts were obtained. NE, newly emerged .

values of NE males represent averages of 250 individuals and other values represent averages of 100 individuals.

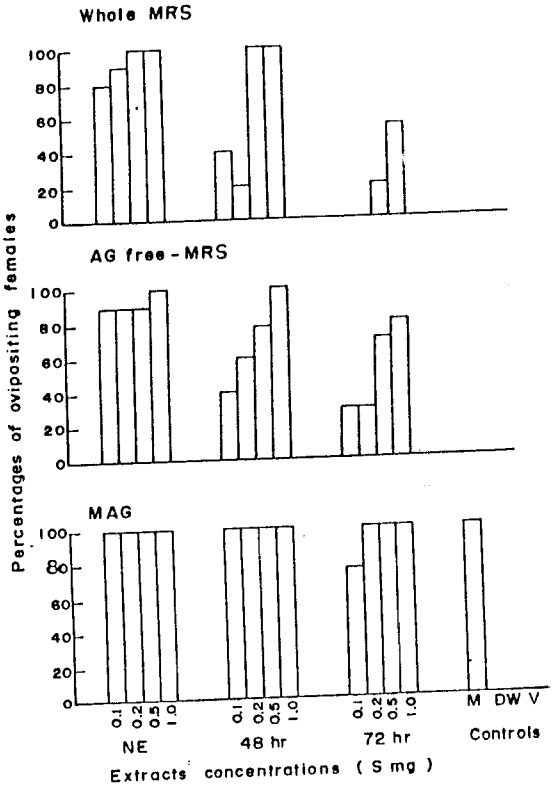

Fig. 2.: Percentage of ovipositing S. littoralis virgin females as response to the injection of different concentrations of male substance extracts obtained from different aged males compared to control females (M, mated; DW, injected with distilled water; V, virgin).

The percentages of ovipositing VFs in the 24 hr following the injection with male substance extracts were determined and data are presented in Fig. 2. MAG extracts proved, when injected, to be the most stimulatory ones since the percentages of ovipositing VFs were 100 % in all concentrations of various groups except that of the lowest concentration, 0.1 S mg, of the 72 hr-old males. The percentage of the ovipositng females was found to increase as the amount of the injected MRS and AG free-MRS extracts increased.

On the other hand, data clearly demonstrate that the younger the male, from which the extracts were obtained, the higher the percentage of the ovipositing VFs injected with these extracts. In this concern, the effect of the whole MRS-72 hr-old extract was found to be weaker than that of the AG free-MRS-72 hr-old extract. However, 100 % of females of the M group were ovipositing within the 24 hr following mating. Within the same period, all females of V and DW groups were unable to deposit any eggs.

Stimulation of ovipositional activity (OA):

Average OAs were obtained at the end of the first 24 hr following treatments. Relative OAs of injected VFs were determined to express the degree of oviposition. They were calculated by relating , as percentages, the number of eggs laid/female, in 24 hr after injection, in each group to the average number of egg laid/female, in the same period , of the M group. Fig. 4 presents the relative OAs.

Data presented in Fig.3 show that the injection with all male substance extracts has stimulatory effects on the OAs of the NE VFs i.e. most of them oviposited in the first 24 hr following the injection. However, females of V and DW groups deposited no eggs during such period of time, but females of M group laid a large number of eggs (580.6 $\pm$ 78.3). The OAs increased as the extract concentration increased. This can be clearly demonstrated in Fig.4 which shows the relative OAs of the injected VFs of all groups.

