STUDIES ON THE ROLE AND REGULATION OF THE MALE ACCESSORY

REPRODUCTIVE GLAND SECRETIONS OF THE COTTON STAINER,

Dysdercus fasciatus SIGNORET (HETEROPTERA : PYRRHOCORIDAE)

By

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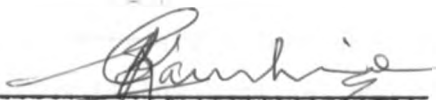
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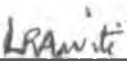
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LIST OF ABBREVIATIONS

AO	-	Aorta
ARG	-	Accessory reproductive gland
B	-	Bucket
C	-	Cotton wool
CA	-	<u>Corpora allata</u>
CC	-	<u>Corpora cardiaca</u>
CG	-	Coalesced parts of the gland
CL	-	Cloth
Cl	-	Clumped cytoplasmic contents
CT	-	Connective tissue
DM	-	Degenerating mesadenes
DVD	-	Duct from the vas deferens
E	-	Epithelium
ED	-	Ejaculatory duct
EL	-	Empty lumen
G	-	Gauze
L	-	Lumen
N	-	Net
n	-	Nucleus
PDM	-	Poorly developed mesadenes
P_i	-	Pars intercerebralis
Pss	-	Poorly staining secretion
rl	-	radial line
S	-	Secretion
SEM	-	Standard Error of the Mean
SG	-	Secretory granules
Sp	-	Sperm
SV	-	Seminal vesicle
T	-	Tray
Tw	-	Twig
VD	-	Vas deferens

ABSTRACT

It has been established that in most insects, reproduction is under the influence of hormones produced by the neuro-endocrine system. The mechanism of such influence, particularly its role in the regulation of the male accessory reproductive glands (ARGs) of the cotton stainers, is not well understood.

In the male cotton stainer Dysdercus fasciatus Signoret, the activities of the corpus allatum (CA) and ARGs were studied morphometrically and histologically to establish their possible functional relationship. Such a relationship would help to explain the unusually long copulatory phenomena characteristic of these bugs.

This study was undertaken to (1) describe the activities of the CA and ARG during sexual maturation in the male D. fasciatus (2) investigate the role of the CA in the functional activities of the ARGs (3) determine the effect of the ARGs secretion on mating behaviour and sexual maturation.

Results from this investigation showed that the CA and ARGs exhibited cyclic secretory activity patterns over a period of 20 days. Two peaks of maximum secretory activity in each gland lasting four days in the first cycle and two to three days in the second cycle were observed. Between these peaks there was another synchronised period of low activity in both glands. Although the peaks of maximum secretory output

were synchronised, they were slightly out of phase with each
other. The activity of the ARGs lagged slightly behind that
of the CA by one day during both the first and second
copulatory cycles. The attainment of maximum secretory
activity by the CA on the third day coincided with the onset of
copulation and release of sperm from the testes. Maximum
secretory activity by the ARGs was attained on the seventh to
8th days when the insects had been in copula for about three
to four days.

Histological observations of the CA showed that, during
the first three days following adult emergence, there was a
remarkable increase in the cytoplasmic content of the CA.The
size of the nuclei remained the same although in the more
sexually mature insects the nuclei were slightly bigger with
more visible intranuclear granules.

The ARGs showed important changes in their histology during
sexual maturation. At adult emergence, the epithelial cuboidal
cells of the mesadenes were thick, forming sacs that contained
no secretion. After the third day, there was a shrinkage
in the size of the epithelia accompanied by an increase in
the size of the glandular lumina, accumulation of granular
secretion and increase in its staining properties. By the
seventh day the glandular secretion stained very intensely and
some of it could be seen in the lower part of the ejaculatory
duct together with a bundle of sperm. On the ninth day, there
was a reduction in size of the mesadenial lumina but the intensely
stained granular secretion within was retained.

Precocene II had potent effects on the activities of CA and ARG. The response to it by the glands varied, with higher doses having greater effects than low doses. Thus applications of 1 µg/1 µl doses had no effect on the activity of the CA or ARG as indicated by the morphometric changes. Doses above 5 µg/1 µl caused a reduction in the volume of the CA which was statistically significant (P ≤ 0.05). It also retarded the development of the ARGs and delayed sperm descent by three days. Concentrations above 10 µg/1 µl led to atrophy of the CA, retarded ARG development and prevented sperm descent. Concentrations of 25 µg/1 µl and above were lethal to the insects after the second day.

Histological observations of the CA and ARG in the precocene treated insects showed that precocene acted on the CA by destroying the nuclear chromatin and synthetic machinery of the cytoplasm. The ARGs were affected by higher doses of precocene as shown by the extremely thin epithelia of the mesadenes. Time of application of the precocene was a critical factor and applications were effective only at adult emergence.

The observed retarded development of the ARGs following precocene treatment indicated that the CA was involved in regulation of the ARGs. The continued secretory capability by the ARGs even at high doses of precocene suggested further that the ARGs were not exclusively under the influence of the CA. The failure of sperm to descend after

precocene treatment indicated that the CA plays role in
male sexual maturation. The observed earlier onset of
copulation and descent of sperm from testes in newly emerged
insects following the introduction of glandular homogenates
of the ARGs from sexually mature male insects further
suggested that the secretions of the ARGs could be playing
a role in sperm maturation and translocation.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1   Occurence and distribution of the genus Dysdercus

Members of the genus Dysdercus, Boisduval, commonly known as the cotton stainers, are heteropteran bugs belonging to the family Pyrrhocoridae and are found distributed in all the cotton growing regions of the tropics. Within these areas, the bugs are known to be closely associated with plants belonging to the order Malvales of which the cotton plant (Gossypium hirsutum) is a member as well as a major host. The presence of many other plant hosts which support the bug population during off-season periods of the cotton crop has aggravated the pest status of Dysdercus species. These host plants are found in various families but the most common ones are Malvaceae, Bombacaceae and Sterculiaceae.

The distribution of various species of cotton stainers in Africa has been documented by several researchers (Le Pelley, 1959; Pearson, 1958). Pearson (1958) reported that there were 10 species of cotton stainers occuring in Africa. Since then only five of them have been recorded in Kenya (Crowe, 1967). These are Dysdercus cardinalis Gerst, Dysdercus fasciatus Signoret, Dysdercus intermedius Dist, Dysdercus nigrofasciatus stall and Dysdercus superstitiosus (F).

Dysdercus species are of considerable economic importance
to the cotton growing areas of Africa since they cause
multiple damages to the cotton plant (Bohlen, 1973). The
main damage is caused by adults which feed on seeds
developing inside the green bolls. On the other hand, the
nymphs suck directly on the ripened seeds. Damage to the
bolls depends on the number of bugs and the time of attack.
Light infestations result in destruction of seed embryos
which cause irregular development of the bolls. Heavy
attacks on young bolls leads to abortion of the bolls.
The bugs also transmit fungal spores particularly those of
Nematospora gossypii which induce internal boll rots (see
Kumar, 1984). This fungus, often in association with other
fungi and bacteria, destroy the inner parts of the young
bolls leading to the development of a slimy memberaneous
mass which gives the lint a brown colour at maturity.
Furthermore, the effects of feeding by the adults as well
as nymphs lowers the germination potential of the seeds and
reduce their quality as a source of oil and seed cake for
livestock.

Many agricultural insect pests including D. fasciatus
are traditionally controlled by use of pesticides. These
methods have become increasingly costly and their use is
being limited by reports on the development of pesticide
resistant insect strains, their toxicity to man and other
animals, and their effects on non-target beneficial
organisms. These and the ecological problems of pesticide

residues in food   chains has led to attempts towards
the development of alternate   measures of insect control
little dependent on the use of pesticides. It is hoped
that studies on the reproductive physiology of the cotton
stainers may contribute towards the discovery of a
possible weakness in the reproductive processes, which
could be exploited in effective design of integrated
control programmes.

1.2 Reproduction in cotton stainers

Some preliminary research has already been carried
out on the reproductive biology of the cotton stainers
as insect pests by Pearson (1958), Odhiambo (1968), Arora
(1971)and Awiti (1976 and 1980). Odhiambo (1968) and
Arora (1971) showed that after a short pre-mating period
of two days after adult emergence, males and females stay
in continuous copula for five to six days before dis-
engaging for the female to oviposit. The female then
lays a batch of 100 or more eggs which are deposited in
a moist spot in debris or soil. They also reported that
each mature female lays an average of about four batches
of eggs with an interoviposition period of four to eight
days. The eggs normally take five to eight days to
hatch into the first instar nymphs. The second to fifth
instar   nymphs feed on ripe seeds in open bolls or
on those that have fallen to the ground. Diapause has
not been reported in pre-adult stages and adults remain
sexually active at all times. However, low temperatures
and prolonged absence of food tend to decrease mating
activities (Awiti, 1976).

According to Loher and Gordon (1968) a period of five
to six days of continuous mating in D. fasciatus is remarkably
longer than that of a related bug Oncopeltus fasciatus which
copulates several times a day. A similar observation in other
insects was reported by Leopold et al., (1971). Furthermore,
investigations by Odhiambo (1968) and Arora (1971) showed that
the interoviposition and pre-oviposition periods in the mated
and virgin females were the same though the mated females
laid more eggs than the virgin ones. They concluded that
the long copulatory period shown by cotton stainers is not
an important factor in egg production. Since then much attention
has been paid to the reproductive biology of the male insects.

In the male D. fasciatus Awiti (1976) reported that
males are usually sexually immature at adult emergence
because the sperm has not descended from the testes into the
seminal vesicles. Sperm descent takes place about three to
four days after adult emergence when females are also sexually
receptive. In addition, the accessory reproductive glands
(ARGs) are not fully developed at this time and do not actively
secrete until the third or fourth day. Awiti also noted that
the final processes of sperm maturation and migration into
seminal vesicles together with the active secretion of the
ARGs were all synchronised suggesting that the male ARGs
could   play   a role in sperm release and transfer.

In another subsequent study, Awiti (1980) demonstrated
that the application of an anti-juvenile hormone Precocene II

to newly emerged male bugs prevented continuous matings up
to eight days following adult-emergence and prevented descent
of sperm from the testes into the seminal vesicles. The
secretory activity of the ARGs was also slowed down and the
the synthetic apparatus appeared degenerated. But the
implantation of sexually mature male ARGs into newly emerged
males brought forward the onset of mating by one to two days.
These studies indicated that in the male D. fasciatus either
the secretory activity of the ARGs or sperm maturation in the
testes or both were under the endocrine control. It is
possible that the final processes of sperm maturation might
have depended on the ARG secretions whose synthesis is controlled
by the corpus allatum (CA).

1.3   The role of male ARG secretions in insect reproduction

Although research on ARG involvement in the male insect
reproduction is still in its infancy, from recent findings,
it is now evident that secretions of such glands play a major
role in insect reproduction. According to Leopold (1976) the
gland secretions transfer and activate sperm, form barriers
to reinsemination and contributes to the survival and
fecundity of the species. He concluded that such functions
appeared unique to the Class Insecta when compared to other higher
animals. In order to understand the part that may be played
by the ARG secretions in the male D. fasciatus, it has been
necessary to examine the role of such secretions in other
insects an account of which is given below.

1.3.1 Involvement with insemination

In insects, sperm transfer during copulation normally takes any one of the following forms: transfer by means of spermatophore; direct transfer in seminal fluids and haemocoelic transfer (Davey, 1960; Hinton, 1964). Thus in Orthopteroid orders the ARGs play a major role in the formation of spermatophore. For instance Loher and Edson (1973) showed that the removal of the ARG Complex in the Cricket Teleogryllus commodus prevented spermatophore formation but did not prevent copulation. Leahy (1973) reported that the removal of ARGs had a similar effect in Schistocerca gregaria. Furthermore, using ultrastructural methods, Kokwaro and Odhiambo (1981) were able to demonstrate that in the dipteran Glossina morsitans morsitans West., spermatophore were partly derived from the male ARG secretions. Similarly in Hemipteran insects Davey (1958) demonstrated that in Rhodnius prolixus, the ARG secretions formed the spermatophores and influenced the migration of sperm.

In insects which do not utilise the spermatophore for sperm transfer, there appears to be a variation in the involvement of ARG secretions in this process. For instance in the dipteran Drosophila melanogaster, males whose ARGs have been depleted due to repeated matings are no longer able to transfer sperm from the seminal vesicles (Lefevre and Johnnson, 1962). In another dipteran Musca domestica, Leopold (1970) was however not

able to observe a similar effect. Another function of the
male ARG secretion which is closely linked with sperm transfer
is sperm activation. This has been reported in Cimex
lectularius (Davis, 1965) Bombyx mori (Omura, 1936, 1938),
R. prolixus (Davey, 1958), Melolontha melolontha (Landa,
1960; 1961) and Ornithodorus moubata (Shepherd, 1982a,b).
In these insects at the time of release from the testes,
or storage in the seminal fluid, sperm are non-motile,
and become motile during copulation or after deposition in
the female (see Review by Leopold, 1976). Furthermore,
in most studies of the initiation of sperm motility, it is
not apparent whether the secretions produce the effect
directly, by providing the sperm with an exogeneous source
of energy, or indirectly by catalyzing the metabolism of
either an exogenous or endogenous substrate. Whether the
ARG secretions could be playing similar functions in D.
fasciatus is not well known.

1.3.2 Formation of barriers to reinsemination

Another function of the male ARG secretions in
insects is to form barriers to reinsemination. The
formation of a copulation or mating plug from secretions
produced by the ARGs has been observed in a number of
insects. For example, in the male bee Apis mellifera,
Laidlaw (1944) reported that a secretion produced
during copulation blocks the genital opening of the
female to prevent loss of semen. Spielman,et al., (1967,
1969) believed that in the mosquito Aedes aegypti this
is achieved by the bursa copulatrix reacting with the
male ARG secretions which cause vacuolization and the

swelling of the cells of the bursa. Gwadz et al., (1972),
however, disagreed with this hypothesis. They suggested
that the barrier to reinsemination is a post-copulatory
change in the mating behaviour of the female rather than
the seminal expulsion from the bursa copulatrix.

The existance of receptivity-inhibiting substances
(RIS) which originate from the male ARG secretions in most
monogamous flies has been reported for M. domestica (Riemann et al.,
1967; Adams, 1968; Terranova and Leopold, 1971), A. aegypti,
(Hiss and Fuchs, 1972), Hylemmya brassicae, (Swailes, 1971),
D. melanogaster, (Merle, 1968 and Burnet et al., 1973) and
Phormia regina, and Cochliomyia hominivorax (Nelson et al.,
1969). The R.I.S. contain an active principle called matrone
(Fuchs et al., 1968) to which the female response varies from
one species to another. For instance in Aedes and Musca the
female becomes permanently refractory to reinsemination (A
review by Leopold, 1976). On the other hand, in Drosophila
the effect of R.I.S. produced by the ARG is short lived and
the   prevention of reinsemination is attributed to the
presence of sperm in spermatheca.

The mode of action of the RIS is not well understood.
Gillot and Friedel (1976) believe that having reached the
reproductive tract of the female, the RIS produced by the
male ARG pass through the wall of the tract and stimulate
it to produce a hormone that affects receptivity. Moreover,
Trumann and Riddiford (1970) reported that in the silkmoth
H. cecropia the brain under the influence of the bursa
factor, inhibits the release of the "calling" hormone from
the corpora cardiaca resulting into the loss of receptivity

by females. Furthermore, Leopold et al., (1971) have established that in Musca the brain is the site of the RIS. Thus in A. aegypti, Gwadz et al., (1971) suggested that the terminal ganglion seemed to be the site of action of matrone. These findings are inadequate to show the relationship between the RIS and juvenile hormone but the work of Manning (1966, 1967), Lea (1968), Spielman et al., (1969) and Gwadz et al., (1971) indicate that the two substances may act antagonistically by competing for the same active site.

1.3.3 Contributions to survival and fecundity

There is as yet no concensus on the nutritional value of the male ARG secretions in the female insect. Among orthopterans and neuropterans, it is speculated that the spermatophore may be removed and eaten by the female after mating (Engelmann, 1970). Moreover, in the grasshopper Gomphocerus rufus (Loher and Huber, 1966) they may be partially digested and totally resorbed as demonstrated in Melolontha melolontha (Landa, 1960). Among the parasitic and semi-parasitic cimicoidae, insemination is haemocoelic. Hinton, (1964, 1974) believes that the semen in this case is digested by the female for nutritional purposes. This is however disputed by Mellanby (1939) who reported that in bed bugs, Cimex, virgin females survived starvation for longer periods than the mated females. Thus in support of this, Davis (1966) observed that starved cimex females displayed an avoidance to mating.

The involvement of male ARG secretions in the stimulation of egg maturation and oviposition has been studied only in a limited number of insects, mostly dipterans. In all cases

studied a fecundity enhancing substance (FES) is passed from the male to the female. However, there is still a controversy about the origin of this FES. For examples, among orthopterans the FES is known to be produced by the testes in _L_. _migratoria_ and _Teleogryllus_ _commodus_ (Loher and Edson, 1973). On the other hand, in _S_. _gregaria_ and _Melanoplus_ _sanguinipes_ the site of FES was demonstrated to be in the ARG (Pickford _et al_., 1969; Gillot and Friedel, 1976b). Furthermore, in the Hemipteran _R_. _prolixus_ (Davey, 1965a) the site of production of the FES is the testes. Among dipterans there is a variation among species but the site of production of the FES is predominantly the ARGs or the ejaculatory duct as in _M_. _domestica_ (Leahy and Lowe, 1966; Baumann, 1974).

The mode of action of the FES like the RIS is poorly understood. It is believed that both substances reach the female reproductice- tract and stimulate it to produce a hormone that alters fecundity or receptivity (Gillot and Friedel, 1976b). For instance working on _H_. _cecropia_, Riddiford and Ashenhurst (1973) proposed that the FES stimulates the production of a bursa-produced factor which then acts on the brain causing it to stimulate the _corpus_ _cardiacum_ which in turn produces an oviposition hormone. They further postulated that the same bursa produced factor influences the brain and inhibits the release of the "calling" hormone from the _corpora_ _cardiacum_, hence switching off the urge to reinsemination in the female. According to this hypothesis in _H_. _cecropia_ the

fecundity enhancing substances and the receptivity substances are more or less one.

Other authors have established that materials of the male ARG secretions influence maturation and release of eggs by the female insect. Thus Leahy (1973) indicated that secretions of the male ARGs of S. gregaria influenced both processes of maturation and egg release from the female. Implantation of male ARGs into either grouped or single females invoked an increase in the number of eggs produced by the female, and control implants of seminal vesicles or sperm filled spermatheca produced no significant effect. Pickford et al., (1969) also showed that ARG secretions of the male acridid Melanoplus sanguinipes, stimulates egg laying. In contrast, studies by Leahy (1973) with S. gregaria show that the male ARG secretions do not affect an increase in fecundity in other orthopteran species. Thus Quo Fu (1959) reported that in L. migratoria marilensis, males deprived of their testesand ARGs elicited a normal rate of oviposition in the females they mated with. In a separate study Benz (1969) postulated a similar mechanism in the moth Zeiraphera diniana. He found out that the ARG secretions facilitated oviposition rather than maturation.

From these examples it is clear that the male ARG secretions of a number of insects control the mating behaviour of the insect species. It is also clear from several publications that most of the work that has been

done on the effect of the male ARG secretions concentrated mainly on female reproduction and relatively little work has been done on the role of the male ARG secretions within the male insect itself (Awiti, 1980; Shepherd et al., 1982a, 1982b, Pinkerton et al., 1982). Working on D. fasciatus Awiti (1980) observed that when ARGs from six day old male insects were implanted into newly emerged males they led to accelerated sexual maturity and reduced the period of the onset of mating by one to two days. This observation suggested that the ARG secretions contained a factor which stimulated accelerated mating behaviour. However, it is also possible that the stimulus could have emanated from the haemolymph which was transfered together with the ARGs during dissection.

1.4 Neuro-endocrine control of reproduction in female insects

The control of ovarian development by the Corpus allatum (CA) in the female Rhodnius prolixus was first reported by Wigglesworth (1936). The regulation of egg maturation by a hormone from the CA in various insects has also been discussed by Weed (1936), Johansson (1954, 1958), Scharrer (1958), Bell (1969), Engelmann (1970), Pratt and Davey (1972), Willis (1974) and Chippendale (1977). Experiments by Wigglesworth (1936) also indicated that after allatectomy the ARGs in the Hemipteran R. prolixus diminished progressively in size and failed to produce their normal secretion. This

suggested that the ARGs were under the CA control.
Similar investigations were carried out in other
insects by other workers such as Scharrer (1946),
Odhiambo (1966, 1971), Manning (1966), De Loof and
Lagasse (1972), Gillot and Friedel (1976a). The
results showed that the size of the CA correlated
positively with their activity.

Parameters such as glandular volume, nuclear
volume or ratio of cytoplasm to nucleus of the CA
cells have been correlated with the intensity of its
endocrine activity (Engelman, 1970; Nayar, 1973,
Boxall, 1976). For example Thomsen (1952) observed
that the glands of Calliphora erythrocephala increase
in size during sexual maturation. Similar investi-
gations were carried out in other insects by Ganagarajah
(1965); Odhiambo, (1966), Boxall (1976). Other
experiments by Unnithan et al., (1977, 1979) showed
that the application of the anti-juvenile hormone
precocene II to newly emerged adult females of the bug
Oncopeltus fasciatus inhibited egg maturation and
induced degeneration of the Corpus allatum. Recently
Collette and Jacques (1984) showed that precocene II
inhibited oocyte maturation and lead to the degeneration
of the Corpora allata in the firebrat Thermobia domestica.
In male insects very limited corresponding work has
been carried out and hence the endocrine functions
in the male insect are still poorly understood in
comparison to the female insect.

1.5 Neuro-endocrine control of reproduction in

   male insects

It has been shown that the process of spermatogenesis
is not entirely controlled by the CA or pars intercerebralis
in R. prolixus (Wigglesworth, 1936), Locusta (Girardie and
Vogel, 1966), S. gregaria (Cantacuzene, 1967), Scatophaga
stercolaria (Foster, 1967), O. fasciatus (Economopoulos
and Gordon, 1971). In female insects, the endocrine
control of oocyte development applies strictly to vite-
llogenesis with oogenesis in the germarium not being
affected by hormones (Engelmann, 1970). Highnam and Hill
(1969) argue that in the male insect spermatogenesis
proceeds without hormonal influence. But Engelmann (1970)
maintains that the CA is involved in sexual maturation
of several male insects.

The CA involvement in male ARG development and
function has been reported in allatectomised males of
R. prolixus (Wigglesworth, 1936), L. maderae (Scharrer,
1946) and S. gregaria (Odhiambo, 1966, 1971). In
contrast Johansson (1958) reported that the growth and
function of the ARGs in O. fasciatus is not affected
by allatectomy. Moreover, Engelmann, (1970) argues that
there is no direct endocrine control of the secretory
activity of the ARGs. Furthermore, the inactivity of the
ARG following the ablation of the CA appears to be a

secondary phenomenon. He suggested that the metabolism
of the animal is affected by the endocrine glands just
as in the females so that in their absence, protein
metabolism may be lowered. This in turn may not allow
the necessary high metabolic activity of the ARGs since
the starting material has not been made available.