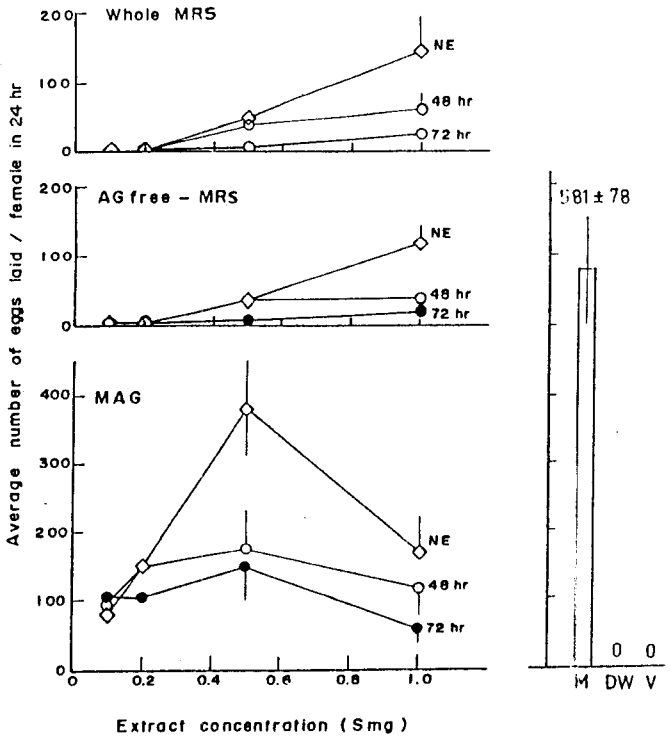

Fig. 3.: Ovipositional activity (OA) of S. Littoralis virgin female injected with different concentrations of aqueous extracts of MRS, AG free-MRS, and MAG obtained from different aged males; OA is expressed as average number of laid eggs/female in 24hr after treatment.

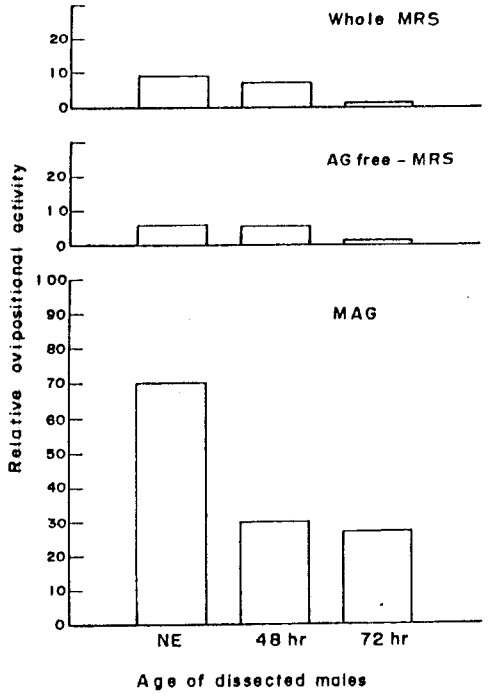

Fig.4 .: Relative OAs of S. littoralis virgin females injected with male substance extracts from different aged males expressed as percentage of OAs of mated females.

(Conc. 0.5 S mg)

Statstical analysis proved that there are highly significant differences in OAs due to the injection of different male substance extracts of the same concentration. Analysis demonstrated that at 0.1 S mg and 0.2 S mg highly significant differences are existed between OAs due to all AG extracts, from different aged males, and those caused by all other extracts. But, the differences between the effects of AG extracts taken from various aged males are found to be insignifncant. The same trend was occured when the concentration 0.5 S mg of various extracts were used, but it should be noticed that OAs caused by the injection of AG extracts from NE males is highly significantly greater than those refered either to AG extracts, from other aged males, or all other male substance extracts. It is noticed also that OAs caused by AG-48 hr and AG- 72 hr extracts are highly significantly or significantly greater than those caused by other male substance extracts.

At the concentration 1 S mg, statistical analysis cleared that OAs caused by AG-NE extract is also the greatest one and it is highly significantly greater than OAs caused by AG free-MRS-48 hr, MRS-72 hr, and AG free-MRS-72 hr extracts; and is significantly greater than AG-72 hr and MRS-48 hr extracts. MRS-NE extract is ~~highly~~ significantly active than both MRS-72hr and AGfree-MRS-72hr extracts and significantly active than AGfree-MRS-48hr. Stimulation of egg-laying resulted from injection of AGfree-MRS-NE and AG-48hr extracts is found to be significantly greater than that resulted from injection of both AGfree-MRS-72hr and MRS-72hr extracts.

It has been demonstrated by several authors in a number of Dipteran, Coleopteran, Lepidopteran, Neuropteran,Hemipteran, and Orthopteran insects that mating results in the stimulation of oviposition. Even the larviparous tsetse Glossina austeni exhibited dependence on mating for ovulation and larviposition stimulation since VF did not ovulate and their mature eggs eventually disintegrated (Ejezie and Davey,1977).

On the other hand, Odhiambo (1968) revealed that there was no influence on egg production or oviposition by mating in the cotton stainer bug Dysdercus fasciatus under laboratory conditions. Contrary, Pathak recently (1982) found, also under laboratory conditions, that the mated females (MFs) of the same insect species lay substantially more eggs than VFs. Similarly, whilst Stockel (1972) reported that VFs of Sitotroga cerealella never lay eggs, Ayertey (1975) revealed that in VFs of the same insect species 98 % laid eggs.