The recent discovery of the antiallatotropin
precocene has facilitated research into the possible control
of reproduction in insects by the CA by replacing the
tedious allatectomy experiments. Precocene was discovered
by Bowers in 1976 and since then it has been used as a
highly potent insect growth regulator. It was isolated
from the bedding plant Ageratum houstonianum and named
precocene by Bowers (1976) because of its induction of
precocious metamorphosis in Oncopeltus fasciatus, Lygaeus
kalmii and Dysdercus cingulatus (Bowers, 1976). In addition
it causes antigonadotropic activity in O. fasciatus and
other species of Heteroptera, Coleoptera, Diptera (Bowers,
1976; Yamada and Yagi, 1984) and in Thysanura (Collette and
Jacques, 1984).

Since 1976, the cytotoxic action of precocene on the
CA has been utilized in biological studies of insect
reproduction. Most of the studies have been carried out in
female insects and the role of the juvenile hormone in
vitellogenesis has been established. Little corresponding
work has been done in the male insects thus making it
difficult to compare their endocrine functions with those of

1.6 Scope and objectives of the present study

Previous researches done by Odhiambo, (1968), Arora (1971), Awiti (1976; 1980) have already illustrated the most important aspects of the reproductive biology of Dysdercus fasciatus. For example, Odhiambo (1968) and Arora (1971) pointed out that the observed long copulatory behaviour in D. fasciatus was not significant in egg maturation and production. Awiti (1976) reported that in D. fasciatus males were sexually immature at adult emergence as shown by lack of sperm in seminal vesicles. Sperm descent occured three to four days after adult-emergence. This was the time when females were sexually receptive. She showed that ARGs were not fully developed and not actively secreting at adult-emergence. These were reported to be fully developed and actively secreting by the third or fourth day after adult-emergence. From these observations it was suggested that in D fasciatus apparently three events are synchronised, namely the onset of mating, the release of sperm from the cyst cells of the testes into the sperm reservoir, and the increased secretory activity of the male accessory reproductive glands.

The application of precocene II to newly emerged male bugs was also shown by Awiti (1980) to prevent the continuous matings upto eight days following adult

emergence. Furthermore, the spermatozoa failed to descend into seminal vesicles suggesting that precocene II interfered with the sexual maturity of the bug. This study, therefore, sought information on the activities of the CA and ARG in the unpaired and paired male D. fasciatus. The objectives were:-

(a)   To investigate the activities of the CA and ARG during sexual maturation in the male D. fasciatus.

(b)   To investigate the role of the CA on the functional activities of the ARGs.

(c)   To determine the effect of the ARG secretion on mating behaviour and sperm descent from the testes.

(d)   To determine the regulatory mechanism of sexual maturation in the male D. fasciatus.

CHAPTER   TWO

MATERIALS   AND   METHODS

2.1   Source of experimental insects

All investigations in this study were carried out in
the Entomology Laboratory of the Department of Zoology,
University of Nairobi.   Insects chosen for this study were
cotton stainer bugs of the genus Dysdercus fasciatus
(Hemiptera: Pyrrhocoridae).   These were obtained
from the existing colony maintained in the departmental
insectary but some were obtained from the field while
feeding on wild shrubs growing on Riverside drive, Chiromo
Campus notably on Vernonia lasiopus and Hibiscus calyphyllus.

In this Chapter the breeding of experimental insects is
discussed in Section 2.2, while condition of experimental
insects is described in Section 2.3.   Section 2.4 describes
the various materials and methods used in studies of the
activities of the CA and ARG during sexual maturation.
Finally section 2.5 gives the materials and methods used
in the study of the effect of the ARG homogenates on mating
behaviour and sperm descent.

2.2   Breeding of the experimental insects

All insects were raised in the departmental insectary
which was maintained at a temperature of about $29 \pm 1^{o}C$ and
a photoperiod regime adjusted to roughly 12 hours of light

and 12 hours of darkness. Light was provided by 40 watt
bulbs placed at 30 cm from the breeding containers. In
this way they also imparted some heat to the containers.

Adult cotton stainers were reared in plastic cylinders
of an approximate capacity of 2.5 cc. The bottoms of these
cylinders were sealed with a fine mesh of mosquito netting
material which allowed eggs to pass through. The cylinders were
placed inside plastic trays the bottoms of which were lined
with wet pieces of cotton gauze on which eggs were collected.
This was moistened every morning to provide the necessary
moisture stimulant for oviposition, and was changed once
every other two days. Tops of each container were covered
with a fine mesh of netting material supported by a rubber
band to prevent bugs from escaping and to allow adequate
ventilation for the bugs (Plate 1).

Food in the form of soaked or half-boiled cotton
seeds was provided once every two days in small petri-
dishes. Some soaked cotton wool was provided with the
seeds to supply the necessary humidity inside the cylinders.

Eggs were collected from the gauze and occasionally
from the food inside the petri-dishes every morning and
placed in small glass vials containing moistened rolls of
cotton-wool, and covered with a dry plug of cotton wool.
Under these conditions hatching took five days.

Plate 1

A plastic cylinder in which adult insects were reared
for mating and oviposition purposes.

Plate 1:

T = Tray on which eggs were laid

G = Gauze on which eggs were oviposited

N = Netting material

Newly hatched nymphs were transfered to clean kilner jars (Plate 2) whose bottoms had been lined with moistened cotton wool to supply moisture requirements. As soon as they moulted into fourth instars, they were transfered into more spacious plastic buckets (Plate 3) which contained an elaborately folded gauze supported by twigs. The gauze served two purposes. It provided the necessary moulting sites as well as shelter to the bugs. This greatly minimised cannibalistic tendencies due to overcrowding (see Boxall, 1976). Each bucket was covered with a thin material of white cloth which prevented bugs from walking out. Like adults nymphs were fed on moist cotton seeds at two day intervals and water was constantly provided in the form of moistened cotton wool.

All insect containers were cleaned thoroughly once a week to remove exuviae and waste remains of the cotton seeds. Under these conditions the life history of the bugs lasted between 30 to 35 days.

Plate 2:

A kilner jar in which the 1st to 3rd instars were raised.

Plate 2:

C = Cotton wool moistened to supply moisture.

TW = Twig used as a moulting site

F = Food   in petri dish

N = Netting material for ventilation

Plate 3:

Plastic bucket used for rearing the 4th and 5th
instar nymphs.

Plate 3
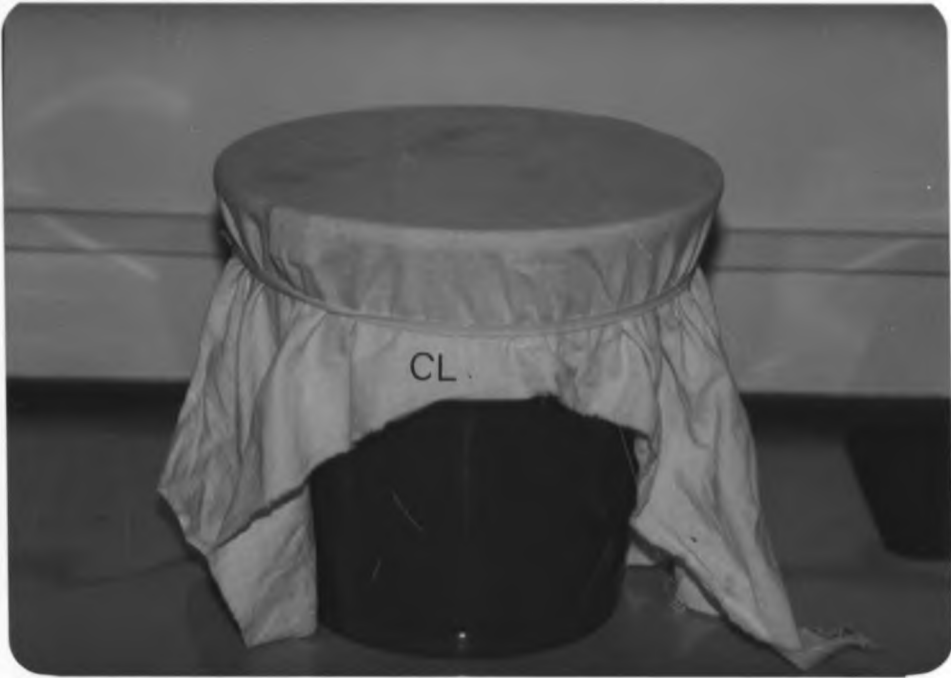
CL = Cloth cover of the bucket

B = Bucket

2.3 Condition of experimental insects

In all experiments the age of the insect was very critical. Hence male insects were isolated as soon as they emerged or a few hours later, but not exceeding 16 hours. They were then placed in clean kilner jars whose bottoms were lined with a dry piece of gauze, provided with a moistened roll of cotton-wool and some soaked cotton seeds in a petri-dish as food.

It was found desirable to use male insects that emerged first from each batch since they appeared to be of a uniform size compared to the males that emerged as the females started emerging. Hence the males that emerged with females were spared from experimental purposes and instead used for breeding. By so chosing insect specimens of the same size, it was assumed that they were all in the same physiological state.

2.4 The activity of the CA and ARG in the male D. fasciatus during sexual maturation

2.4.1 Morphometric studies

In the unpaired insects, the newly emerged male insects were isolated and placed in kilner jars prepared as described in 2.3. From these containers the insects were drawn for dissections which were done on consecutive days from day 0 to day 20.

The paired male and female insects were placed in
one container which had been provided with shelter, food
and water. Dissections of such male insects were performed
from the day when all insects had been in copula for
approximately one day upto the 13th day when it was assumed
all the male insects had gone through the first copulatory
cycle.

The insects were killed by quick immersion in
absolute alcohol. Before dissection, the wings and legs
of the freshly killed bugs were removed and the insect
pinned with dorsal side uppermost in a dissecting dish.
Dissections were performed in a Dysdercus Ringer medium
(Berridge, 1965) under an M5A Wild Herrbrugg Stereo
dissecting microscope at low power.

A longitudinal slit was made through the centre of
the head capsule from the posterior part of the pronotum
to the anterior apex of the head. By using a pair of fine
forceps (size 4) the slit was teased apart exposing part
of the brain. The remains of head sclerites, pronotum and
fat were cut away to expose the entire brain with its
retrocerebral complex hanging at its base on the aorta.
The Aorta is joined very closely to the oeosophagus.

The CA and Corpora cardiaca (CC) are in very close
proximity to each other with the CC lying anterior to the
CA. The CC is a blue whitish structure while the CA is a

transparent structure in newly emerged insects and pale-yellowish in mature male insects. The CA is basically a sphere when observed from its dorsal aspect, but ventrad, it is partially bisected medially to form two lobes which are sometimes of unequal sizes.

To expose the male reproductive system, two longitudinal slits were made at the periphery of the abdominal tergites with the help of a fine needle. Using a fine pair of forceps, the entire dorsal tergites together with the digestive system were pulled backwards to expose the male reproductive system. This comprised of the orange-yellowish testes joined by the seminal vesicles and vas-deferens to the paired ARGs. A crozier shaped ejaculatory duct lay between the pair of the ARGs.

An ocular micrometer was used in all measurements of linear dimensions of the CA and ARG. It was inserted in the eye piece chamber of the dissecting microscope and calibrated using a stage micrometer scale (CAT 1400). The number of microns per each ocular scale unit were worked out at various magnifications. This gave rise to the following calibration table:-

Magnification	Microns per ocular scale unit
x 90	100
x 180	50
x 375	25
x 750	13

Measurements of the CA and ARG were taken in vitro in Dysdercus Ringer solution on a cavity slide. To measure the CA, the Aorta was detached from the brain and orientated to its ventral aspect where the CA could be seen clearly as an oval structure partially bisected to form two lobes. In this aspect the length and width of the CA was taken at high power with minimum optical illusions. Linear measurements of the ARGs were taken after the entire reproductive system had been detached from the supporting connective tissue and the sorrounding fats removed. The longest side of the bean shaped ARG was measured as the maximum length while the shortest central region was measured as the maximum width.

Finally all the linear measurements of both the CA and ARG which had been previously expressed in microns were later converted into millimetres. From these, their volumes were worked out using the formula of Mial and Mordue (1980) where:-

$$\text{Volume} = \tfrac{4}{3}\pi \, a \times b^2$$

Where π = 3.14

     a = Mean length of the gland

     b = Mean width of the gland

2.4.2 Histological methods

(a) Histology of the CA

To ease handling during processing, the entire brain with its retrocerebral complex was dissected out intact and fixed overnight in Masson's modification of aqueous Bouin's fixative (Ewen, 1962). Fixation varied from 12-16 hours at room temperature. After fixation, specimens were dehydrated in 70% and 80% ethanol (overnight) and then passed through two changes of 90% ethanol each lasting 6 hours and lastly 100% ethanol (2-4 hours). Following dehydration, the speci-mens were cleared in three changes of celloidin in methyl-benzoate for a total of 48 hours and finally in two changes of benzene each lasting about 20 minutes. Subsequently the specimens were transfered into a 1:1 mixture of benzene and paraplast   at $60^{o}C$ for 30 minutes followed by infiltration with two changes of pure molten paraplast wax at $60^{o}C$ (1 hour each). Finally the specimens were embedded in pure paraplast wax. Blocks of embedded specimens were trimmed with a short scalpel blade and mounted on cuboidal wooden blocks and sectioned serially at 5-7 um on a Jung Heidelberg microtome. Sections were transfered to warm slides smeared lightly

with a mixture of egg albumen and glycerine on a warm plate
and allowed to stretch out in water.

Prior to staining, the sections were deparaffinised
by passage through two changes of xylene (5 minutes in
each), then through one change of absolute ethanol followed
by graded series of 95%, 80%, and 70% (3 minutes each).
Finally they were rinsed in distilled water for 30 minutes
to remove the yellow colour of the fixative.

Staining of the sections was carried out as described
by Ewen (1962). Briefly, hydrated sections were passed
through acid permanganate (1 minute), rinsed in distilled
water and passed in sodium bisulphate to decolourise.
They were quickly rinsed in distilled water followed by
dehydration in 30% and 70% ethanol (1 minute in each) and
then stained in aldehyde fuchsin for 10 minutes. After
staining the sections were hydrated by passing through 95%
ethanol, acid alcohol, 70% and 30% ethanol (30 seconds each).
Finally they were passed through distilled water to complete
the hydration. Next, the serial sections were passed
through a mordant of phosphomolybdic-Phosphotungstic acid
which was followed by a rinse in distilled water. They
were then counterstained in Halmi's counter stain for 1
hour, passed through a solution of 2% acetic acid in 95%
ethanol, then through absolute ethanol before they were
finally cleared in two changes of xylene. The sections
were mounted on slides in DPX and left to dry in air.

Subsequent observations on the serial histological preparations were made with a Leitz Laborlux 12 compound microscope and photographed using a Leitz Laborlux 12 microscope on which a Wild MPS 15 Semiphotomat Camera was attached.

(b) Histology of the ARGs

Dissection of the ARGs for histological work was carefully done to include the testes with their connecting seminal vesicles and vas deferens. Upon dissection the organs were fixed overnight in Masson's modification of Bouin's fixative. Dehydration was done in a graded series of 50%, 70%, 95% and absolute ethanol (30 minutes in each), followed by clearing through two changes of xylene for a total of 40 minutes. They were subsequently infiltrated with wax through two changes of pure molten paraplast at $60^{o}C$ (45 minutes in each) before finally being embedded in paraplast. Embedded glands were trimmed, mounted and sectioned serially as described above (2.4.2 a).

Prior to staining, sections were deparaffinised and hydrated as described above for the CA. Hydrated sections were stained in Meyer's or Erlich's haematoxylin for 15 minutes. Stained sections were washed for 10 minutes in running water, blued in Scotts solution (three minutes) and rinsed in distilled water for three minutes. Counter staining was done in aqueous Eosin (5 minutes) followed by

dehydration in 70%, 95% and absolute ethanol.

The same general principles of clearing and mounting under coverslips with DPX were performed and preparations were left to air dry. These were later observed in a Leitz Laborlux compound microscope and photographed as described for the CA above.

2.4.3 Precocene studies

Each experiment in this study was carried out on a set of 20 newly emerged male insects of the same age. Half the number of bugs were treated with 1, 5, 10, 15 and 20 µg/1 µl concentrations of Precocene II (6, 7 dimethoxy 2, 2 dimethyl Chromene, Calbio Chemicals Lot 800461 A Grade) dissolved in acetone. Control males received topical applications of 1 µl pure acetone. Before the various doses of Precocene were used, they were kept in vials, tightly sealed with parafilm and kept in the freezer till required for use.

A single topical application of 1 µl for each dose was drawn in a micropipette attached to a rubber dispenser and applied on the ventral surface of the thorax of the unanaesthetized insects. The insects were allowed to dry before they were released into observation jars. Observations for the effect of Precocene were started a day after each application till the 10th day. Observations included linear measurements of the CA and ARGs and a

quick inspection of the seminal vesicles and vas deferens to
check sperm descent.

2.5 The effect of ARG homogenates on mating behaviour
     and sperm descent

Under this study, ARGs were dissected from sexually
mature 6 day old male insects, transfered in a homogenizer
containing 0.5 ml Dysdercus Ringer solution. This was
placed in a vessel containing Ice-cubes to prevent deterioration
of the material. Using a Hamilton microliter syringe (No. 701),
10 μl of the homogenate was injected through the third dorsal
intersegmental membrane of the abdomen. Control insects
received an equal quantity of saline alone. The wound made
by the syringe was lightly treated with Streptomycin sulphate
(Dawa pharmaceuticals) and sealed with molten dental wax.
Both experimental and control insects were paired with the
females of the same age and size and relevant observations
started a day later.

CHAPTER THREE

RESULTS

3.1 Morphometric changes of the CA and ARGs in the
male D. fasciatus during sexual maturation

3.1.1 Changes in the size of the CA in the
unpaired male D. fasciatus

In this study the changes in the size of the CA
was used as a criterion for the state of its activity.
The CA was small at adult emergence (Figure 1) with a
mean volume of $4.60 \pm 0.20 \times 10^{-3}$ mm^3. During the first
three days following adult emergence, its volume
increased to $7.52 \pm 0.28 \times 10^{-3}$ mm^3. The highest rate
of volume increase was recorded between day 0 and day 1
where as much as 34% of the total volume change was observed.
This was the highest rate of increase recorded on a
single day. By the 3rd day the gland had attained its
maximum size.

After the 3rd day the volume of the gland was stable
upto the 6th day when the highest value was obtained. This
stable and high state of activity is denoted as P_1 (Figure 1)
representing a period of the first highest CA activity.
After the 6th day the CA volume started to fall gradually upto
the 8th and 9th days,then sharply from the ,9th to 11th day
when the lowest mean volume was recorded (Appendix I). It
represented a drop of volume of the gland by 43% over that

recorded at peak activity on day 6 and much smaller than the volume at adult emergence.

After the 11th day the changes in the volume of the gland were repeated. These showed lower values than those previously observed. There was a sharp increase in the volume of the CA from the 11th day to the 12th day by 46%. Again this was the highest rate of volume increase recorded in one day. On the 14th day a second climax P_2 (Figure 1) with a mean volume of $6.55 \pm 0.51 \times 10 x^{-3}$ mm^3 was attained. Unlike P_1, P_2 was short-lived staying on only upto the 15th day and falling off gradually after that.

These results show that the secretory activity of the CA is cyclical. It started at a low level at adult emergence, rose gradually and reached a peak on the 3rd day. This peak was maintained for about four days and dropped between the 7th and 11th days. After the 11th day, similar changes in the volume of the CA were noted.

3.1.2 Changes in the size of the ARGs in the unpaired
       male D. fasciatus

Like the CA the ARGs activity was determined in terms of volume changes (Figure 2 and Appendix II). The results show that at adult emergence the mean volume of the ARGs was small, measuring $9.24 \pm 0.22 \times 10^{-1}$ mm^3. This increased to $25.64 \pm 3.29 \times 10^{-1}$ mm^3 by the 5th day -an increase of

Figure 1: Graph showing changes in the volume of the CA in the
unpaired D. fasciatus during sexual maturation. At
adult emergence the volume of the gland is small but
increases sharply reaching its first peak (P_I) on the
3rd day. This is maintained with minor fluctuations
till the 7th day followed by a steep decrease in volume
attaining a minimum value on the 11th day. Starting
from the 11th upto 12th day there is another abrupt
increase in the glandular volume attaining its 2nd
peak ($P2$) on the 14th day. This lasts until the
15th day when the volume falls off again. Note that
value $P2$ is smaller than P_I.

Figure 1.
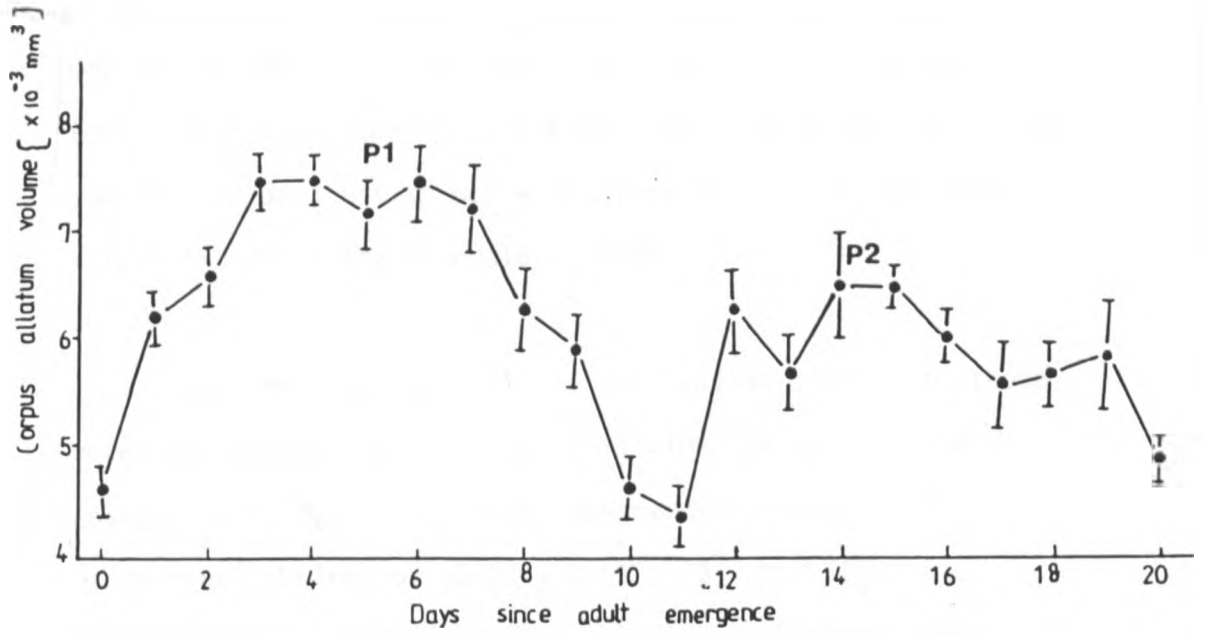
Vertical bars represent Standard Error of the Mean (S.E.M.)

177% over that recorded on the day of adult-emergence. After the 5th day, there was little increase in the glandular volume resulting into a maximum volume of $28.24 \pm 1.60 \times 10^{-1}$ mm^3 (P$_1$) on the 7th day. Between the 5th and 8th days there was a fairly stable activity of the ARGs (Figure 2). On the 8th day the volume of the ARGs started to fall again and the lowest mean was recorded on the 11th day. A comparison of the lowest mean on the 11th day with that on the 7th day shows a fall of 27% in the glandular volume.

From the 11th day there were increases similar to those observed between day 0 and 5. This time the mean volume changes were higher than those observed previously. A maximum mean volume of $33.07 \pm 3.06 \times 10^{-1}$ mm^3 (P$_{II}$) was attained on day 15 representing an increase in the volume of the gland by 61% from that observed on the 11th day when the gland activity was low (Figure 2). P$_{II}$ was shorter lived than P$_I$ previously observed. This was immediately followed by a rather abrupt fall in the volume on the 16th, 17th and 18th days. On the 19th day a small increase in the volume of the gland was observed but started falling off by the 20th day.

These results show that the volume of the ARG increases from the day of adult-emergence upto the end of the second copulatory cycle, which comes after the 18th day. In

Figure 2:   A graph showing changes in the volume of the ARGs
during sexual maturation.

At adult emergence the volume of the gland is small
but gradually increases reaching its first peak (P_I
between the 6th-8th days. This is followed by a
gradual fall in the glandular volume up to the
11th day, when a minimum value is obtained.
Beginning from about the 12th day, there is a
renewed increase in the mean volume up to the 15th
day when a second peak (P_{II}) is obtained. After
the 15th day there is a gradual fall which attains
its lowest value on the 18th day. On the 19th day
a renewed increase in the volume of the gland starts
to show again.

Figure 2.
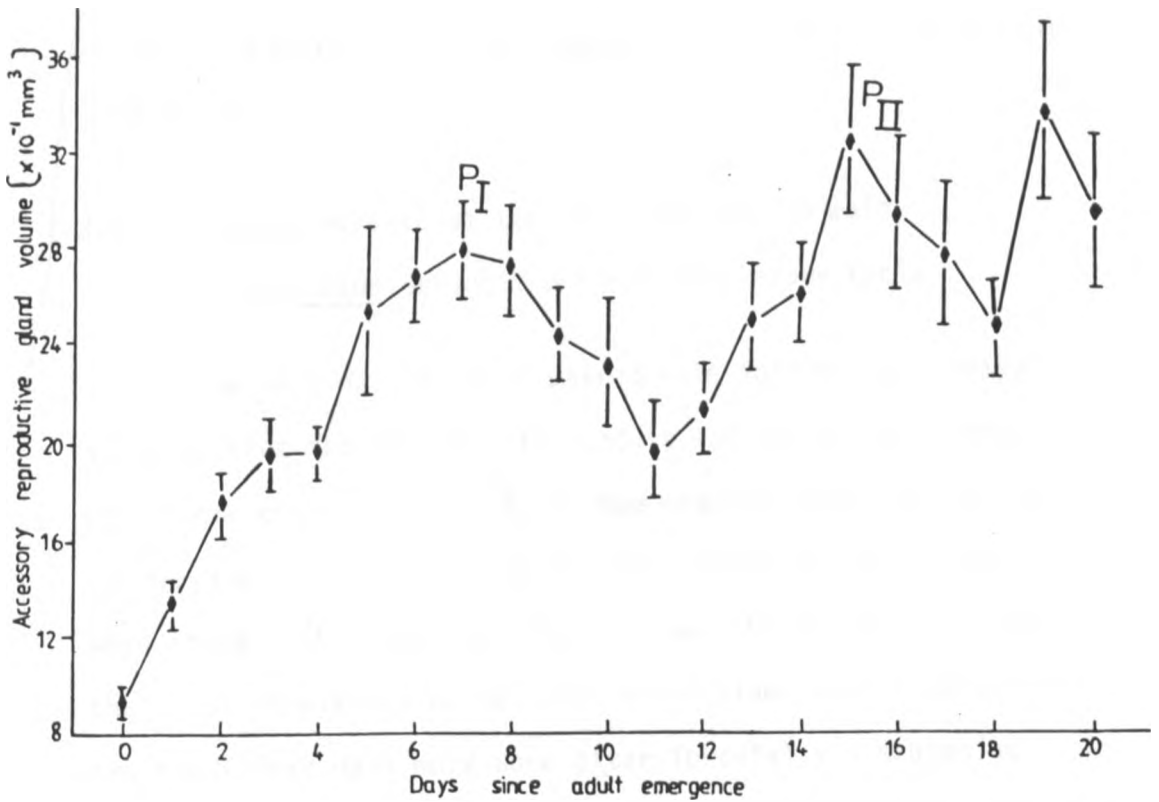
Vertical bars represent Standard Error of the Mean (S.E.M.)

between this time, there is a major slump in the glandular
volume which suggests cyclicity of its secretory activity.
The pattern of volume change of the ARG is similar to that
of the CA (Figure 1). This suggests a functional relationship
between them.

3.1.3 Changes in size of the CA in the paired male
       D. fasciatus during the first copulatory cycle

The size of the CA in paired male insects were measured
starting from the 4th day after adult emergence, when about
90% of the experimental insects had entered into copular for
at least one day. Measurements were continued for 10 days
when it was safely assumed that all insects had gone through
the first copulatory cycle. All dissections performed during
the first five days were done after forcefully disengaging
the male from the female. Other dissections following the
first five days of copulation were performed on insects that
had mated.

Results obtained in this study basically followed a
trend observed in the unpaired male insects (3.1.1.).
The mean volumes of the CA in the mating pairs of male insects
(Appendix III) were on the whole smaller than those of the
unpaired insects. On the 5th day when insects had been
in copula for one day, there was a decrease in the volume
of the CA by 43% (Figure 3a). There was a further drop
in volume on the 6th day after which it remained fairly

Figure 3a:

Changes in the volume of the CA in the mating male D. fasciatus
during the first copulatory cycle. The volume of the gland is
low compared with that of the unpaired male insects in Figure 1.
Note also the very low volumes of the gland on the 11th to the 13th
day when mating is completed.

Figure 3b:

Changes in the volume of the ARG in the mating male D. fasciatus
during the first copulatory cycle. The fluctuations in the
volume changes previously not observed in the unpaired insects
(Figure 2) shows that the gland is being depleted.

The low volumes of the gland during the first three days of mating
and during the 10th-11th days when mating is complete are clearly
shown.
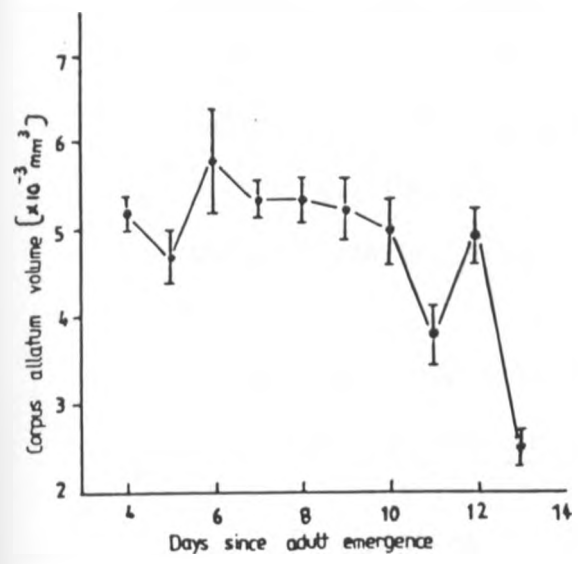
Figure 3a.   Figure 3b.
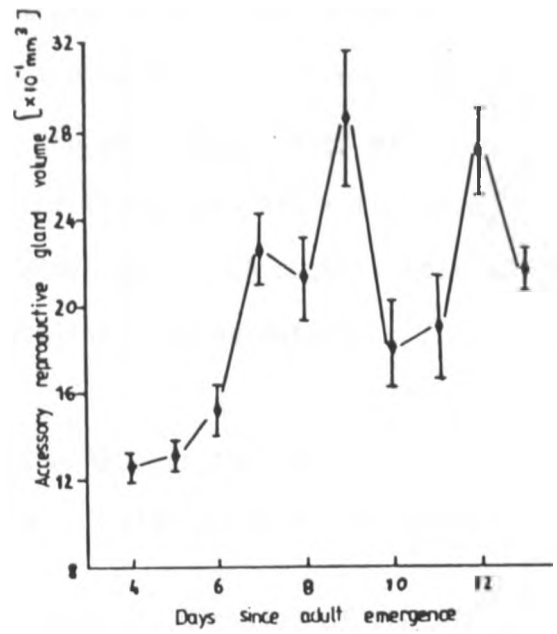
Vertical bars represent Standard Error of the Mean (S.E.M.).

stable up to the 10th day. The period between the 4th and
10th day was also a period when the insects were in continous
copulation.

These results show that there is a slight change of
pattern of the CA secretion in the mated insects compared
with the virgin insects. This change is associated with
mating since it is occuring about three days after the onset
of mating. The smaller mean values of the CA suggest that
the contents of CA are being utilised during mating.

3.1.4 Changes in the size of the ARGs in the paired
       male D. fasciatus during the first copulatory cycle

The pattern of volume changes in the size of the ARGs
in paired male insects was characterised by fluctuations
which had not previously been observed in the unpaired insects.

The size of the ARGs on the 4th day after adult
emergence followed by a day of copulation showed a mean
volume of $12.65 \pm 0.67 \times 10^{-1} mm^3$ (Appendix IV). This was
lower than the volume of the ARGs in the unpaired individuals
of the same age (Appendix II). Although this difference
was not statistically significant ($P \leq 0.05$) it represented
a drop of 34% in the glandular volume. Apparently a lot of
secretory materials were depleted from the ARGs when the
insects had remained in copula for one day. This low volume
was boosted gradually on the 5th and 6th days. From the 7th

day it increased rapidly reaching a maximum on the 9th day - a rise of over 126% compared with that observed at the onset of mating.

Between the 9th and 10th days there was a very sharp fall in the glandular volume - about 36% of the glandular secretion was lost. At this time the majority of insects were disengaged. These results suggest that there were two pronounced periods in the cycle in which a lot of the glandular secretion was released namely, towards the onset of copulation and towards the end of copulation.

3.1.5 Sperm descent in the unpaired male D. fasciatus

A visual inspection of the vesicular seminalis was done during the first three days to check sperm descent. On the day of adult-emergence no sign of spermatozoa or secretion could be seen. On the 1st and 2nd days after adult-emergence the vesicular seminalis was still empty (Plate 4) even when pulled and teased apart in Dysdercus Ringers solution. At the end of the 2nd day and beginning of the 3rd day, a bundle of spermatozoa was observed leaving the testes and entering the vesicular seminalis. This was observed in 70% of the insects examined and by the 4th day, all insects had their seminal vesicles full of sperm which could be seen beating in a wave-like fashion at low power. At this time the entire vesicular seminalis had become 'opaque showing a creamy white colour with a streak of greyish-reddish material on one side coming from the testes. By the 6th day the entire

Plate 4:

The male reproductive system of a two day old
insect. Note the empty seminal vesicles (S.V.)
posterior to the testis (T).

Plate 4:

T   = Testis
SV = Seminal vesicle
ARG = Accessory reproductive gland
ED = Ejaculatory duct

vesicula seminalis was filled with a lot of sperm and looked
bigger than the testes (Plate 5). When the vesicula seminalis
was at this time teased on a depression slide, it revealed
a mass of spermatozoa whose extremely long entertwined tails
were immobile although a few could be seen swaying about.

The ejaculatory duct, on the other hand, was empty
from the 1st day to the 5th day. From the 6th day, however,
an intermittent flow of globular fat-like droplets were seen
oozing out of the tiny sperm conduit tube within the ejaculatory
duct. These were glandular secretions of the ARGs. When
the mesadenial sacs were pierced with a fine needle, similar
materials oozed out. On the 7th day, and for the first time,
a mass of sperm together with the secretions of the ARGs could
be seen oozing out of the ejaculatory duct. On the 8th, 9th
and 10th days, no sperm could be seen in the sperm conduit but
the ARG secretions continued to ooze in a few preparations.

These results show that the release of sperm from the
testes into the seminal vesicles starts at the end of the
2nd day. The sperm passes through the ejaculatory duct four
days later when there is active flow of the glandular secretion
of the ARGs. From these observations it seems to me that
sperm transfer possibly takes place on the 7th day after
the bugs have been in copula for 3-4 days. This is indeed
a remarkably long period compared to other insects. What may
be happening during this time is not known.

Plate 5:

The reproductive structures of a 6 day old male.

Note the size of the seminal vesicles (S.V.) full
of sperm and the relatively empty testis (T).

Plate 5:

T = Testis

SV = Seminal vesicle   filled with sperm

ARG = Accessory reproductive gland

3.2 Histological changes of the CA and ARG activities in the
     male D. fasciatus during sexual maturation

3.2.1 Histology of the CA

The CA in the male D. fasciatus is enclosed by a thin
layer of connective tissue. The structure of the CA is cellular
with no evidence of syncytium. On the day of adult emergence
the CA cells are small with densely packed nuclei (Plate 6).
The entire gland seems to possess one basic cell type which
stains homogeneously brown with Paraldehyde-Fuchsin-Halmi
stains (PFH). Each cell contains a pale brownish or reddish
ovoid nuclei which encloses two or three purplish intranuclear
granules. In between the cells some radial lines   are   seen.
These become wider with age. The peripheral cells of the gland
havea higher affinity for the PFH stain and appear   darker
than the core cells.

From the 3rd day, some changes take place in the CA.
Starting from the outside, the thin layer of connective tissue
which have unclear outline become more pronounced and the cells
increase in size. This increase appears to be mainly due to an
increase in the volume of the cytoplasm. The nuclei are also
slightly bigger (Plate 7), more dispersed within the gland and
contain more intranuclear granules.

The colour of the cytoplasm changes from brown to
 pale-greenish   which is maintained in all the sexually
mature insects. The region of the CA which is closely associated
with the Aorta (where the aorta wall fused with the envelope of

the gland) has a thinner connective tissue (Plate 7). There are also similar regions on the remaining sides of the CA that are freely exposed to the haemolymph. Radial lines from the interior of the gland are oriented towards these areas.

During the first two days after adult emergence the gland appeared inactive. This was shown by the staining properties of the cells which showed a brown colour in contrast to that of the mature 6 day old insects which were stained green.

From the 3rd day, however, the increase in the volume of cytoplasm and intranuclear granules suggests an increase in the secretory capacity of the CA cells. The change in colour of the cytoplasm from brown to green also suggests that a new secretory product is being released within the central regions of the gland. These areas have thinner walls which permit diffusion of the secretion to pass through.

Plate 6

A photomicrograph taken from a longitudinal section of
the CA-CC complex of the newly emerged D. fasciatus.
The CA shows crowded nuclei, indistinct cell membranes
and little cytoplasm in the cells. Thin radial lines
can be seen emanating from the centre of the gland
to peripheral areas.

(PFH stain x 400).

Plate 6:
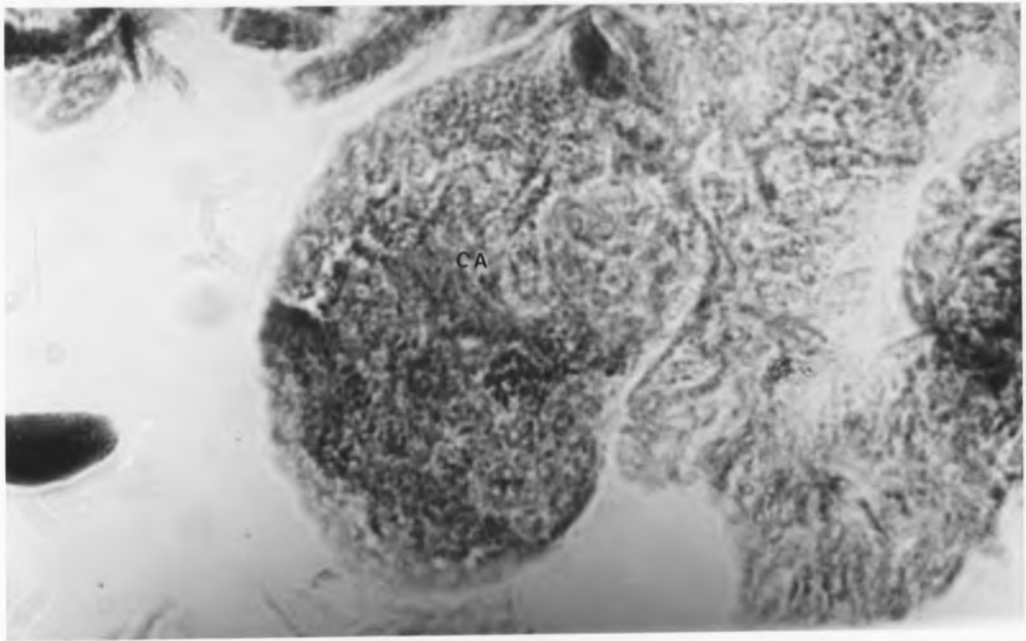
CA = Corpus allatum.

Plate 7:

A photomicrograph of a longitudinal section of the CA of a three day
old insect. Note the clear outline of the connective tissue
surrounding the gland, the increased size of the CA cells, and the
radial lines running through them. The nuclei are bigger, more
dispersed and with more granules. The region of the CA which is
closely associated with the aorta has a thinner connective tissue in
comparison to other boundaries.

Note similar regions on the remaining sides of the CA that are freely
exposed to haemolymph. Radial lines from the interior of the gland
are oriented to these areas.

(P.F.H.   x   1000).

Plate 7
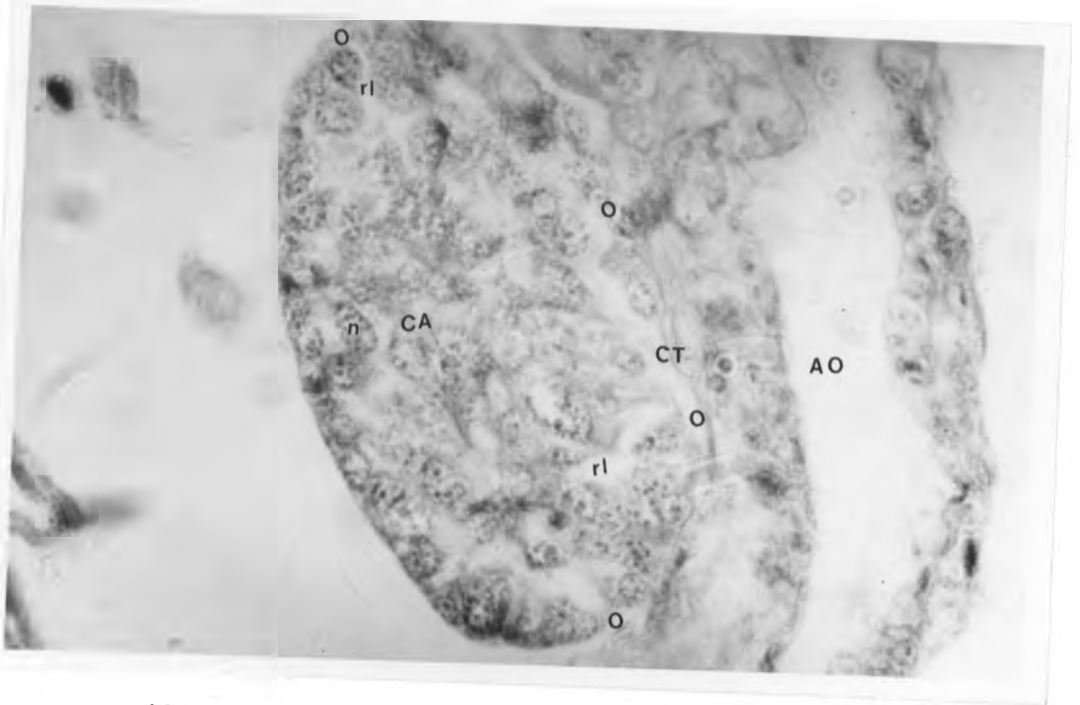
AO = Aorta

n   = Nucleus

CA = Corpus Allatum

CT = Connective tissue

rl = Radial line

O   = Areas of the CA a with a thin connective
       tissue.

3.2.2 Histology of the ARGs

To have a better understanding of the activity of
the ARGs, longitudinal sections of the glands were taken.
This made the assessment of the lumina size of the glandular
sacs, nuclei and quantity of secretory product possible.
Furthermore, through such serial sections, it was possible
to observe the two mesadenes and the ejaculatory duct
through which materials from the gland pass.

Serial longitudinal sections of the mesadenes show
densely packed saccular pockets of irregular shape and
size, whose lumina are confluent. Each pocket possesses
an epithelium made up of a single row of cuboidal cells
sitting on a common winding basement membrane. Each cuboidal
cell consists of a large nucleus containing highly dispersed
granular material. The luminal borders of each pocket
has a wavy outline indicating the presence of a stereo-cilial
brushborder. The outer surface of each mesadene is lined
by a thin membraneous sheath with flattened nuclei scattered
at various intervals along it.

At adult emergence the epithelial lining of the sacs
is thick and encloses a small lumen devoid of any secretion
(Plate 8). Starting from the 1st day, some scanty luminal
contents begin to be seen in the lumina occupying a central
position surrounded by a clear space (Plate 9).

From the 3rd day the mesadenial glands begin to show
several changes in their microscopical anatomy. Most
notable of these changes is the increase in the size of
lumina which suggests an increase in the secretory capacity
of the mesadenes. By this time all mesadenes contain a
fine granular secretion appearing pale pinkish or purplish in
colour with Haematoxylin and Eosin (H & E) stains. In addition
to the increase in size of the lumina, sizes of the cuboidal
epithelial cells decrease. They become flattened due to the
increased internal pressure on them arising from the increased
luminal volume (Plate 10). The basement membrane also
becomes inconspicuous and its nuclei can hardly be recognised.
The nuclei of the epithelia can, however, be seen quite easily
and appear   to increase in size with age.

In the 7 day old male bugs that have been in copula
for 3 days, there is a remarkable increase in the size of
the lumina which cause considerable stretch on the epithelial
cells. The fine granular secretion within the lumina also
become intensely concentrated and purple in colour. In these
insects longitudinal sections passing through the lower end
of the vesicular seminalis as well as the ejaculatory
duct reveal   the presence of secretion in these ducts. In one.
a   bundle of sperm surrounded by the granular secretion
could be seen in the lower part of the ejaculatory duct
(Plate 11).

In the 9 day old bugs which have gone through the first
copulatory cycle, the mesadeinal pockets remain large and
their luminal secretions are intensely stained.   However, the
epithelial linings appeared thicker than those in the 7 day
old bugs indicating that there is less pressure on the
wall of the pockets which suggets a loss of some contents
of the glandular secretion (Plate 12).