However, Brath and Lester (1973) reported that mating has the effect of accelerating the development of the eggs especially in the insects with a short life-span, preventing resorption of the eggs, and stimulating the major oviposition in insects with a short life-span. OAs of the mated and virgin control females; groups V,DW and M; in the present study show that mating has the same influence on the release of egg-laying in S. littorals , the fact which confirms the previous findings on other insect species.

The active factor(s) which increases egg release is produced in the male paragonia or MAGs. Implantation or injection of MAG material, or its analogue, into VFs will greatly induce oviposition in a number of species belonging to different insect orders. The transplants of the MAG or the injection of its extract stimulated the oviposition in Drosophila melanogaster (Kummer, 1960 c.f. Leopold,1976) , Musca domestica (Rieman and Thorson, 1969) and in mosquitoes (Leahy,1967). Concerning Lepidopterans, Truman and Riddiford(—— 1974); Riddiford and Ashenhurst (1973)(c.f. Yamaoka and Hirao, 1977) presented evidence that in Hyalophora cecropia the reception of sperm by bursa copulatrix triggered the release of an oviposition stimulating hormone from the intrinsic cells of the c.c. Injection of an extract of the whole MRSs, including AGs, into VFs stimulates egg-laying in B. mori (Yamaoka and Hirao, 1977) and S. littoralis (Hashem and Megahed

Metwally,1985; Hashem,1986).

Oviposition in VFs was stimulated, in the present investigation, by the injection, into haemocoel, of the MRS, MAG and AGfree-MRS extracts containing the male substance. We have considered, herein, only the injected MRS materials and have not taken into consideration any secretion by the female. Possibly the ovipositional response could involve an interaction of contributions of both the male and female (Karpenko and North, 1973). However all females used in these experiments were NE and unmated. Even those of DW group showed no oviposition stimulation. Thus, the only variable measured was that of the male substance and the discussion of our results must , therefore, be limited to the function of the male. On the other hand, the availability of a suitable oviposition site, or conditions which favour egg-laying, can stimulate oviposition (Engelmann,1970). The oviposition data presented herein were obtained in the absence of such conditions.

The present results strongly demonstrate that stimulation of oviposition in S. littoralis is caused by secretory material(s) exists in the MAG, other factors play, at most a minor role.

The majority of male stimulatory factor(s) in S. littoralis seems likely to be concentrated into the MAG since the AG extract was about 8 and 11 times more active than the MRS and AGfree-MRS extracts, respectively.

All male substance extracts of NE males stimulated oviposition more than those of older males when injected into the VFs. Effects of either concentrations were also more pronounced as well and caused proportional responses. However, the maximum stimulatory activity on egg-laying was brought about by 0.5 S mg MAG extract. AG extracts of various male ages were distinctly stimulative to OAs in VFs at all concentrations. But effeccts of both MRS and AGfree- MRS extracts of 48hr-old males were obvious only at concentration 0.5 S mg or higher, whereas similar

extracts of 72hr-old males caused only very weak stimulations when administered at 1 S mg.

There was no maximum stimulation in OAs due to MRS or AGfree-MRS-NE extracts even when 1 S mg was injected into VFs. It is highly probable that further increase in oviposition can be achieved when still more concentrated extracts suspensions are used for injection. Relative activities of oviposition as responses to injection of male substances showed the same aforementioned features.

Present results demonstrate that stimulation of OAs are mainly due to AGs and are also function of male age. At lower amounts, 0.1 and 0.2 S mg, insignificant differences were found in the stimulant effects due to the male age. Despite the increase in the S mg values of the male substance extracts in older dissected ones(Fig. 1), results proved that extracts from younger males were more potent in releasing egg-laying. This led us to suggest that in S. littoralis it is probable that the active factor(s) in the MAG accumulated secretion may undergo some changes or resorption by storage, into living males, for prolonged period of time. These changes may result in a reduction in its potency when extracted and injected into VFs. However, the degree of accuracy of such hypothesis, and whether there is a single active component in the fluid of the MAG, will probably be determined only by the use of purified extracts.

Present results suggest that the ovipositional stimulation brought about by such male substance(s) appeared to be at least quantitative rather than all or none for each female. This stimulatory activity is concentration-dependent since injection of more concentrated extracts gave distinctly larger increase in egg-deposition.

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