It is therefore clear from these results that during
the first two days after adult emergence   important changes
take place in the ARGs, which lead to the synthesis of the
ARG secretion. It is, however, difficult to say exactly
when the release of the secretion starts or give an estimation
of how much secretion is lost daily. Furthermore, it is
diffucult to verify the cyclical release of the secretory
materials through histological studies of this kind. However,
since the structure of the mesadenes appeared to be the same
in all the insects above 5 days old, it can be assumed
that there is probably a continuous secretory process which
balances with the loss.

Plate 8:

A photomicrograph of a longitudinal section of the ARGs
taken from 0-day old male D. fasciatus.

There are thick epithelial cells lining the mesadenial
sacs.   The lumena are small and lack secretions.

(H and E stains x 160).

Plate   8

L   =   Lumen

E   =   Epithelium

Plate 9:

A photomicrograph of a longitudinal section of ARGs
taken from a two day old insect showing secretion
beginning to form in the lumina.

(H and E stain x 160).

Plate 9
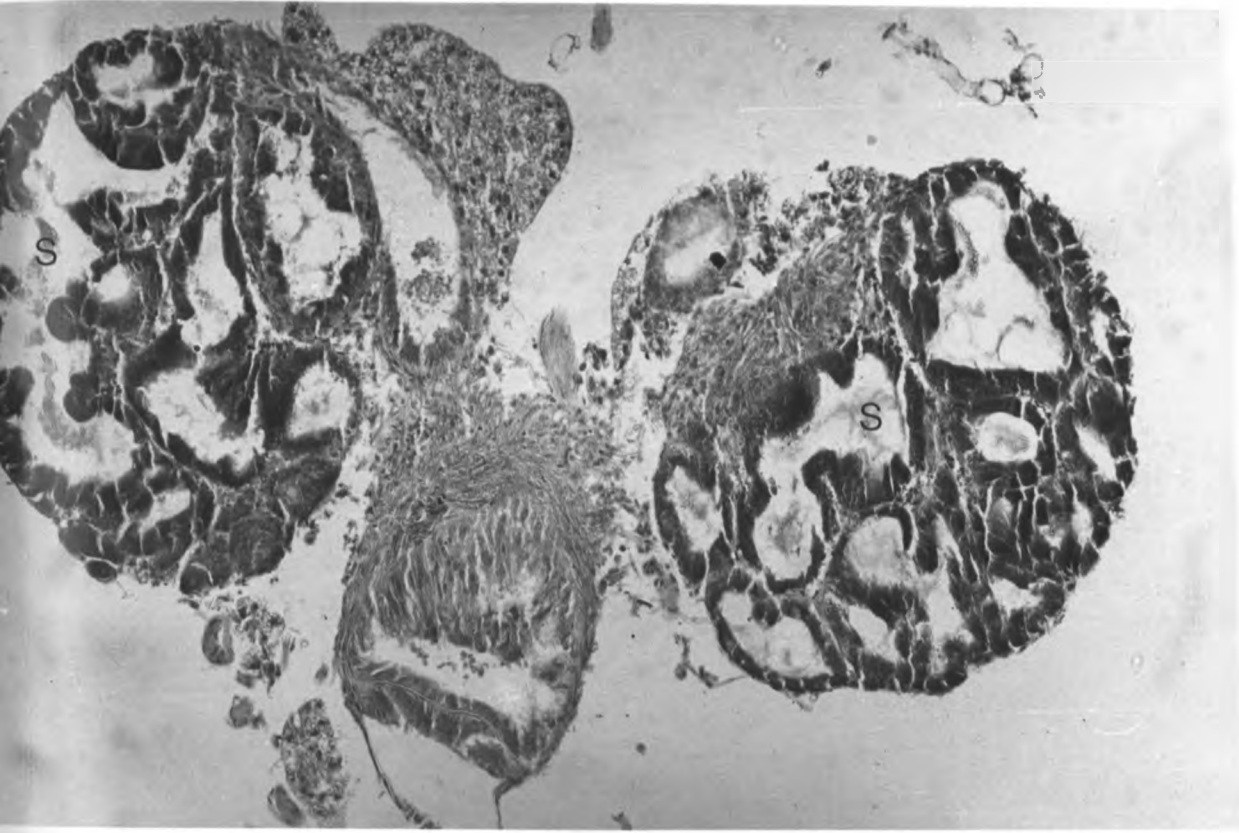
S = Secretion

Plate 10:

A photomicrograph of a longitudinal section of ARGs
from a 6 day old male D. fasciatus.
The size of lumina containing a fine granular secretion
has increased and that of the cuboidal epithelium
especially those of the peripheral sacs decreased.
The duct from the vas deferens is still empty.

(H and E stains x 160).

Plate 10
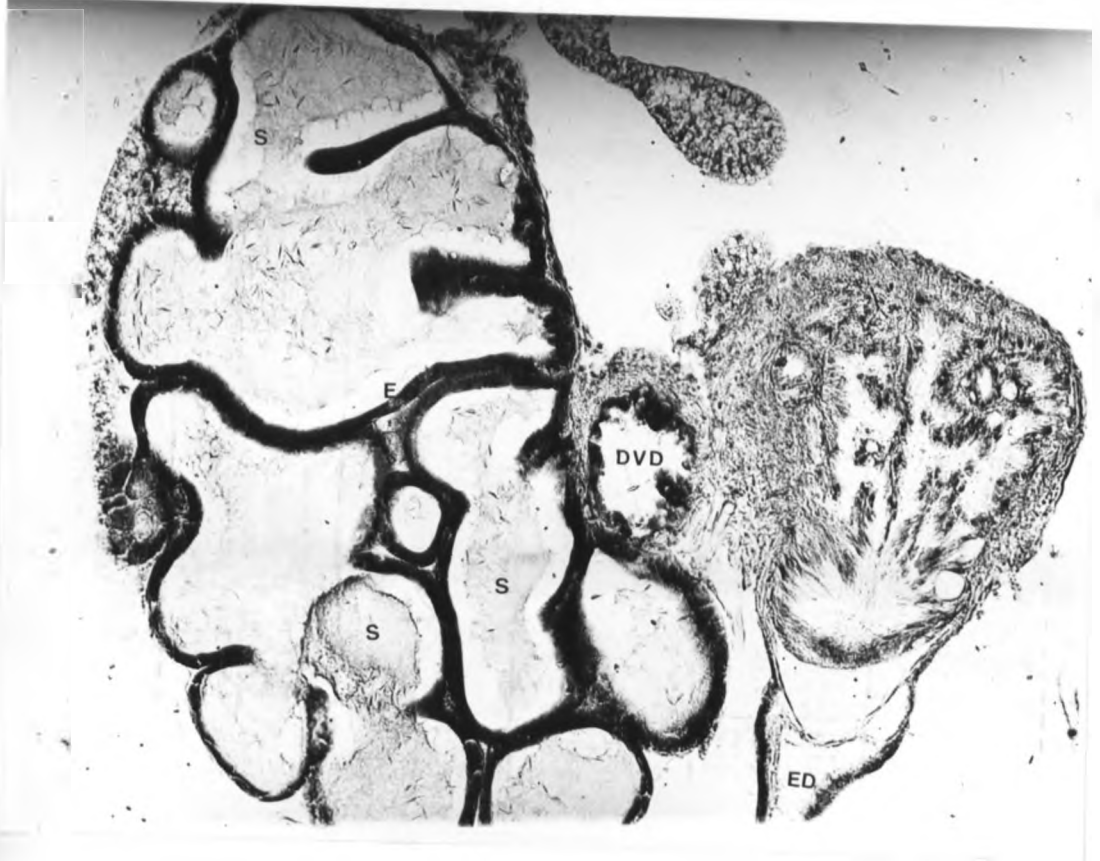
S   = Secretion

DVD = Duct from the vas deferens

ED = Ejaculatory duct

E   = Epithelium

Plate 11:

A photomicrograph of a longitudinal section through the
ejaculatory duct and ectadenes of a 7-day old male
insect in copulation. There is sperm in both the
Vas deferens (V.D.) and ejaculatory duct (ED).

(H and E stains x 160)

Plate 11
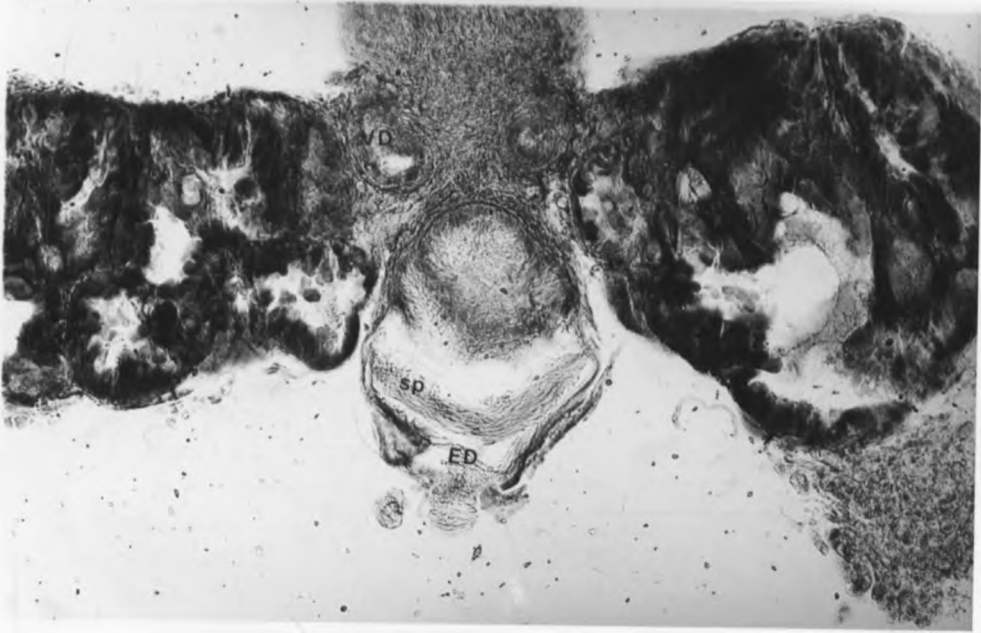
VD = Vas Deferens

ED = Ejaculatory duct

Sp = Sperm

Plate 12:

A photomicrograph of the longitudinal section through the ARG of a 9 day old male insect.

Note   the relatively thick epithelium and heavy accumulation of the granular secretion.

(H and E stains x 160)

Plate 12
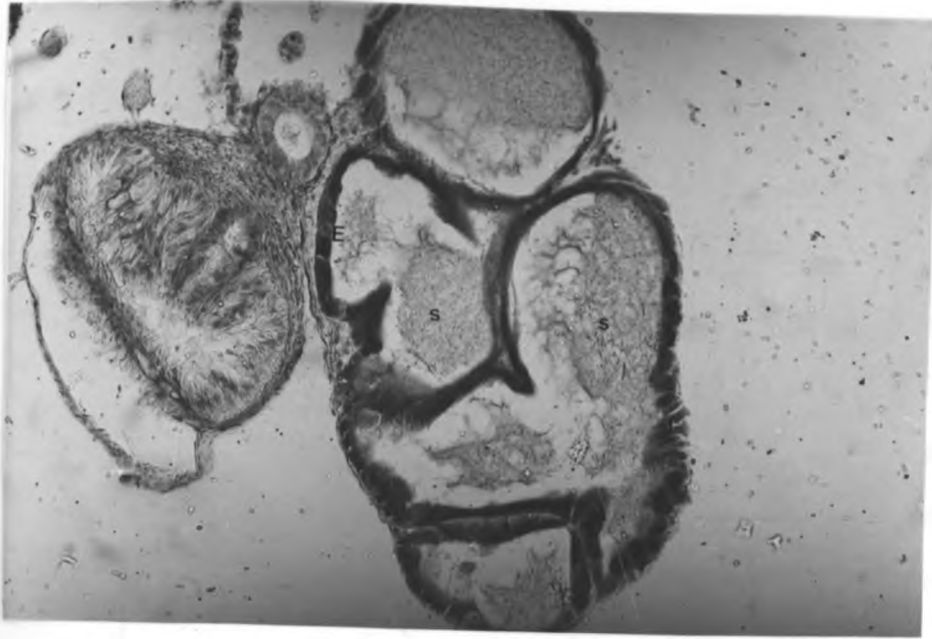
S = Secretion

E = Epithelium

3.3 The effects of Precocene II on the activities of the
     CA and ARGS

3.3.1 The effect of Precocene II on the activity of the CA

As a result of a series of trials, it was discovered
that the application of precocene was most effective in
disrupting the activity of the CA when it was applied soon
after adult-emergence. This was when the cuticle was still
soft thus probably enabling easy penetration of precocene.
The freshly emerged male D. fasciatus bugs were treated
topically with 1, 5, 10, 15, 20 and 25 μg Precocene II
dissolved in acetone. Control insects received acetone only.

Preliminary trials showed that a single application of
25 μg/1 μl dose of precocene on the day of adult emergence
killed all treated animals within the first three days. For
the purposes of this study the dose was considered lethal and
was discontinued. However, the application of 1-20 μg doses
were not lethal to the bugs although doses between 15-20 μg
caused some mortality in each application. In some cases a
few insects died. At other times many insects died and
sometimes none died.

Table 1 shows the percentage differences in the mean volumes
of the CA between the precocene treated insects and the
acetone controls following the application of various doses
of Precocene II. The table shows that the volume of the CA

varied with the dosage applied, 1 ug dose having the least effect and 20 μg dose having the greatest effect.   Topical applications of 1 μg/1 μl doses has no significant effect (t = - 1.58, P ≤ 0.05) on the size of the CA (Appendix V). But as shown in Table 1, the overall activity of the CA in the experimental insects was slowed down by a loss of 6.6% in the overall mean volume (Figure 4a).

With 5 μg doses of precocene II, significant reductions (t = - 6.25, P ≤ 0.05) in the volume of the CA was observed (Appendix VII) with a loss in the mean volume of the CA by 33.4% (Table 1). Although there was a small increase in volume during the first three days (Figure 5a) this dropped abruptly on the 4th day by 39% and remained fluctuating at this level till about the 9th day when it began to recover. The pattern of change amongst the control insects however compared well with the untreated insects.

On the other hand, precocene doses above 10 μg/1 μl seemed to have very potent effects on the CA causing consi-derable shrinkage in the size of the CA, which had a chara-cteristic imbalance in the size of the lobes - one appearing smaller than the other. This sometimes made volume measurements difficult. With 10 μgs, significant reductions in the volume of the CA were induced (t = - 8.07, P ≤ 0.05) (Appendix IX), and for the first time the pattern of volume changes started to differ from that previously observed with 1 and 5 μg doses

respectively. In these lower doses there was an initial increase in volume followed by an unusual fall. However, with the dose of 10 μg the volume of the CA started falling (Figure 6a) within the 1st day of treatment and the lowest fall was recorded on the 4th day. Later there was an apparent recovery in the activity but it remained lower than that at emergence up to the 10th day. As shown in Table 1, there was a loss of 35.6% in volume of the CA in the experimental insects compared to the control insects.

Precocene doses between 15 μg and 20 μg induced similar effects on the CA causing highly significant ($P \leq 0.05$) reduction in the size of the CA as shown in Figures 7a and 8a. In both cases precocene reduced the volume of the CA which did not show any activity at all. There was a decrease of 48.2% in the volume of the CA with 15 μg and 48.8% with 20 μg dose suggesting that 15 μg dose was optimal.

TABLE 1: Percentage differences in the mean volumes of the CA between precocene treated insects and acetone controls after the 10th day following the application of various doses of precocene II.

Precocene dose μg/1 μl	Mean volume of the CA mm^3 x 10^{-3}			
	Acetone controls	Precocene treated	Difference in volume	% difference in mean volume
1	5.69	5.31	- 0.38	- 6.6
5	5.68	3.78	- 1.90	- 33.4
10	5.47	3.52	- 1.95	- 35.6
15	5.31	2.75	- 2.56	- 48.2
20	5.59	2.86	- 2.73	- 48.8

-: A decrease in volume.

3.3.2 The effect of precocene II on the activity of
the ARGs

Following the application of precocene II, there was
a decrease in the volume of the ARGs in all the treated
insects. However, all these losses, in contrast to those
of the CA, were not statistically significant (P≤ 0.05) as
shown in Appendices VI, VIII, X, XII and XIV. Figures 4b,
5b, 6b, 7b and 8b show that whereas no significant differences
were found between the precocene treated insects and acetone
treated controls, there was a gradual decrease in the volume
of the ARGs of the various precocene treated insects. This
decrease in volume intensified with increasing concentration
of precocene. As shown in Table 2, the 15 µg and 20 µg
doses had the severest effects on the size of the ARGs,
inducing 23% and 22% reductions in the mean volumes
respectively. Low doses of precocene had minor effects on
the activity of the ARGs with 1 µg dose having the least
effect. One common feature observed amongst the precocene
treated insects was the poor development of one of the
pair of mesadenes. This feature interfered with linear
measurements of the two glands.

The pattern of activity of the ARGs was probably the
best measure of the effect of precocene on the ARGs.
During the first six days, the pattern of volume changes

of the ARGs in the precocene treated insects were similar
to that of the acetone controls except for their retarded
rates. From the 6th day however, there were profound
differences in the pattern of activity in the precocene
treated insects. Figure 4b shows that 1 µg does of
precocene resulted into peak activity on the 6th day.
In the control insects, this peak was noted on the 7th day
as was the case in the untreated insects (Figure 2).
Clearly, there is a disturbance that has interfered with the
normal secretory pattern.

With 5 µg doses, there was a further deviation in the
pattern of activity. There was a delayed release of
secretory materials till the 9th day (Figure 5b) when
a small quantity was released. With 10 µg doses the effect
of the antiallatotropin appeared to reach a climax. There was
a very gradual and slow increment in the glandular volume
which lacked the usual sharp increases shown in Figure 6b among
the control insects. Similar trends were observed with 15 µg
(Figure 7b) and 20 µg doses (Figure 8b) each showing a
retarded and irregular pattern of the glandular activity.

Since the control insects showed a normal trend of glandular
volume changes unlike the precocene treated insects, it
appears reasonable to assume that precocene II has some
effect on the ARG secretory activity. Since periodicity
of the ARG activity suggests hormonal influence it is then true to

suggest that the hormonal influence of the ARGs is being interfered with by the precocene. The observed poor development of one of the pair of mesadenes with higher doses of precocene is a proof of this hypothesis. The results also suggest that 15 µg dose is an optimal dose for inducing antiallatotropic effects on the male D. fasciatus. These observations indicate that the development of the ARGs is under the influence of hormonal factors released by the Corpora allata.

Table 2: Percentage changes in the mean volumes of the ARGs between precocene treated insects and acetone controls 10 days after the application of various doses of Precocene II

Precocene dose (μgs/1 μl)	Mean Volumes of the ARGs mm^3 x 10^{-3}			
	Acetone controls	Precocene treated	Differences in volume	% Differences in mean volume
1	21.15	18.80	- 2.34	- 11.0
5	20.90	17.30	- 3.60	- 17.2
10	19.39	15.87	- 3.52	- 18.1
15	19.05	14.51	- 4.54	- 23.8
20	19.11	14.87	- 4.24	- 22.1

Figure 4a:

Graph showing changes in the volume of the CA in the male
D. fasciatus topically treated with 1 µg/1 µl Precocene II
at adult emergence.

Note the small difference between the Precocene treated
insects and acetone treated controls.

Figure 4b:

Graph showing changes in volume of the ARGs in the male
D. fasciatus topically treated with 1 µg/1 µl Precocene II
at adult-emergence. There is a reduction in volume of
the ARGs of the Precocene treated insects compared with
the acetone treated insects.

Figure 4a.
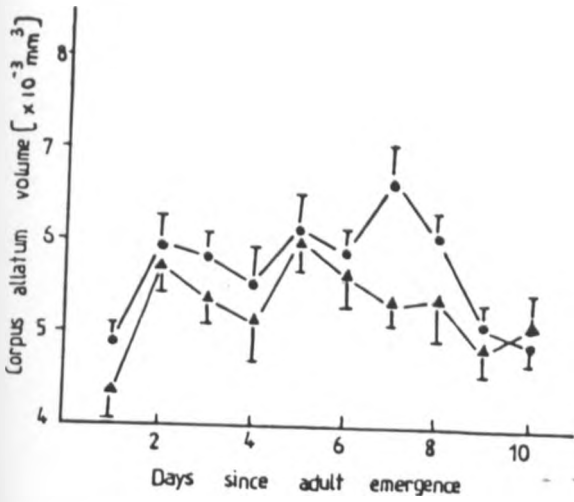
Figure 4b.
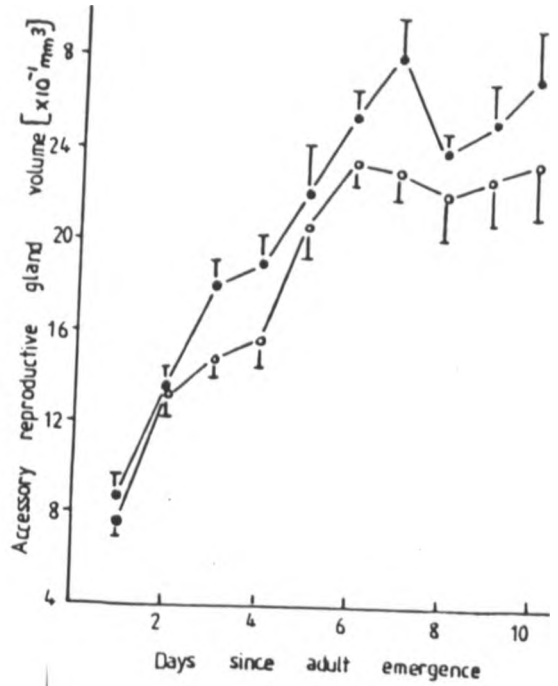
▲———▲Precocene treated insects

●———●Acetone treated control insects

○———○ Precocene treated insects

●———● Acetone treated control insects

Vertical bars represent SEM (Standard error of the mean).

Figure 5a

Graph showing changes in volume of the CA in the male
D. fasciatus treated with 5 µg/1 µl precocene II at
adult emergence. The Acetone treated control insects
show a normal trend while the precocene treated insects
show a comparatively low initial increase during the
first 3 days followed by a   drop in volume till the
10th day when signs of recovery are apparent.

Figure 5b

Graph showing changes in the volume of the ARGs in the
male D. fasciatus treated with 5 µg/1 µl precocene II
at adult-emergence. The reduced rate of growth of ARGs in
the precocene treated insects with respect to the control
insects is clearly shown.

Figure 5a.
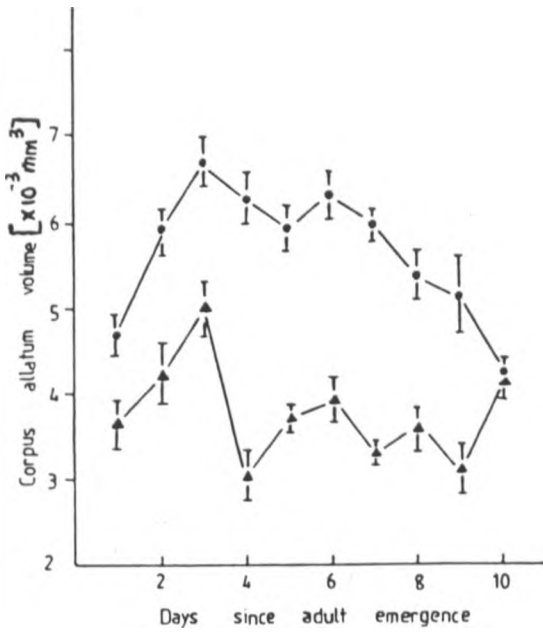
Figure 5b.

▲ ——▲ Precocene treated insects

● ——● Acetone treated control
　　　　insects
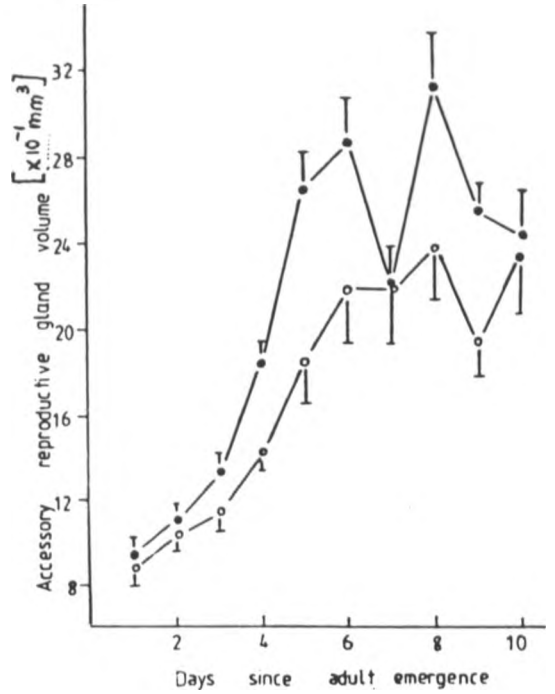
○ ——○ Precocene treated insects

● ——● Acetone treated control
　　　　insects

Vertical bars: SEM

Figure 6a

Graph showing changes in the volume of the CA in the
male D. fasciatus treated with 10 μg/1 μl precocene II.
The difference between the precocene treated insects
and acetone controls is clear. In precocene treated
insects there is an immediate reduction in volume of the
CA starting from the 1st day after treatment with no
signs of improvement. The trend of events is indicative of
atrophy of the gland.

Figure 6b

Graph showing changes in the volume of the ARGs in the
male D. fasciatus topically treated with 10 μg/1 μl
precocene II. Note the reduced and altered pattern of
the glandular development which is much reduced in
treated insect.

Figure 6a.
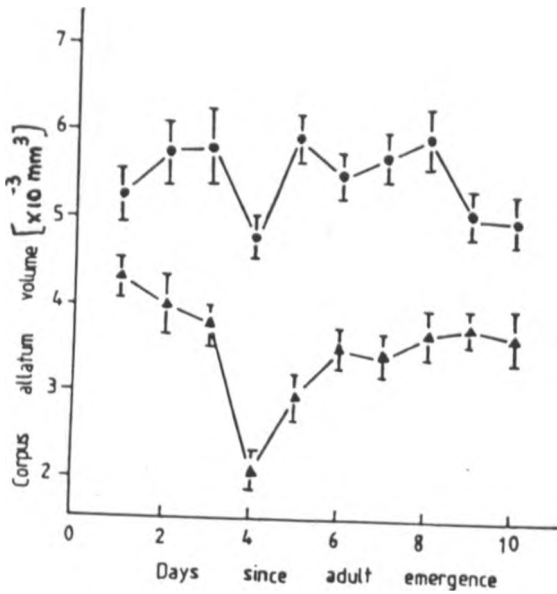
Figure 6b.
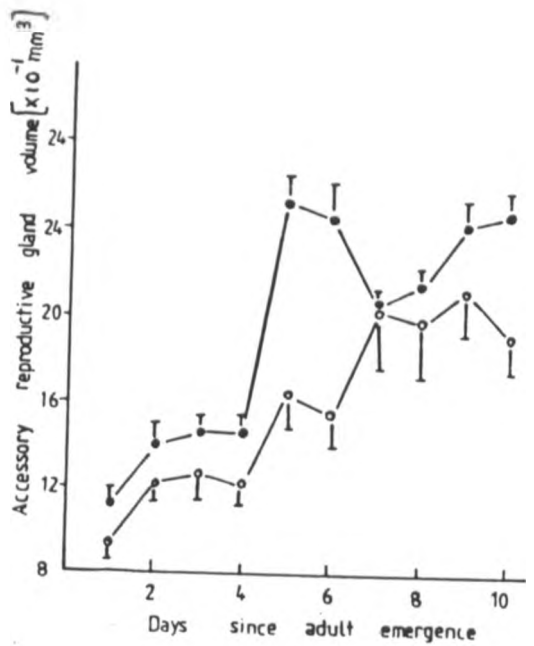
▲──▲Precocene treated insects

●──●Acetone treated control insects

●──●Acetone treated control insects

○──○Precocene treated insects

Vertical bars : SEM

Figure 7a

Graph showing changes in the volume of the CA in the
male D. fasciatus treated at adult emergence with
15 µg/1 µl precocene II.
Note that precocene reduced the volume of the CA rendering
it almost inactive.

Figure 7b

Graph showing changes in the volume of the ARGs in the male
D. fasciatus treated topically at adult emergence with
15 µg/1 µl precocene II. The rate of growth as well as
development deviates from that of the control insects.
They are reduced in treated insects.
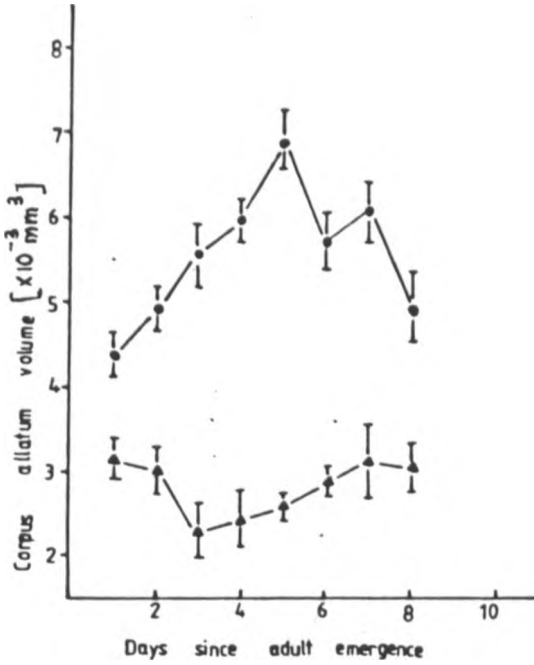
Figure 7a.   Figure 7b.
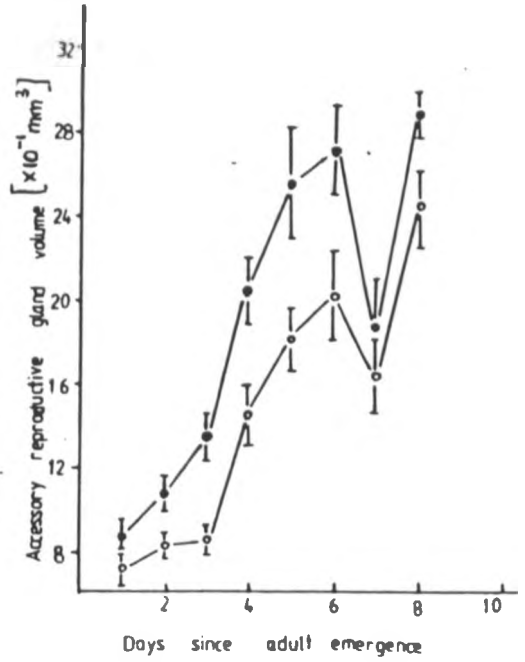
▲——▲ Precocene treated insects   o——o Precocene treated insects

●——● Acetone control insects   ●——● Acetone control insects

Vertical   bars :   SEM.

Figure 8a

Graph showing changes in the volume of the CA in the
male D. fasciatus treated with 20 µg/1 µl precocene II
at adult emergence. The precocene treated insects show
an instant reduction in volume which remains at a similar
level throughout the first copulatory cycle.

Plate 8b

Graph showing changes in the volume of the ARGs in the
male D. fasciatus treated topically at adult emergence
with 20 µg/1 µl precocene II. A retarded pattern of
development is shown in the precocene treated insects.

Figure 8a.
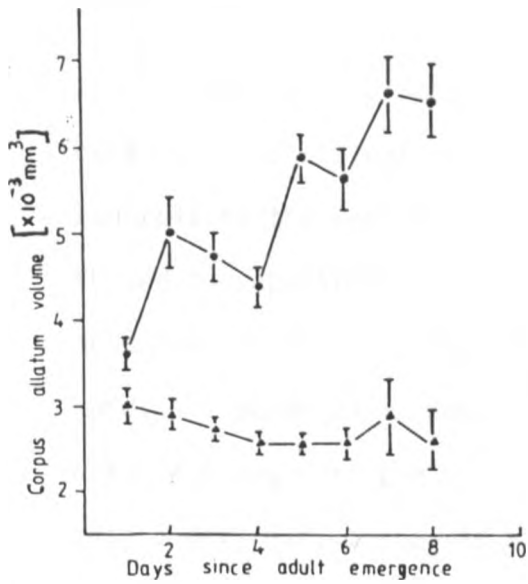
Figure 8b.

▲——▲ Precocene treated insects

●——● Acetone control insects
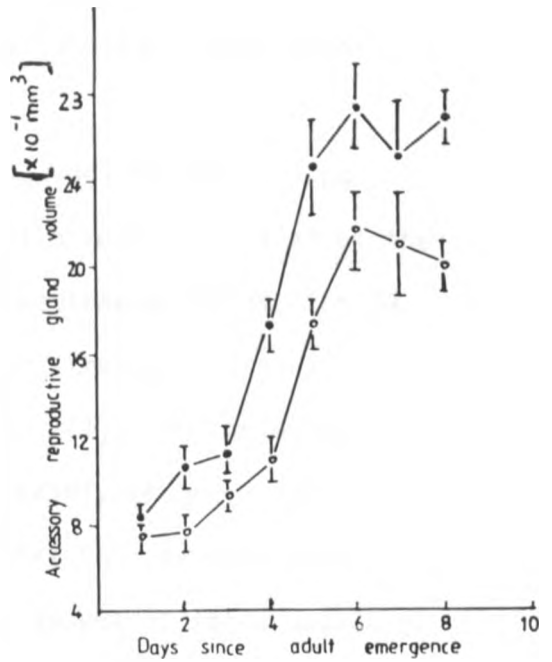
o——o Precocene treated insects

●——● Acetone control insects

Vertical bars : SEM.

3.3.3 The effect of precocene II on the cellular

structure of the CA

As revealed in the morphometric studies, histological
studies undertaken after the application of precocene II
showed that the effects of the antiallatotropin were graded.

The histology of the CA after the application of 1 µg
dose of precocene was not modified with respect to that of the
controls on the same day. Thus in the treated males, the CA
showed the appearance of the cellular structure already
described in the non-treated males (3.2.1). On the other
hand, precocene doses above 5 µg certainly induced some
notable changes in the histology of the CA. In bugs less
than 5 days old, there was a reduced amount of intranuclear
granules and cytoplasm in the gland. In older bugs, the
cells within the gland looked shrunken with densely crowded
nuclei (Plate 13) indicating a failure by the gland to
secrete more cytoplasmic material.

With higher doses of precocene, these effects were more
pronounced. For example in one day old insects treated
with 15 µg/1 µl, there was a wide spread formation of dark
bodies (Plate 14) of various sizes and shapes. In such glands,
the nuclei of the cells could harldy be seen and the remains
of what used to be radial lines could be seen running only short
distances without any access to the periphery of the gland.

This indicated that the exit of secretory materials from
the gland has been blocked. In older insects the radial
lines in the gland had disappeared altogether (Plate 15)
and the gland showed extensive coalescence of cytoplasmic
material with an apparent rupture of the peripheral
cells lining the gland. This was an indication of
the degenerative changes taking place in the gland.

Plate 13:

A photomicrograph of a longitudinal section of the CA
of a five day old male D.fasciatus treated with 5 µg/l µl
precocene II at adult emergence.

The nuclei of the CA cells contain no intranuclear
granules and the individual nuclei tend to be crowded
with little evidence of accumulated cytoplasm.

(PFH   stain   x   400).

Plate   13
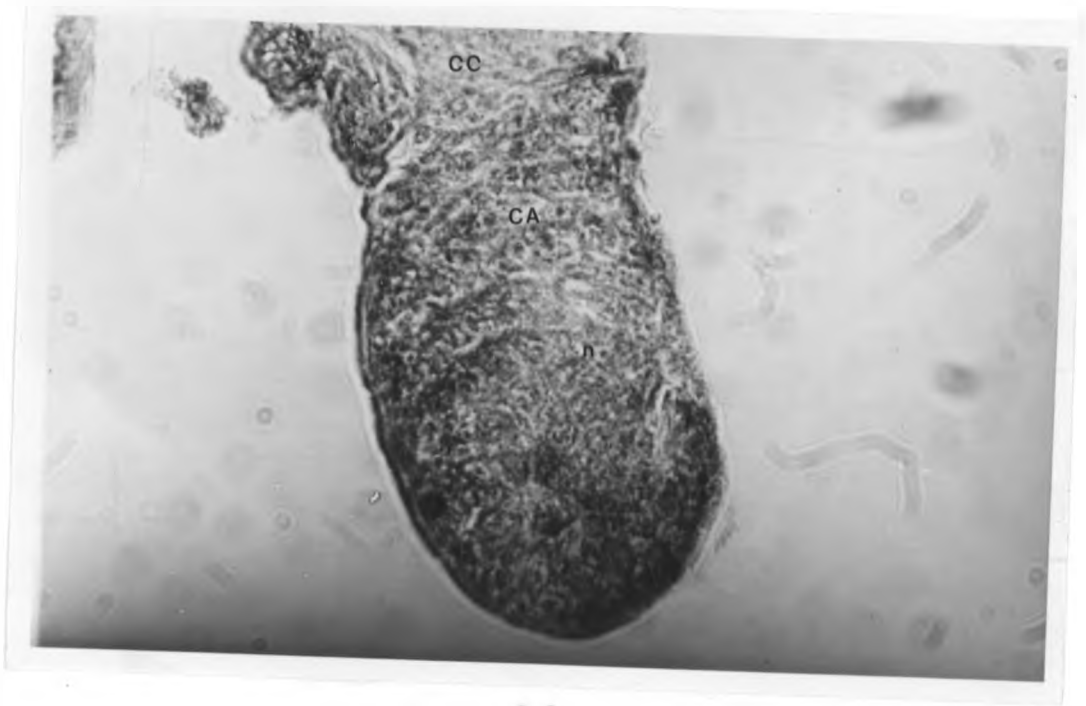
CA   = Corpus allatum

CC   = Corpus cardiacum

n   = Nucleus

Plate 14:

A photomicrograph of a transverse section of the CA in the
3 day old male D. fasciatus topically treated with 15 μg/1 μl
Precocene II. There is widespread formation of dark-
bodies of various sizes and shapes. The nuclei are hardly
visible and the remains of what used to be radial lines
can be seen running short distances with no apparent
access to the periphery.

(PFH stain x 400).

Plate 14
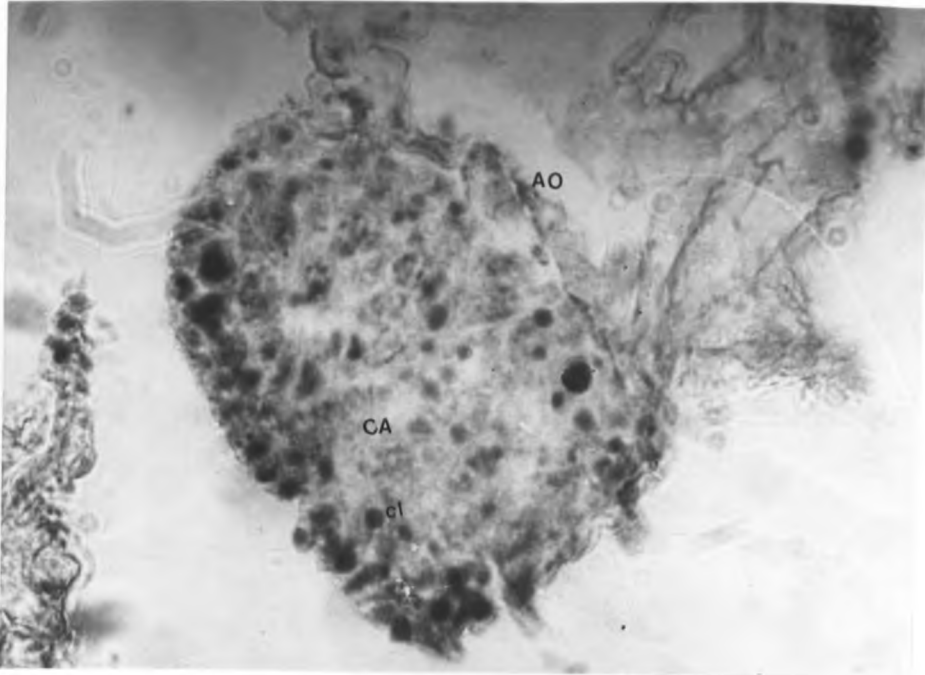
Cl = Clumped cytoplasmic contents

CA = Corpus allatum

AO = Aorta

Plate 15:

A photomicrograph of a longitudinal section of the CA
of a 5-day old insect treated with 15 µg/1 µl precocene
II. Here the radial lines in the gland have disappeared
and the gland shows extensive coalescence of cytoplasmic
material into dark staining bodies.
Note also that the peripheral cells of the CA are ruptured.

(PFH x 1000).

Plate 15
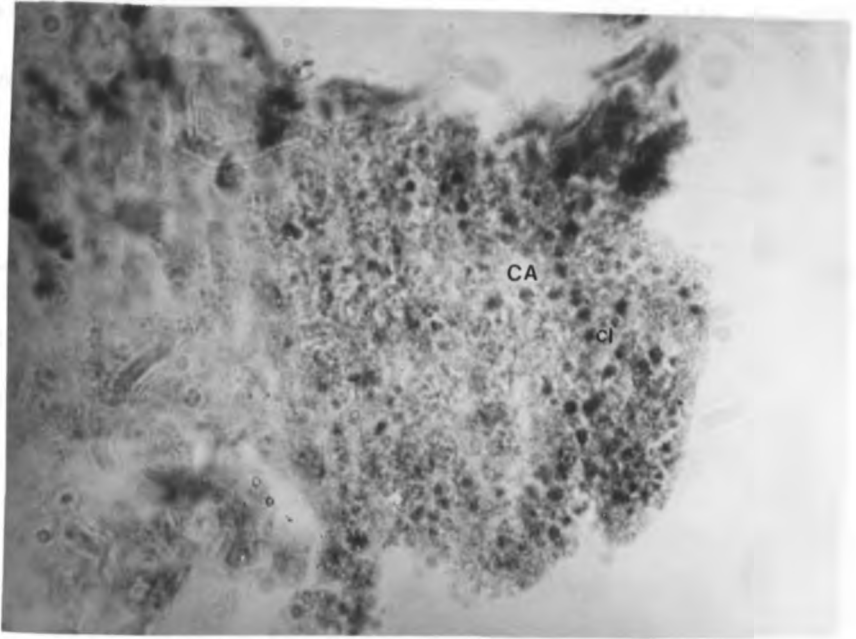
CA = Corpus allatum - ·

CI = Clumped cytoplasmic contents

3.3.4 <u>The effect of Precocene II on the cellular</u>
       <u>structure of the ARGs</u>

The histology of the ARGs following the application of
various doses of precocene II corraborated with the retarded
development of the glands already described morphometrically.

With 1 µg and 5 µg doses of precocene the cellular
structure of the ARGs appeared normal in the majority of
sections examined during the first three days after adult
emergence (Plate 16). In the six day old insects the effect of
the precocene on the ARG became more pronounced. In most
sections there was an imbalance in the development of the
mesadenia with one of the pair of   glands appearing less
developed than the other (Plate 17 and 19) and snowing smaller
mesadenial sacs. In these sacs the secretion was faintly
stained suggesting that its chemical composition could have
been altered. In some sections there was abnormally little
secretion in the lumina of the mesadenial sacs. In such
sections the secretion was localised towards the edges of
the luminal sacs close to the epithelia (Plate 18).   In
these sections it was also common to find empty mesadenial
sacs.

Precocene doses of 10, 15 and 20 µgs seemed to have
had a greater effect on the cellular structure of the ARGs.
There was an obvious poor development of the mesadenial
pockets of one of the pair of the glands which showed a complete

contraction of the mesadenial pockets (Plate 19) containing no secretion. The epithelial lining of these glands appeared very thin and their nuclei could hardly be traced(Plate 20). The basement membrane too had become extremely thin and broken in many places. With 20 µg doses, the mesadenial pockets appeared more irregular with peripheral sacs looking smaller than the interior sacs. In some of them the epithelial lining appeared folded and lacked the rounded structure common in the normal mesadenes (Plate 21). These observations suggest that the secretory activity of the ARGs are controlled by a factor mediated by the CA.

Plate 16:

A longitudinal photomicrograph of the ARG from a two
day old male insect treated with 1 μg/1 μl precocene II
The gland is secreting normally and shows no signs of
retarded development.

(H and E x 160).

Plate 16

S = Secretion.

E = Epithelium

Plate 17:

A photomicrograph of a longitudinal section of a six
day old male D. fasciatus treated topically with
1 μg/1 μl precocene II at adult emergence.

The mesadenial sacs in one gland are small than the
other and the secretion in them is faintly staining.

(H and E stain x 160).

Plate 17
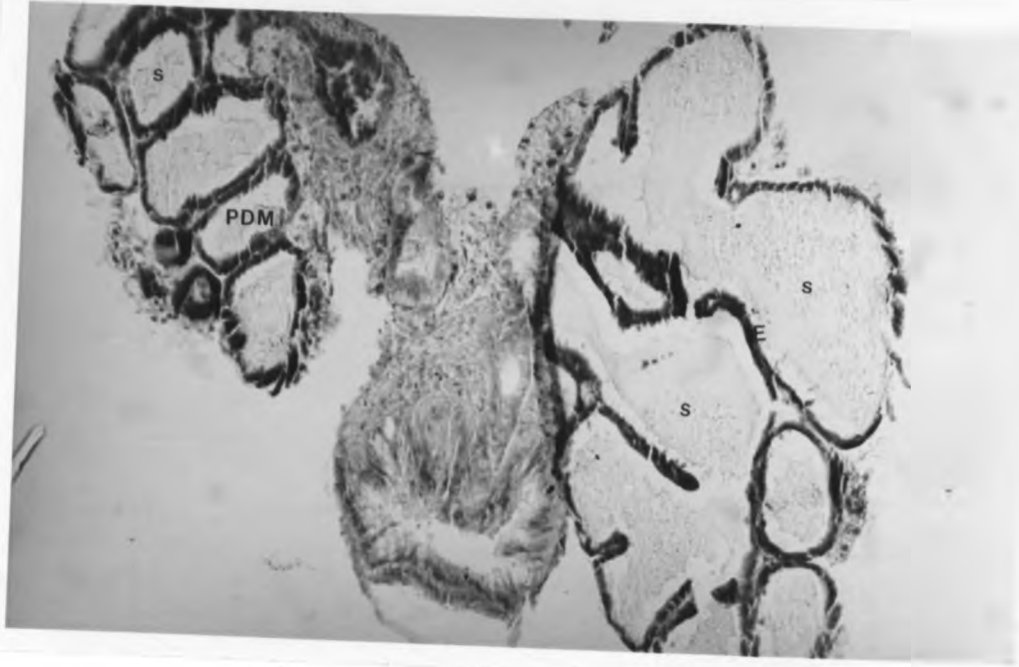
PDM = Poorly developed mesadenial sacs

S   = Secretion

E   = Epithelium

Plate 18:

A photomicrograph of a longitudinal section of the mesadenes of a four day old male D. fasciatus treated topically with 5 µg/1 µl Precocene II at adult emergence.

Note the abnormally little secretion in the lumena. Some sacs contain no secretion at all.

(H and E stain x 160)

Plate 18
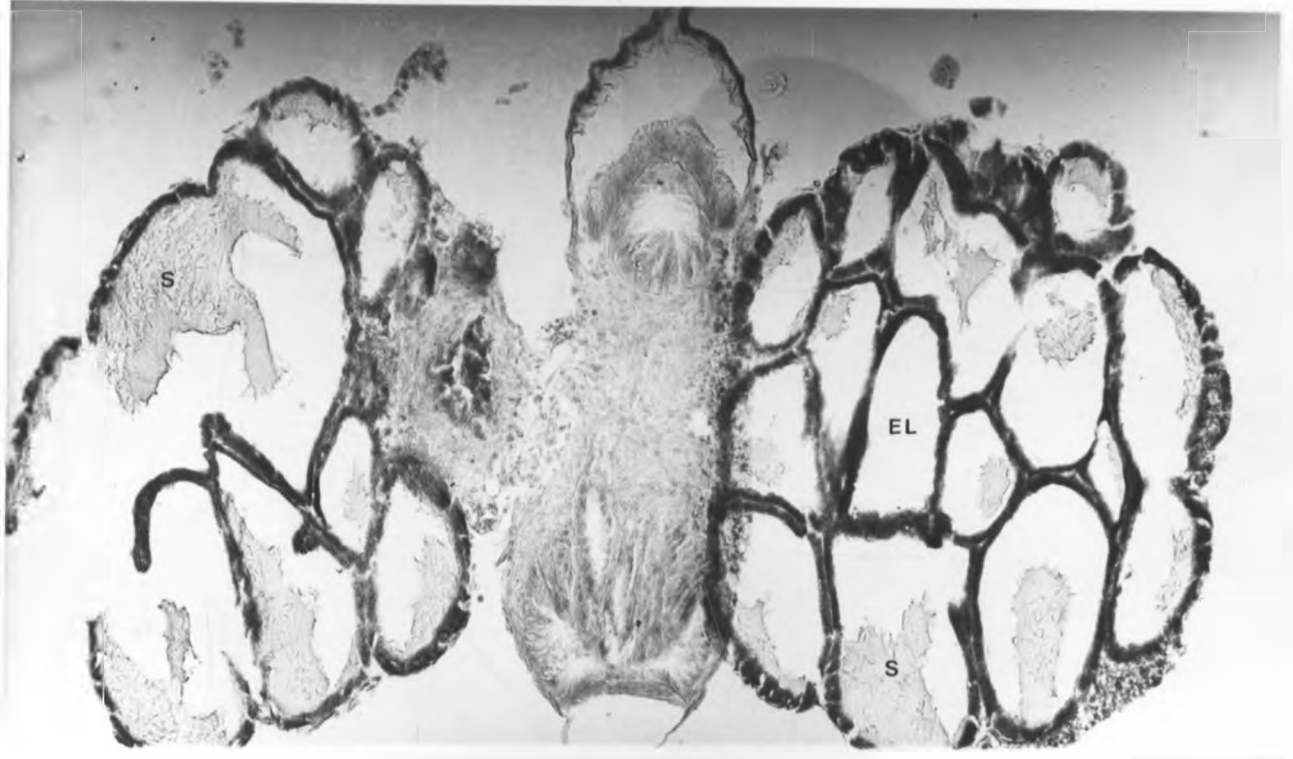
EL = Empty lumen

S   = Secretion

Plate 19:

A photomicrograph of a longitudinal section of the mesadenes
of a three day old male D. fasciatus treated topically
with 10 µg/1 µl Precocene II at adult-emergence.
One of the pair of mesadenes is totally degenerated showing
no secretion in the sacs. The other pair seems to be well
developed but its secretion is still poorly stained.

Note absence of sperm in the vasdeferens.

(H and E stains x 160).

Plate   19
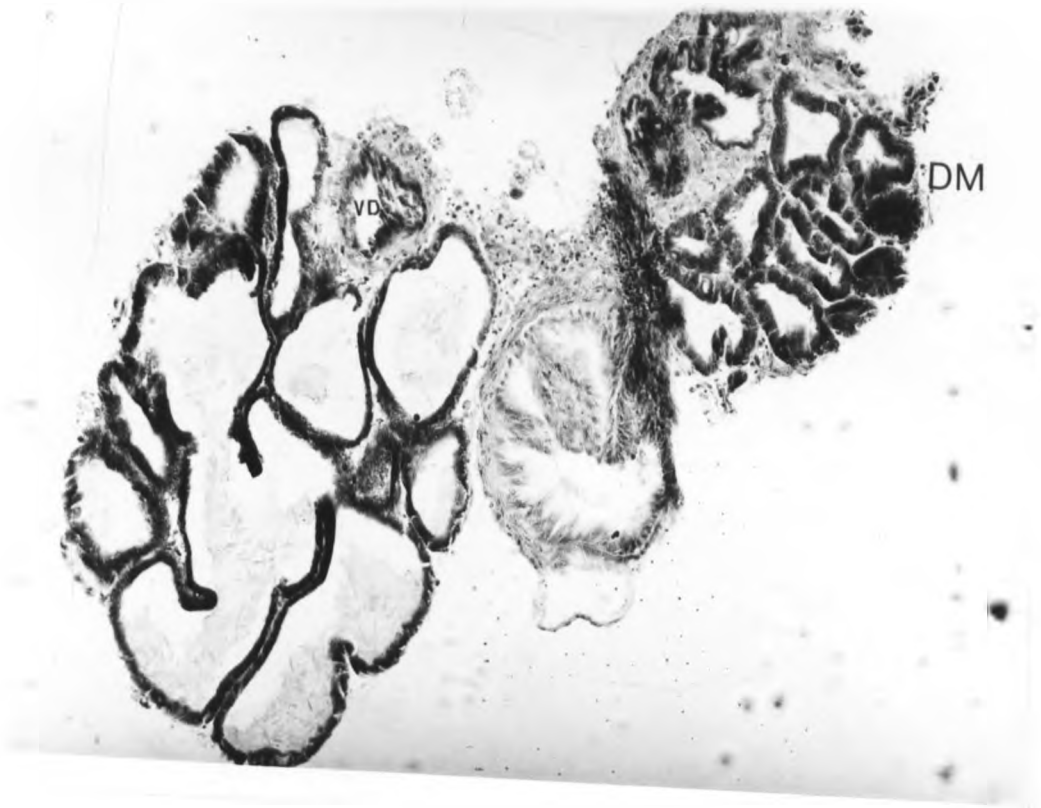
VD = Vas deferens

DM = Degenerated mesadenes

Plate 20:

A photomicrograph of a longitudinal section of the
mesadenes of a five day old insect treated topically
with 15 µg/1 µl precocene II at adult emergence.

The epithelial lining of the mesadenes are extremely
thin containing a poorly stained secretion. There is
poor development of one of the pair of the glands.

(H and E stains x 160).

Plate 20
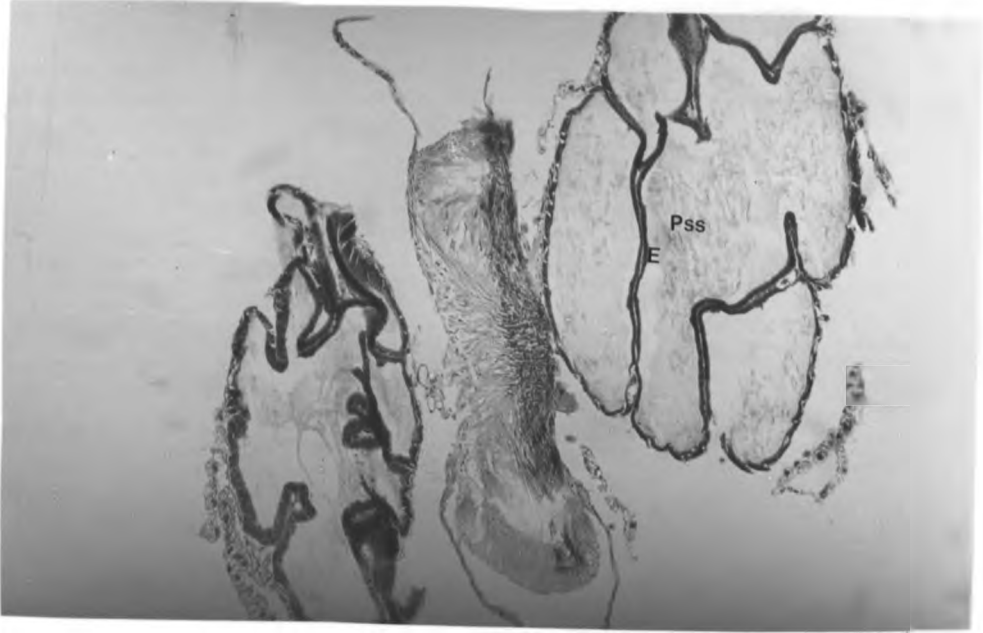
E   = Epithelium

Pss = Poorly stained secretion

Plate 21:

A photomicrograph of longitudinal section of the
masedenes of a six day old male D. fasciatus treated
topically with 20 µg/1 µl precocene II after adult
emergence. All epithelia are showing folds with parts
of the gland coalesced. The secretion has poor
staining effect in this case.

Plate 21
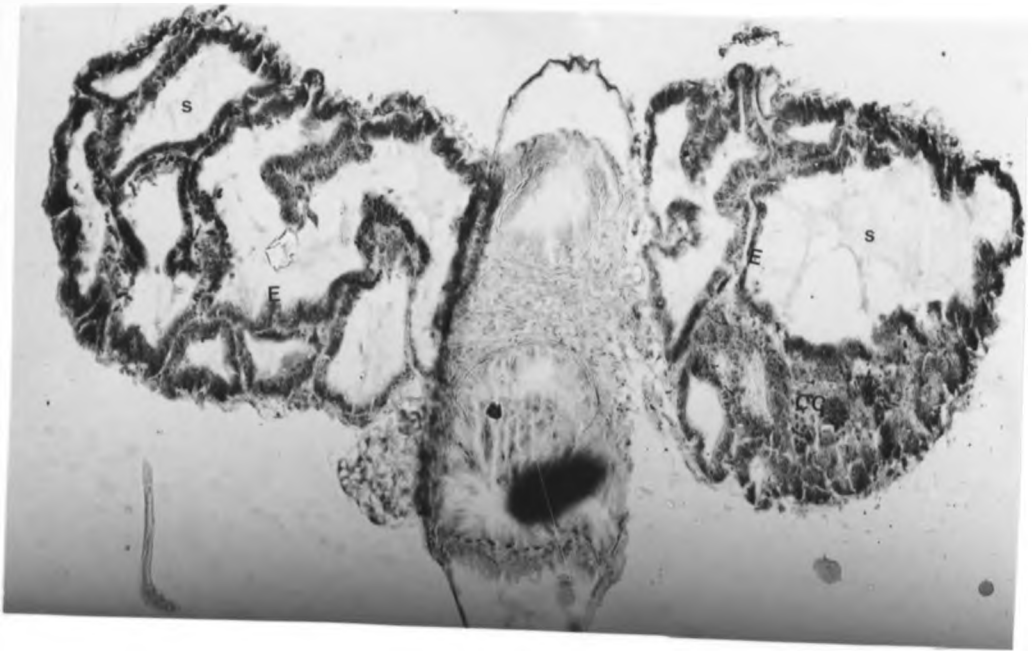
S = Secretion

CG = Coalesced parts of the gland

E = Epithelium

3.4 Effect of Precocene II on sperm descent in D. fasciatus

In this study, it was observed that in the unpaired male
D. fasciatus, sperm descent from the testes into seminal
vesicles takes place on the 3rd day after adult-emergence.
A complementary study of this phenomenon using precocene II
was undertaken to get an insight into the factors responsible
for the regulation of sperm translocation.

The results showed that the normal trend cited above
were interfered with. The extent of this interference varied
in degree depending on the dosage and time of application of the
antiallatotropin. There was a delay in sperm descent until the
4th day when 1 μg doses were applied. Even so the rate was so
slow that upto the 7th day the seminal vesicles were not as
full as those in the control insects. With 5 μg doses sperm
descent was delayed upto the 7th day and by the 10th day the
seminal vesicles had little sperm. In some insects it was
common to see sperm in one seminal vesicle and the other
completely empty. Precocene doses of 10, 15 and 20 μg applied
soon after adult emergence prevented sperm descent (Appendix XV)
and altered the shape of the testes from cylindrical to spherical
with their proximal ends having a very thin neck joining them
to the seminal vesicles.

Time of application of the precocene was a very
critical factor. Every effort was therefore made to apply
the precocene soon after adult-emergence and the results
obtained were satisfactory. Insects which were about five

hours old gave very inconsistent results. In some of them, there was sperm descent even with higher concentrations thus indicating the significance of time of application on the action of precocene II. This study did not establish whether this was due to the inactivity of the CA or easy cuticular penetration by precocene at the time of adult emergence.

The failure of sperm to descend from the testes to the seminal vesicles with high doses of precocene suggested that this compound induced sterility in this insect.

3.5   The effect of ARG homogenates on mating behaviour
      and sperm descent

Table 3 shows the effects of the ARG homogenates on
mating behaviour. When ARG crude homogenates from sexually
mature male insects were injected into the newly emerged
male insects, there was an alteration in the normal mating
behaviour.

It was observed that as early as the 1st day 6% of the
insects which had received crude homogenates of the ARG had
enetered into copula and none of the control insects which
were injected with Dysdercus Ringers solution had done so.
On the 2nd day mating was observed in both the experimental
as well as the control insects. However, the experimental
insects showed a higher percentage of mating couples (23.0%)
compared with the control insects which showed only 17%.
On the 3rd day the percentage of male insects in copula in the
experimental insects had gone up to 64% while that of the
control insects was 60%. On the 4th day a majority of
insects in both groups were in copulation but those injected
with the crude homogenates still showed a higher percentage
of copulating couples.

A visual inspection of sperm descent from the testes
into the seminal vesicles during the first two days after
adult-emergence agrees with the above findings. As early as
the 1st day, some of the insects that received the glandular

TABLE 3 :   Percentage comparison of the effect of ARG homogenates
on mating behaviour.

Days after adult-emergence	Percentage of experimental bugs in copulation	Percentage of control bugs in copulation
0	0	0
1	6	0
2	23	17
3	64	61
4	91	80

homogenates showed sperm descent in their seminal vesicles.
A similar random dissection of a similar number of control
insects failed to show any evidence of sperm descent on the
1st day. On the 2nd day, more male bugs among those that had
received the crude homogenates showed a more expanded seminal
vesicle indicating descent of sperm. The control insects on
the other hand, showed no sperm descent at all even when the
seminal vesicles were teased in Dysdercus Ringer. On the
3rd day the control insects started to show sperm descent.

The observed early tendency to mate combined with the
earlier descent of sperm seems to indicate that there is a
factor within the ARGs that induces early mating behaviour
and sperm-descent in otherwise sexually immature insects.

CHAPTER   FOUR

DISCUSSION   AND   CONCLUSIONS

4.1 The activity of the <u>Corpora allata</u> (CA) in the male

<u>D</u>. <u>fasciatus</u> during sexual maturation

During postembryonic development of the adult pterygote
insects, the thoracic glands disappear during or soon after
the last moult. Consequently, of the organised insect
endocrine system, only the cerebral neurosecretory system
and the <u>Corpora allata</u> (CA) remain as the only possible
sources of hormones   controlling reproductive development.
Early investigators such as Nayar (1958), Strangways-Dixon
(1961), Engelman (1957), Scharrer and Von Harnack (1958)
and Ganagarajah (1965) have studied in detail the activities
of the CA of various female insects and have reported the
presence of cyclic changes of the volume of the CA during
the gonotrophic cycles. They observed an increase in the
volume of the CA during the initial phase of yolk deposition
and a decrease in its volume as the oocytes grew, reaching
a minimum value when the eggs were fully developed. They
concluded that the CA hormone was necessary for egg development.
Inspite of all these findings, detailed studies of a similar
kind in male insects are still scanty and have not been
studied fully.

During investigations reported here, the activity of
the CA in the male <u>D</u>. <u>fasciatus</u> showed a cyclical secretory

pattern with distinctive phases of high and low glandular output. This pattern correlated closely with the reproductive changes taking place in the insect. For example, the periods of high activity coincided with the onset of mating while periods of low activity coincided with the intermating periods. It was also revealed that, the paired male insects showed smaller glandular volumes than those of the virgin ones. This suggested that the contents of the CA were instrumental in the reproductive activity of the insect. These contents appeared to sustain the insect during the prolonged mating activity.

Cyclical changes of the secretory capacity of the CA have been worked out in a number of female species. (Reviews by Highnam, 1964 and Wigglesworth, 1964) and this has been used as a measure of hormonal activity of the gland. On the otherhand, in the male insects, no cyclical changes in the size of the CA have been observed (Johansson, 1958; Scharrer and Von Hannack 1958; Odhiambo, 1966). These authors even dismiss its possibility although Odhiambo (1966) cautions that there is a great need for experimental confirmation of this trend in the male insects. Thus compared to other insects the observed cyclical pattern of glandular activity in the male D. fasciatus is unique. This could be reflecting the peculiar mating behaviour of this insect which is also unique among insects.

4.2 The activity of the Accessory reproductive glands
(ARGs) in the male D. fasciatus during sexual
maturation

In her studies on the structure of the male reproductive
system and spermatogenesis in the cottonstainer D. fasciatus
Awiti (1976) gave an account of the development of the ARGs
in the nymphal stages of the insect. She showed that the
mesadenia in this insect arose from the posterior bulbous
end of the vas-deferens and differentiation of the gland
commenced in the 4th instar and was completed in the 5th
instar. It was also observed that the secretory activity
of the cells of the mesadenia started towards the end of
5th instar so that at adult emergence, the luminal contents
were still scanty but secretion increased with the age of
the bug. This study however did not demonstrate the daily
changes in the volume of the glands although it was
demonstrated that the time of active secretion of the
mesadenia coincided with the time of mating.

The morphometric as well as the histological investigations
obtained during the present study show that the ARGs in the
male D. fasciatus exhibit a cyclical secretory pattern which
like that of the CA was closely correlated with the
reproductive behaviour of the insect. From the time of
adult emergence, there was a steady increase in the volume of
the ARGs in both the virgin and mated insects.   This

reached a climax after about seven days but four days later, the volume of the glands dropped gradually to a very low level and this coincided with the intermating period. In the virgin male insects where the study was carried up to the 20th day, a similar trend of activity was repeated although higher glandular mean volumes than those of the mated insects were observed. This difference in volume although not statistically significant suggested that during mating a remarkable depletion and utilization of the glandular secretion took place. The cyclical secretory pattern of the ARGs in the male insects has not been reported before. This is not surprising since mating lasts for very brief moments in most of them. The long copulatory periods shown in the male D. fasciatus therefore seem to require that these glands build up a certain level of secretion required for sperm transfer during this period and hence the cyclical release pattern.

4.3 Correlation between the activity of the CA and ARGs in the male D. fasciatus during sexual maturation

The CA of adult insects has been reported to secrete a hormone which influences the reproductive performance of the insect species (Manning, 1966; Gillot and Friedel, 1976a; Engelmann, 1970; Chaudhury et al., 1981). This gonadotropic function is more clearly defined in the female than in the male insects (Nayar, 1973). Thus in

many female insects, the CA has been shown to control
the development and secretory activity of the ARGs
(Wigglesworth, 1936, Weed, 1936; Johansson, 1954; 1958;
Scharrer, 1958; Bell, 1969; Engelmann, 1970; Pratt and
Davey, 1972; Willis, 1974; Chippendale, 1970). It has
been shown that after allatectomy the ARGs are small
and thin and increase in volume only if the CA are
subsequently reimplanted. However, in the male insects,
the regulation of the ARGs by the CA is still poorly
understood. Nevertheless in some male insects studied so
far, the growth and evelopment of the ARGs is controlled
by the CA hormone in a manner similar to that of the
female insects (Highnam and Hill, 1969, Nayar, 1973).
Thus the CA involvement in the development of the male
ARG has already been reported in R. prolixus (Wigglesworth,
1936) Melanoplus (Weed Pfeiffer, 1936), Leucophae
(Scharrer, 1946) Schistocerca (Cantacuzene, 1967,
Odhiambo, 1966, 1971) and L. decemlineata (DeLoof and
Lagasse, 1972). This is, however, not true in O.
fasciatus (Johansson, 1958) which is closely related
to D. fasciatus.

The distinctive cyclical secretory activities of the
CA and ARGs observed during this study showed synchronised
peaks of activity occuring at a time when the insect
was engaged in active copulation. There were similar
synchronised periods of low activity coinciding with the
intermating period in the insect. These cyclical activities

of the CA and ARGs were however slightly out of phase
with each other with the peaks and low periods of
activities of the ARGs coming one to two days after
that of the CA. This is the first time such activities
have been reported in the male insect. This observation
suggests a functional relationship between these glands.
It suggests that there is a stimulating effect by the
materials of the CA to which the ARGs respond. This
was later confirmed by the application of precocene II
which reduced the sizes of both glands indicating that
both organs were being affected either directly or
indirectly leading to poor growth and function. Thus
these observations suggest that in the male D. fasciatus
the CA is involved in the regulation of the ARGs just
like in those insects where this function has been
confirmed.

4.4 Allatotoxic effects of precocene II and its
     consequences on the activity of the ARGs

Precocene has been reported to induce symptoms
of juvenile hormone deficiency in many insect species
especially in Hemiptera (Bowers et al., 1976) and
Orthoptera (Pener and Orshan, 1977). These compounds
have also been reported to possess antigonadotropic
activity in Oncopeltus fasciatus and other species of
Heteroptera, Coleoptera, (Bowers 1976; Bowers et al.,
1976), and recently in a Thysanura Thermobia domestica
(Collette and Jacques, 1984).

These effects result from a cytotoxic action on the
CA which inhibits the biosynthesis of the juvenile hormone.
During studies reported here, precocene II was found to
have considerable effects on the developments and activities
of both the CA and ARGs, and these effects were dependent
on the concentrations used. Thus, concentrations below
5 μg/1 μl precocene II had no effects on the two glands.
On the other hand, precocene doses between 5 μg and 20 μg
induced statistically significant (P ≤ 0.05) reductions in
the volume of the CA and a dose of 25 μg per male was lethal.
Reduction in volume resulted from the destruction of the
cytoplasmic contents of the CA cells. There was a wide-
spread coalescence of parts of the cytoplasm which formed
dark bodies. No cell or nuclear membranes could be
visualised in these glands suggesting that degenerative
changes (atrophy) had taken place.

Allatotoxic effects of precocene II have been
reported in various species of Exopterygota such as
Oncopeltus fasciatus (Bowers et al., 1976; Unnithan
et al. 1977) Locusta migratoria (Schooneveld, 1979a,b)
Schistocerca gregaria (Unnithan et al., 1980) and in
Diploptera punctata (Feyereinsen et al., 1981). There is
still, however, a controversy about the time when
precocene is most effective on the CA. Thus in O. fasciatus
precocene is reported to be only effective in the actively
synthesizing CA (Unnithan and Nair, 1979; Masner et al.,
1979) while in Diploptera punctata, precocene is effective

both in the active and inactive glands (Feyereinsen
et al., 1981a). During the present study, precocene II
was found to be allatotoxic when applied two hours
after adult emergence when wings were not fully developed
and expanded. On the other hand, insects treated after
about five hours following adult emergence did not seem
to respond to the precocene. This investigation did
not, however, establish precisely whether the effectiveness
of precocene in less than five hours after adult emergence
was due to the inactivity of the CA or easy cuticular
penetration of precocene at this stage. On the other
hand, if size is considered to be an indicator of activity
then the Corpus allatum of those insects that were less
than five hours old were inactive. Thus, results from
this study indicate that precocene is allatotoxic to
the non-actively synthesizing CA.

The application of precocene II to the newly
emerged male insects did not have any significant
reductions (P $\leq$ 0.05) on the volume of the ARGs
compared with the control insects which received acetone
only. However, the cyclical secretory activity of the
glands was seriously disturbed resulting in a slow and
delayed volumetric changes. There was also reduced
growth of the mesadenes in the majority of the precocene
treated insects. This was a clear indication that
precocene was retarding the ARG development and possibly

altering their chemical composition. These observations

suggested functional relationships implying that the

ARGs were under the control of factors released by the

CA. These observations are in conformity with previous

observations by other researchers (Wigglesworth, 1936;

WeedPfeiffer, 1936; Scharrer 1946, Cantacuzene 1967)

who using allatectomy demonstrated that the ARG secretory

activity was controlled by the CA.

4.5 The effect of precocene II on sexual maturation

in the male D. fasciatus

Precocene II has been demonstrated to induce

sterility in many female insects (Bowers and Martinez-Pardo,

1977; Masner et al., 1979; Landers and Happ 1980;

Samaranayaka-Ramasamy and Chaudhury, 1982). In the male

insects little has been reported on the regulation of

sexual maturation. Thus in the male Glossina morsitans

morsitans the application of precocene II by Samaranayaka

and Chaudhury (1982) failed to impair the mating and

insemination ability. According to these authors, this

trend appeared to be a normal case since as previously

reported, sexual maturation in most male insects is

independent of control by the CA. However, in the

male D. fasciatus studies by Awiti (1976) demonstrated

that at the time of adult-emergence, males, of the

Dysdercus species were not sexually mature since sperm

had not descended from the testes into the seminal

vesicles. Mating occured about three to four days after

adult emergence and sperm descent into the seminal
vesicles took place at the same time. In another
subsequent study Awiti (1980) showed that precocene II
prevented continuous matings up to eight days following
adult emergence and prevented descent of sperm from the
testes to the seminal vesicles.

Results obtained from this investigation agree with
the findings of Awiti (1980). They show that precocene II
disturbs the sequence of reproductive events leading to
sterility of the male insects. The response however
varied with the time of application and dose applied.
With 1 μg doses of precocene, normal descent of sperm was
observed. Doses of 5 μg and above delayed sperm
descent by three to four days before it finally started
to come in trickles. Higher doses (above 10 μg), however,
made the insects completely sterile and sperm failed to
descend. Thus in the male D. fasciatus sexual maturation
seems to be under the control of factors from the CA;
and this is contary to what has been observed in other
male insects such as Glossina morsitans morsitans
(Samaranayaka-Ramasamy 1981), Locusta migratoria
(Pener et al., 1972), Leucophae maderae (Scharrer 1946,
Engelman, 1970) and Melanoplus sanguinipes (Gillot and
Friedel, 1976). This would imply that the whole issue
of regulation of reproductive physiology would heed to
be thoroughly examined before conclusive statements can
be made about it.

4.6 Effects of the male ARG homogenates on sexual
maturation in the male D. fasciatus

Early investigations have shown that the ARG
secretions of the male insects serve multifunctional
roles such as involvement with insemination (Omura,
1936; 1938; Davey, 1958; Landa, 1960, 1961; Davis, 1965, Lum,
196I; Loher and Edson, 1973; Leahy 1973; Shepherd et al. 1982; Pollock,
1974; Kokwaro and Odhiambo, 1981), formation of barriers
to reinsemination (Laidlaw 1944; Davey, 1960; Leferre and
Johnsson, 1962; Hinton, 1964; Spielman et al., 1967, 1969;
Riemann, 1967; Adams, 1968; Merle,1968; Nelson et al.,
1969; Terranova and Leopold, 1971; Swailes, 1971; Hiss
and Fuchs 1972; Burnet et al., 1973; Borut and Fedman
1976; Gillot and Friedel, 1976) and contributions to the
survival and fecundity of the species (Landa, 1960; 1961;
Hinton 1964, 1974; Loher and Huber, 1966; Pickford et al.
1969; Benz, 1969; Gillot and Friedel 1976b).

In the female D. fasciatus Odhiambo (1968) stated that
the act of mating did not influence the longevity,
length of the preoviposition period, duration of the
interoviposition period and total number of eggs laid.
This implied that the ARG secretions from the male
insects did not play any major role in influencing
the reproductive physiology of the female insects,
leaving the male insect as the only option. Thus,
investigations by Awiti (1980) showed that the

implantation of two to six day old male ARGs into
abdomens of the sexually immature $\underline{D}$. fasciatus males,
brought forward the onset of mating from the normal
three to four days to one to two days following adult
emergence. This showed that the recipients of the
glands were quite ready for mating even at such an
early age. Furthermore, the duration of mating in
recipients compared quite well with those of the normal
mating pairs.

Results obtained in this study using the ARG homogenates
agree with those of Awiti (1980). It was noted that the
newly emerged male insects which received the actively secreting
homogenates entered into copula on the 1st and 2nd days
after adult emergence. Furthermore, visual inspection
of the seminal vesicles of these insects revealed that
sperm descent had taken place, indicative of enhancement
of sexual maturity. These observations suggest that a
factor(s) from the ARGs may be involved in the final
processes of sperm maturation in addition to sperm transfer
and this is the first time such findings have been
reported in the male insect. However, how widespread
this function of ARGs may be among insects calls for
more investigations.

REFERENCES

ADAMS,T.S.and NELSON, D.R. (1968). Bioassay of crude extracts
        for the factor that prevents second matings in
        female Musca domestica.

        Ann. Entomol. Soc. Am., 61: 112-16.

ARORA, G. (1971). A study of occyte development in the adult
        Pyrrhocorid bugs of the genus Dysdercus.
        M.Sc. Thesis, University of Nairobi.

AWITI, L.R.S. (1976). Studies on the structure of the male
        reproductive system and spermatogenesis in the
        male cottonstainer, Dysdercus fasciatus Signoret.
        M.Sc. Thesis, University of Nairobi.

AWITI, L.R.S. (1980). Ultrastructural and experimental
        studies on the structure and function of the
        male accessory reproductive glands of Dysdercus
        fasciatus Signoret.
        Ph.D. Thesis, University of Nairobi.

BAUMANN, H. (1974). Biological effects of paragonial
        substance PS-I and PS-2 in females of Drosophila
        funebris.
        J. Insect. Physiol., 20: 2347-62

BELL, W.J. (1969). Dual role of juvenile hormohe in the
        control of yolk formation in Periplaneta
        americana.
        J. Insect Physiol., 15: 1279-1290.

BENZ, G. (1969). Influence of mating, insemination, and
other factors on oogenesis and oviposition
in the moth Zeiraphera diniana.
J. Insect Physiol., 15: 55-71.

BERRIDGE, M.J. (1965). The physiology of excretion in the
cotton stainer Dysdercus fasciatus.
J. Exp. Biol., 44: 553 pp.

BOHLEN, E. (1973). Crop pests in Tanzania and their control
(Ed. by Federal Agency for Economic Cooperation)
Verlag Paul, Parey, Berlin.

BORUT, S.and FELDMAN-MUHSAM, B. (1976). A new observation on
spermateleosis in ticks.
J. Parasitology, 62: 318-320.

BOWERS, W.S. (1976). In: The juvenile hormones (Ed. by
Gilbert, L.I.) Plenum, New York. pp. 394-408.

BOWERS, W.S., OHTA, T., CLEERE, J.S. and MARSELLA, P.A.
(1976). Discovery of Insect antijuvenile hormones
in plants.
Science, 193: 542-547.

BOWERS, W.S. and MARTINEZ-PARDO, R. (1977). Antiallatotropins
Inhibition of Corpus allatum development.
Science, 197: 1369-1371.

BOXALL, R.J. (1976). A morphological study of the
        retrocerebral complex of the adult cottonstainer,
        Dysdercus nigrofasciatus (Stal).
        M.Sc. Thesis, University of Nairobi.

BURNET, B., CONNOLY, K., KEARNEY,M.and COOK, R. (1973).
        Effect of male paragonial gland secretion on
        sexual receptivity and courtship behaviour of
        the the female Drosophila melanogaster.
        J. Insect. Physiol., 19: 2421-31.

CANTACUZENE, A.M. (1967). Histologie des glandes annexes
        males de Schistocerca gregaria, F. (Orthopteres)
        Effect de l'allatectomie sur leur structure et
        leur activite.
        C.R. Acad. (Paris), 264: 93-96.

CHAUDHURY, M.F.B., DHADIALLA, T.S. and KUNYIHA, R.W. (1981).
        Evidence of neuroendocrine relationships between
        mating and ovulation in the Tsetse fly Glossina
        morsitans morsitans.
        Insect. Sc. Applications, 1: 161-166.

CHIPPENDALE, G.M. (1977). Hormonal regulation of larval
        diapause.
        Ann. Rev. Ent., 22: 121-38.

COLLETTE, B. and JACQUES, B. (1984). Antigonadotropic and
        antiallatal effects of precocene II in the Firebrat
        Thermobia domestica (Thysanura, Lepismatidae).
        J. Insect. Physiol., 30: 463-470.

CRAIG, G.B. (1967). Mosquitoes: Female monogamy
        induced by male accessory gland substance.
        Science, 156: 1499-1501.

CROWE, T.J. (1967). The recognition and control of the
        more important pests of Agriculture in Kenya.
        Kenya Department of Agriculture Handbook, Nairobi.

DAVEY, K.G. (1958). The migration of spermatozoa in
        the female of Rhodnius prolixus Stal.
        J. Exp. Biol., 35: 694-701.

DAVEY, K.G. (1960). The evolution of spermatophore in
        insects.
        Proc. R. Entomol. Soc. Lond. Ser. A., 35: 107-13.

DAVEY, K.G. (1961). The release by feeding of a pharma-
        cologically active factor from the Corpus
        cardiacum of Periplaneta americana.
        J. Insect. Physiol., 8: 205-208.

DAVEY, K.G. (1965). Reproduction in the insects.
        Freeman, San Franscisco.

DAVIS, N.T. (1965). Studies of the reproductive physiology
of Cimicidae. II: Artificial insemination and
the function of the seminal fluid.
J. Insect. Physiol., 11: 355-66.

DAVIS, N.T. (1966). Reproductive physiology.
Entomol. Soc. Am. 7: 167-78.

DELOOF,A.and LAGASSE, A. (1972). The Ultrastructure of the
male accessory reproductive glands of the colorado
beetle.
Z. Zellforsch., 130: 545-552.

ECONOMOPOULOS,A.P.and GORDON, N.T. (1971). Growth and
differentiation of the testes in the large
milkweed bug Oncopeltus fasciatus Dalla.
J. Insect. Zool., 177: 391-406.

ENGELMANN, F. (1957). Die steurung der ovarfunktion bei
der ovoviviparen schabe Leucophae maderae (Fabri).
J. Insect. Physiol., 1: 257-278.

ENGELMANN, F. (1970). The Physiology of Insect Reproduction
Pergamon, Oxford.

EWEN, A.B. (1962). Improved aldehyde fuchsin staining
technique for neurosecretory product in Insects.
Trans. Amer. Mic. Soc., 81: 94-96.

FEYEREISEN, R., JOHNSON, G., KOENER, J., STAY, B. and TOBE,
        S.S., (1981a). Precocenes as pro-ollatocidins
        in adult female <u>Diploptera punctata</u>.
        J. Insect. Physiol., <u>27</u>: 855-868.

FOSTER, W. (1967). Hormone mediated nutritional control
        of sexual behaviour in male dung flies.
        Science, <u>158</u>: 1596-7.

FUCHS, M.S., CRAIG, G.B.Jr. and HISS, E.A. (1968). The
        biochemical basis of female monogany in mosquitoes -
        1. Extraction of the active principle from <u>Aedes</u>
        <u>aegypti</u>.
        Life Sci., 7: 835-39.

FUCHS, M.S., CRAIG, G.B.Jr. and DESPOMMIER, D.D. (1969).
        The protein nature of the substance inducing
        female monogamy in <u>Aedes</u> <u>aegypti</u>.
        J. Insect. Physiol., <u>15</u>: 701-09.

FUCHS, M.S. and HISS, E.A. (1970). The partial purification
        of the protein components of matrone from <u>Aedes</u>
        <u>Aegypti</u>.
        J. Insect. Physiol. <u>16</u>: 931-39.

GANAGARAJAH, M. (1965). The neuroendocrine complex of
        adult <u>Nebria</u> <u>brevivolis</u> (F.) and its relation to
        reproduction.
        J. Insect. Physiol., <u>11</u>: 1377-1387.

GILLOT,C and FRIEDEL, T. (1976a). Development of accessory
reproductive glands and its control by the Corpus
allatum in the adult male Melanoplus sanguinnipes.
J. Insect. Physiol., 22: 365-372.

GILLOT,C and FRIEDEL, T. (1976b). Fecundity enhancing and
receptivity-inhibiting substances produced by male
insects: A review.
Adv. Invert. Reprod., I: 199-218.

GIRARDIE,A and VOGEL, A. (1966). Etude du controle neuro-
humoral, de l'activite sexualle male de Locusta
migratoria (L.).
C.R. Acad. Sci. Paris, 263: 543-546.

GWADZ, R.W., CRAIG,G.B.Jr. and HICKEY, W.A. (1971). Female
sexual behaviour as the mechanism for rendering
Aedes aegypti refractory to insemination.
Biol. Bull. Woods Hole., 140: 201-214.

GWADZ, R.W. (1972). Neuro-Hormonal regulation of sexual
receptivity in female Aedes aegypti.
J. Insect. Physiol,, 18: 259-66.

HIGHNAM,K.C.and HILL, L. (1969). Comparative Endocrinology
of the invertebrates.
Arnold, London.

HINTON, H.E. (1964). Sperm transfer in insects and the
    ovulation of haemocoelic insemination.
    In: Insect Reproduction.
    Symp. R. Entomol. Soc. London, 2nd Ed.: 95-107.

HINTON, H.E. (1974). Accessory functions of seminal
    fluids.
    J. Med. Entomol., 11: 19-25.

HISS,E.A.and FUCHS, M.S. (1972). The effect of matrone
    on oviposition in the mosquito Aedes aegypti.
    J. Insect. Physiol., 18: 2217-27.

JOHANSSON, A.S. (1954). Corpus allatum and egg production
    in the starved milkweed bugs.
    Nature, 174: 89.

JOHANSSON, A.S. (1958). Relation of nutrition to endocrine-
    reproductive functions of the Milkweed bug
    Oncopeltus fasciatus Dallas (Heteroptera:
    Lygaeidae).
    Nytt. Mag. Zool., 7: 5-132.

KOKWARO, E.D. and ODHIAMBO, T.R. (1981). Spermatophore
    of the tsetse, Glossina morsitans morsitans
    Westwood: An Ultrastructural study.
    Insect. Sci. Applications, 1: 185-190.

KUMAR, R. (1984). Insect pest control with special
        reference to African Agriculture.
        Edward Arnold, London.

LAIDLAW, H.H. (1944). Artificial insemination of the
        queen bee (Apis mellifera L.): Morphological
        basis and results.
        J. Morphol., 74: 429-65.

LANDA, V. (1960). Origin, development and function of the
        spermatheca in cockchafer (Melolontha melolontha L.)
        Acta. Soc. Entomol. Cech., 57: 297-316.

LANDA, V. (1961). Experiments with artificial spermatophore
        in cockchafer (Melolontha melolontha L.)
        Acta. Soc. Entomol. Czechoslov., 58: 296-301.

LANDERS, M.H. and HAPP. P (1980). Precocene inhibition
        of vitellogenesis in Drosophila melanogaster.
        Experentia, 36:   619-620.

LEA, A.O. (1968). Mating without insemination in virgin
        Aedes aegypti.
        J. Insect. Physiol., 14: 305-308.

LEAHY, M.G. and LOWE, M.L. (1966). Extraction of a substance
        from male accessory glands that enhances oviposition
        in Diptera.
        Bull. Ent. Soc. Amer., 12: page 302.

LEAHY, M.G. (1973). Oviposition of virgin Schistocerca
        gregaria (Forskal) after implant of the male
        accessory complex.
        J. Entom. Ser. A., 48: 69-78.

LEFEVRE, G.and JOHNSON, V.B. (1962). Sperm transfer, storage
        displacement and utilization in Drosophila
        melanogaster.
        Genetics, 47: 1719-1736.

LEOPOLD, R.A. (1970). Cytological and cytochemical studies
        on the ejaculatory duct and accessory secretion
        in Musca domestica.
        J. Insect. Physiol., 16: 1859-1872.

LEOPOLD, R.A., TERRANOVA, A.C., THORSON,B.J.and DEGRUGILLIER,
        M.E. (1971). The Biosynthesis of the male housefly
        accessory secretion and its fate in the mated
        female.
        J. Insect. Physiol., 17: 987-1003.

LEOPOLD, R.A. (1976). The role of male accessory glands
        in insect reproduction.
        Ann. Rev. Entomol., 21: 199-221.

LE PELLEY, R.H. (1959). Agriculture insects of East
        Africa; A list of East African plant feeding
        insects and mites.
        E. A. High Commission, Nairobi.

LOHER,W.and GORDON, H. (1968). The maturation of sexual
        behaviour in a new strain of the large milkweed
        bug, Oncopeltus fasciatus.
        An. Entomol. Soc. Am., 61: 1566-1572.

LOHER,W.and HUBER, F. (1966). Nervous and endocrine
        control of sexual behaviour in a grasshopper
        (Gomphocerus rufus L.)
        Symp. Soc. Exp. Biol., 20: 381-400.

LOHER,W.and EDSON, K. (1973). The effect of mating on
        egg production and release in the cricket
        Teleogryllus commodus.
        Entomol. Exp., Appl. 16: 483-90.

LUM, P.T. (1961). The reproductive system of some Florida
        mosquitoes. II: The male accessory glands and
        their role.
        Ann. Entomol. Soc. Am., 54: 430-33

MANNING, A. (1966). Corpus allatum and sexual receptivity
        in female Drosophila melanogaster.
        Nature, 211 : 1321-1322.

MANNING, A. (1967). The control of sexual receptivity in
        female Drosophila.
        Animal Behaviour: 15: 239-250.

MASNER, P., BOWERS, W.S., KALIN,M.and MUHLE, T. (1979).
        Effects of precocene II on the endocrine
        regulation of development and reproduction in
        the bug Oncopeltus fasciatus.
        Gen. Comp. Endocr., 37: 156-166.

MELLANBY, K. (1939). Fertilization and egg-production
        in the bed bug Cimex lectularius L.
        Parasitol., 31: 193-211.

MERLE, J. (1968). Fonctionnement ovarian et receptivite
        sexuelle de Drosophila melanogaster apres
        implantation de fragments de l;apparejl genital
        male.
        J. Insect Physiol., 14: 1159-68.

MIAL, R.C. (1980). The morphological and behavioural
        effects of precocene II on locust.
        J. Insect. Physiol., 26: 607

MIAL,R.C.and MORDUE, W. (1980). Precocene II has juvenile
        hormone effects in the 5th Instar Locusta
        migratoria.
        J. Insect Physiol., 26: 361-364.

NAYAR,K.K. (1958). Studies on the neurosecretory system
        of Iphita limbata Stal. - Part V. Probable
        endocrine basis of oviposition in the female
        insect.
        Proc. Indian Acad. Sci. B., 47: 233-251.

NAYAR, K.K. (1973). Elements of insect endocrinology.
        Prentice Hall, New York

NELSON, D.R., ADAMS, T.S. and POMONIS, J.G. (1969). Initial
        Studies on the extraction of active substance
        inducing monocoitic behaviour in Musca domestica,
        Phormia regina, and Cochliomyia hominivorax.
        J. Econ. Entomol., 62: 634-39.

ODHIAMBO, T.R. (1966c). Growth and hormonal control of

sexual maturation in the male desert locust,

Schistocerca gregaria (Forskal).

Trans. R. ent. Soc. Lond., 118: 393-412.

ODHIAMBO, T.R. (1968). The effect of mating on egg

production in the cottonstainer, Dysdercus

fasciatus.

Ent. Exp. and Appl., 11: 379-388.

ODHIAMBO, T.R. (1971). The architecture of the accessory

reproductive glands in the male desert locust. 5:

Ultrastructural study during maturation.

Tissue and Cell, 3: 309-324.

OMURA, S. (1936). Artificial insemination of Bombyx mori

J. Fac. Agric. Hokkaido Imp. Univ. Sapporo,

38: 135-50.

OMURA, S. (1938). Studies on the reproductive system of

the male Bombyx mori. II: Post testicular

organs and post testicular behaviour of the

spermatozoa.

J. Fac. Agric. Hokkaido Imp. Univ. Sappora, 40:

129-70.

PEARSON, E.O. (1958). The insect pests of cotton in
          Tropical Africa.
          Empire cotton growing cooporation and
          Commonwealth Institute of Entomology,
          London.

PENER, M.P., GIRARDIE, A. and JOLY, A. (1972).
          Neurosecretory and Corpus allatum controlled
          effects on mating behaviour and colour change
          in adult Locusta migratoria migratorioides
          males.
          Gen. Comp. Endocrinol., 19: 494-508.

PENER, M.P. and ORSHAN, L. (1977). Morphogenetic
          effect of precocene II, an antijuvenile
          hormone in the African migratory locust
          Locusta migratoria migratorioides.
          Israel J. Zool., 26: 262-263.

PENER, M.P., ORSHAN,L.and De WILDE, J. (1978).
          Precocene II causes atrophy of CA in
          Locusta migratoria.
          Nature, 262: 272-353.

PICKFORD, R., EWEN,A.B.and GILLOT, C. (1969). Male
        accessory gland substance. An egg-laying
        stimulant in Melanoplus sanguinipes (F).
        Can. J. Zool., 47: 1199-203.

PINKERTON, A.M., JUDY, D. HALL and SHEPHERD, J. (1982).
        Scanning electron microscopy of post ejaculatory
        spermiogenesis in the Tick Ornithodorus moubata.
        Tissue and Cell, 14 (4): 785-797.

POLLOCK, J.N. (1974). Male accessory secretions, their
        use and replenishment in Glossina (Diptera
        Glossinidae).
        Bull. Ent. Res., 64: 533-539.

PRATT, G.E. and DAVEY,-K.G. (1972). The Corpora allata
        and oogenesis in Rhodnius prolixus (Stal).
        The effects of allatectomy.
        J. Exp. Biol., 56: 201-214.

PRATT, G.E. and PENER, M.P. (1963). Precocene sensitivity
        of Corpora allata in adult female Locusta
        migratoria after electrocoagulation of the pars
        intercerebralis.
        J. Insect. Physiol., 29: 33-39.

QUO FU   (1959). Studies on the reproduction of the oriental
         migratory locust: The physiological effect of
         castration and copulation.
         Acta. Entomol. Sinica., 9: 464-76.

RIDDIFORD,L·M.and WILLIAMS, C.M. (1971). Role of the
         corpora cardiaca in the behaviour of saturniid
         moth. I: Release of sex-pheromones.
         Biol. Lab., 140: 1-7.

RIDDIFORD,L·M.and ASHENHURST, J.B. (1973). The Switch over
         from virgin to mated behaviour in female Cecropia
         moths: The role of the bursa copulatrix.
         Biol. Bull. Mar. Biol. Lab., 144: 162-171.

RIEMANN, J.G., MOEN,D.J.and THORSON, B.J. (1967). Female
         monogamy and its control in houseflies.
         J. Insect Physiol., 13: 407-18.

RIEMANN, J.G. and THORSON, B.J. (1969). Effect of male
         accessory material on oviposition and mating
         by female houseflies.
         Ann. Ent. Soc. Am., 62: 828-834.

SAMARANAYAKA-RAMASAMY, M. (1981). Influence of feeding and
         hormonal factors on sexual maturation in male
         Glossina morsitans morsitans West Wood (Diptera:
         Glossinidae).
         Insect Sci. Appl., 1: 273-280.

SAMARANAYAKA-RAMASAMY, M. and CHAUDHURY, M.F.B. (1982).

        Precocene induced sterility in F_1 Generation

        of Glossina morsitans morsitans.

        J.Insect Physiol., 28: 559-569.

SCHARRER, B. (1946). The relationship between Corpora

        allata and reproductive organs in the adult

        Leucophae maderae (Orthoptera)

        Endocrinology, 38: 46-55.

SCHARRER, B. (1958). Proceedings of the second International

        symposium on neurosecretion. (Springer, Berlin)

        79-84-L-Strong.

        J. Insect Physiol., 11: page 271.

SCHARRER, B. and VON HARNACK, M. (1958). Histophysiological

        studies on the Corpus allatum of Leucophae

        maderae. I: Normal life cycle in male and

        female adults.

        Biol. Bull. Woods hole., 115: 508-520.

SCHOONEVELD, H. (1979a). Precocene induced collapse and

        resorption of Corpora allata in nymphs of Locusta

        migratoria.

        Experentia, 35: 363-364.

SCHOONEVELD, H. (1979b). Precocene induced necrosis and
        haemocyte-mediated breakdown of Corpora allata
        in nymphs of Locusta migratoria.
        Cell Tiss. Res., 203: 25-33.

SHEPHERD, J., STEWART,L.and JUDY D. HALL, (1982a).
        Maturation of tick spermatozoa in vitro.
        Inter. J. Inv. Repr., 4: 311-321.

SHEPHERD, J. OLIVER,J.H.and JUDY D. HALL (1982b). A
        polypeptide from male accessory glands which
        triggers maturation of tick spermatozoa.
        Inter. J. Inv. Repr., 5: 129-137.

SMITH, J.M. (1956). Fertility, mating behaviour and sexual
        selection in Drosophila subobscura.
        J. Genet., 54: 261-279.

SPIELMANN, A.M., LEAHY, G., and SKAFF, V. (1967). Seminal
        loss in repeatedly mated female Aedes aegypti.
        Biol. Bull. Woods Hole, 132: 404-412.

SPIELMANN, A., LEAHY,M.G and SKAFF, V. (1969). Failure
        of effective insemination of young female Aedes
        aegypti. mosquitoes.
        J. Insect Physiol., 15: 1471-79.

STRANGEWAYS-DIXON, J. (1961). The relationship between
        nutrition, hormones and reproduction in the blow fly
        Calliphora erythrocephala Meig. I: Selective feeding
        in relation to the reproduction cycle, the Corpus
        allatum volume and fertilization.
        J. Exp. Biol., 38: 225-235.

SWAILES, G. (1971). Reproductive behaviour and effects of
        male accessory gland substance in the cabbage
        Hylemya brassicae.
        Ann. Entomol. Soc. Am., 64: 176-79.

TERRANOVA,A.G.and LEOPOLD, R.A. (1971). A bioassay
        technique for investigating mating refusal in
        female houseflies.
        Ann. Entomol. Soc. Am., 64: 263-66.

TERRANOVA, A.C., LEOPOLD, R.A., DEGRUGILLIER, M.E. and
        JOHNSON, J.R. (1972). Electrophoresis of the male
        accessory secretion and its fate in the mated
        female.
        J. Insect Physiol., 18: 1573-91.

THOMSEN, E. (1952). Functional significance of the neuro-
        secretory brain cells and the Corpus cardiacum in
        the female blow fly Calliphora erythrocephala.
        Meig, J. Exp. Biol., 29: 137-72.

TRUMAN, J.W. and RIDDIFORD, L.M. (1970). Neuroendocrine
control of ecdysis in silk moths.
Science, N.Y. <u>167</u>: 1624-1626.

UNNITHAN, G.E., NAIR,k.k.,and BOWERS, W.S. (1977). Precocene
induced degeneration of the <u>Corpus allatum</u> of adult
females of <u>Oncopeltus fasciatus</u>.
J. Insect. Physiol. <u>23</u>: 1081-1094.

UNNITHAN,G.E,and NAIR, K.K. (1979). The influence of
<u>Corpus allatum</u> on the susceptibility of <u>Oncopeltus</u>
<u>fasciatus</u> to precocene.
Ann. ent. Soc. Am., <u>72</u>: 38-40.

UNNITHAN, G.C., NAIR, K.K., and SYED, A. (1980). Precocene
induced metamorphosis in the desert locust <u>Schistocerca</u>
<u>gregaria</u>.
Experentia, <u>36</u>: 135-36.

WEED PFEIFFER, I. (1936). Removal of corpora allata on
egg production in the grasshopper <u>Melanoplus</u>
<u>differentialis</u>.
J. Exp. Zool., <u>82</u>: 439-461.

WEED, I.G. (1936). Removal of corpora allata on egg production
in grasshopper <u>Melanoplus differentialis</u>.
Proc. Soc. Exp. Biol. Med., <u>34</u>: 883-85.

WIGGLESWORTH, V.B. (1936). The function of the Corpus allatum
        in the growth and reproduction of Rhodnius prolixus
        (Hemiptera).
        Quart. J. Microscop. Sci., 79: 91-121.

WIGGLESWORTH, V.B. (1964). The hormonal regulation of growth
        and reproduction in insects.
        Adv. Insect. Physiol., 2: 247-336.

WIGGLESWORTH, V.B. (1965). The principles of Insect Physiology
        Sixth Edition, Methuen, London.

WILLIS, J.H. (1974). Morphogenetic action of insect hormones
        Ann. Rev. Entomol., 19: 97-115.

YAMADA, S.and YAGI,S.   (1984). Induction of precocious
        metamorphosis in the oriental cinch bug Cavalerius
        saccharivorus Okajima (Hemiptera Lygaeidae) by
        precocene II. A short communication.
        Appl. Ent. Zool., 19 (4): 521-523.

APPENDIX I: Changes in size of the CA in the unpaired male
            D. fasciatus during sexual maturation

Age (days)	Mean volume $\pm$ S.E. $(x\ 10^{-3}\ mm^3,\ n = 10)$
0	4.60 $\pm$ 0.20
1	6.20 $\pm$ 0.20
2	6.64 $\pm$ 0.30
3	7.52 $\pm$ 0.28
4	7.53 $\pm$ 0.28
5	7.27 $\pm$ 0.32
6	7.58 $\pm$ 0.36
7	7.25 $\pm$ 0.43
8	6.35 $\pm$ 0.39
9	5.91 $\pm$ 0.29
10	4.61 $\pm$ 0.30
11	4.30 $\pm$ 0.36
12	6.31 $\pm$ 0.42
13	5.70 $\pm$ 0.38
14	6.55 $\pm$ 0.51
15	6.50 $\pm$ 0.20
16	6.14 $\pm$ 0.25
17	5.63 $\pm$ 0.43
18	5.79 $\pm$ 0.33
19	5.91 $\pm$ 0.53
20	4.83 $\pm$ 0.24

$\bar{X}$ = 6.14   S.D. = 0.98   S.E.   = 0.21

APPENDIX II: Changes in size of the ARGs in the unpaired male
D. fasciatus during sexual maturation

Age (days)	Mean volume $\pm$ S.E. ($\times 10^{-1}$ mm^3, n = 10)
0	9.24 ± 0.22
1	13.42 ± 0.79
2	17.54 ± 1.34
3	19.90 ± 1.09
4	19.27 ± 1.02
5	25.63 ± 3.29
6	27.38 ± 1.92
7	28.24 ± 1.60
8	27.92 ± 2.25
9	24.72 ± 1.70
10	23.61 ± 2.63
11	20.45 ± 3.71
12	21.98 ± 1.84
13	25.51 ± 2.15
14	26.60 ± 2.03
15	33.07 ± 3.06
16	29.87 ± 3.10
17	28.28 ± 3.15
18	25.29 ± 1.79
19	34.31 ± 3.63
20	30.08 ± 3.15

$\bar{X}$ = 24.39   S.D. = 6.16   S.E. = 1.34

APPENDIX III: Changes in size of the CA in paired male D. fasciatus during the 1st copulatory cycle

Age (days)	Period of copulation (days)	Mean volume $+$ S.E. $(x\ 10^{-3}\ mm^3,\ n = 10)$
4	1	5.23 ± 0.15
5	2	4.74 ± 0.31
6	3	5.81 ± 0.62
7	4	5.36 ± 0.21
8	5	5.36 ± 0.25
9	6	5.29 ± 0.36
10	-	5.00 ± 0.35
11	-	3.81 ± 0.38
12	-	4.98 ± 0.31
13	-	2.50 ± 0.19

$\bar{X} = 4.80$ S.D. = 0.96   S.E. = 0.31

- = Period after the insects have disengaged.

APPENDIX IV: Changes in size of the ARG in the paired male
D. fasciatus during the 1st Copulatory cycle

Age (days)	Period of copulation (days)	Mean volume + S.E. $(x\ 10^{-1}\ mm^3,\ n = 10)$
4	1	12.65 + 0.67
5	2	13.19 + 0.50
6	3	15.34 + 1.36
7	4	22.77 + 1.71
8	5	21.29 + 2.12
9	6	28.67 + 3.07
10	-	18.12 + 2.33
11	-	19.09 + 2.58
12	-	27.17 + 2.07
13	-	21.65 + 0.94

$\bar{X}$ = 19.99   S.D. = 5.42   S.E. = 0.31

- = Period after the insects have disengaged.

APPENDIX V: Changes in size of the CA in the male D. fasciatus treated topically with 1 µg/1 µl Precocene II at adult emergence

Period after treatment (days)	Mean volume ± S.E. ($\times 10^{-3}$ mm^3, n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
1	4.35 ± 0.35	4.82 ± 0.23
2	5.76 ± 0.32	5.89 ± 0.31
3	5.43 ± 0.35	5.77 ± 0.29
4	5.11 ± 0.42	5.58 ± 0.41
5	6.05 + 0.39	6.18 ± 0.43
6	5.65 ± 0.36	5.83 ± 0.26
7	5.38 ± 0.26	6.72 ± 0.43
8	5.41 ± 0.47	6.12 ± 0.28
9	4.85 ± 0.29	5.11 ± 0.23
10	5.14 ± 0.27	4.89 ± 0.23

(a) $\bar{X}$ = 5.313   S.D. = 0.48   S.E. = 0.15 $P < 0.05$ (not significant)

(b) $\bar{X}$ = 5.69   S.D. = 0.60   S.E. = 0.19

APPENDIX VI: Changes in size of the ARGs in the male $\underline{D.}$ $\underline{fasciatus}$ treated topically with 1 µg/1 µl Precocene II at adult emergence.

Period after treatment	Mean volume $\pm$ S.E. (x 10^{-1} mm^3, n = 10)	
	Experimental[a]	Control[b]
(days)	Precocene treated	Acetone treated
1	7.65 $\pm$ 0.47	8.80 $\pm$ 0.76
2	13.41 $\pm$ 1.83	13.32 $\pm$ 0.90
3	15.01 $\pm$ 0.95	18.35 $\pm$ 1.11
4	15.71 $\pm$ 1.23	18.13 $\pm$ 1.20
5	20.73 $\pm$ 1.54	22.27 $\pm$ 2.02
6	23.61 $\pm$ 1.04	25.65 $\pm$ 1.08
7	23.28 $\pm$ 2.23	28.24 $\pm$ 1.60
8	22.27 $\pm$ 2.03	24.04 $\pm$ 0.76
9	22.87 $\pm$ 1.80	25.45 $\pm$ 1.55
10	23.64 $\pm$ 2.44	27.28 $\pm$ 2.27

(a) $\bar{X}$ = 18.81   S.D. = 5.54   S.E. = 1.75

(b) $\bar{X}$ = 21.15   S.D. = 6.38   S.E. = 2.02 $P < 0.05$ (not significant)

APPENDIX VII: Changes in size of the CA in the male <u>D. fasciatus</u> treated topically with 5 µg/1 µl Precocene II at adult emergence

Period after treatment	Mean volume $\pm$ S.E. $(x\ 10^{-3}\ mm^3,\ n = 10)$	
	Experimental[a]	Control[b]
(days)	Precocene treated	Acetone treated
1	3.67 $\pm$ 0.31	4.73 $\pm$ 0.28
2	4.18 $\pm$ 0.41	5.96 $\pm$ 0.19
3	5.04 $\pm$ 0.24	6.71 $\pm$ 0.31
4	3.05 $\pm$ 0.36	6.28 $\pm$ 0.35
5	3.75 $\pm$ 0.12	5.96 $\pm$ 0.26
6	3.96 $\pm$ 0.28	6.38 $\pm$ 0.28
7	3.33 $\pm$ 0.10	6.09 $\pm$ 0.16
8	3.61 $\pm$ 1.95	5.42 $\pm$ 0.30
9	3.12 $\pm$ 0.29	5.16 $\pm$ 0.36
10	4.15 $\pm$ 0.11	4.18 $\pm$ 0.15

(a) $\bar{X} = 3.78$ S.D. = 0.58   S.E. = 0.18

(b) $\bar{X} = 5.68$ S.D. = 0.79   S.E. = 0.25

$P \leq 0.05$ (significant)

APPENDIX VIII: Changes in size of the ARG in the male <u>D</u>. <u>fasciatus</u> treated topically with 5 µg/1 µl Precocene II at adult emergence

Period after treatment (days)	Mean volume $\pm$ S.E. ($\times 10^{-1}$ mm^3, n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
1	8 97 $\pm$ 1.14	9.47 $\pm$ 0.73
2	10.19 $\pm$ 0.80	10.08 $\pm$ 0.97
3	11.19 $\pm$ 0.93	13.32 $\pm$ 0.70
4	14.33 $\pm$ 0.60	18.46 $\pm$ 1.03
5	18.59 $\pm$ 2.05	26.35 $\pm$ 1.66
6	21.87 $\pm$ 2.77	26.64 $\pm$ 2.27
7	21.86 $\pm$ 3.15	21.93 $\pm$ 1.65
8	23.70 $\pm$ 2.55	31.28 $\pm$ 2.64
9	19.13 $\pm$ 1.98	25.49 $\pm$ 1.23
10	23.23 $\pm$ 2.71	24.23 $\pm$ 2.28

(a) $\bar{X}$ = 17.3   S.D. = 5.60   S.E. = 1.79

(b) $\bar{X}$ = 20.9   S.D. = 7.70   S.E. = 2.45

$P \leq 0.05$ (not significant)

APPENDIX IX: Changes in size of the CA in the male D. fasciatus
treated topically with 10 μg/1 μl Precocene II at
adult emergence

Period after treatment	Mean volume $\pm$ S.E. ($\times 10^{-3}$ mm^3, n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
(days)		
1	4.32 $\pm$ 0.18	5.27 $\pm$ 0.34
2	4.08 $\pm$ 0.34	5.75 $\pm$ 0.38
3	3.17 $\pm$ 0.23	5.79 $\pm$ 0.45
4	2.03 $\pm$ 0.08	4.74 $\pm$ 0.27
5	2.95 $\pm$ 0.22	5.92 $\pm$ 0.28
6	3.52 $\pm$ 0.21	5.51 $\pm$ 0.28
7	3.41 $\pm$ 0.28	5.73 $\pm$ 0.25
8	3.70 $\pm$ 0.22	5.96 $\pm$ 0.35
9	3.76 $\pm$ 0.19	5.06 $\pm$ 0.30
10	3.69 $\pm$ 0.20	5.00 $\pm$ 0.29

(a) $\bar{X}$ = 3.52   S.D. = 0.64   S.E. = 0.20 $P \leq 0.05$

(b) $\bar{X}$ = 5.47   S.D. = 0.42   S.E. = 0.13   (significant)

APPENDIX X : Changes in size of the ARGs in the male D. fasciatus treated topically with 10 µg/1 µl Precocene II at adult emergence

Period after treatment	Mean volume $\pm$ S.E. $(x\ 10^{-1}\ mm^3,\ n = 10)$	
	Experimental[a] Precocene treated	Control[b] Acetone treated
(days)		
1	9.39 $\pm$ 0.54	10.29 $\pm$ 0.45
2	12.09 $\pm$ 0.47	14.03 $\pm$ 1.06
3	12.65 $\pm$ 1.21	14.58 $\pm$ 0.61
4	12.12 $\pm$ 0.96	14.57 $\pm$ 0.73
5	16.38 $\pm$ 1.70	25.33 $\pm$ 1.31
6	15.47 $\pm$ 1.51	24.59 $\pm$ 1.61
7	20.55 $\pm$ 1.94	20.24 $\pm$ 0.81
8	19.94 $\pm$ 2.12	21.46 $\pm$ 0.83
9	21.12 $\pm$ 2.06	24.03 $\pm$ 1.37
10	19.01 $\pm$ 1.23	24.87 $\pm$ 1.12

(a) $\bar{X}$ = 15.87   S.D. = 4.17   S.E. = 1.32 $p < 0.05$

(b) $\bar{X}$ = 19.39   S.D. = 5.54   S.E. = 1.75   (not significant)

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APPENDIX XI: Changes in size of the CA in the male <u>D. fasciatus</u>
treated topically with 15 µg/1 µl Precocene II
at adult emergence

Period after treatment (days)	Mean volume $\pm$ S.E. ($\times 10^{-3}$ mm^3, n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
1	3.00 $\pm$ 0.22	3.59 $\pm$ 0.14
2	2.97 $\pm$ 0.17	4.99 $\pm$ 0.41
3	2.77 $\pm$ 0.10	4.79 $\pm$ 0.28
4	2.59 $\pm$ 0.07	4.40 $\pm$ 0.20
5	2.58 $\pm$ 0.06	5.91 $\pm$ 0.23
6	2.57 $\pm$ 0.14	5.66 $\pm$ 0.29
7	2.97 $\pm$ 0.42	6.62 $\pm$ 0.40
8	2.59 $\pm$ 0.23	6.57 $\pm$ 0.34

(a) $\bar{X}$ = 2.755   S.D. = 0.19   S.E. = 0.04 $P \leq 0.05$

(b) $\bar{X}$ = 5.31   S.D. = 1.06   S.E. = 0.37   (significant)

APPENDIX XII: Changes in size of the ARGs in the male <u>D. fasciatus</u>
treated topically with 15 μg/1 μl Precocene II
at adult emergence

Period after treatment	Mean volume $\pm$ S.E. (x 10^{-1} mm^3, n = 10)	
	Experimental[a]	Control[b]
(days)	Precocene treatment	Acetone treated
1	7.57 + 0.50	8.32.+ 0.42
2	7.77 + 0.62	10.94 + 0.81
3	9.44 + 0.63	11.55 + 1.25
4	11.05 + 1.02	17.45 + 1.13
5	17.48 + 1.01	24.92 + 0.99
6	21.75 + 1.90	27.50 + 2.10
7	21.01 + 2.98	25.05 + 1.56
8	20.08 + 0.93	26.68 + 1.13

(a) $\bar{X}$ = 14.51   S.D. = 6.16   S.E. = 2.21 $P \leq 0.05$

(b) $\bar{X}$ = 19.05   S.D. = 7.92   S.E. = 2.81 (not significant)

APPENDIX XIII: Changes in size of the CA in the male D. fasciatus treated topically with 20 µg/1 µl Precocene II at adult emergence

Period after treatment	Mean volume $+$ S.E. ($\times 10^{-3}$ mm^3 n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
(days)		
1	3.18 + 0.26	4.39 + 0.26
2	3.09 + 0.29	4.97 + 0.27
3	2.27 + 0.34	5.59 + 0.36
4	2.45 + 0.36	6.00 + 0.28
5	2.61 + 0.15	6.96 + 0.38
6	2.80 + 0.22	5.76 + 0.31
7	3.35 + 0.28	6.13 + 0.31
8	3.13 + 0.24	4.99 + 0.38

(a) $\bar{X}$ = 2.86   S.D. = 0.38   S.E. = 0.13 $p < 0.05$
(b) $\bar{X}$ = 5.59   S.D. = 0.80   S.E. = 0.28   (significant)

APPENDIX XIV: Changes in size of the ARGs in the male D. fasciatus treated topically with 20 µg/1 µl Precocene II at adult-emergence

Period after treatment (days)	Mean volume $\pm$ S.E. ($\times 10^{-1}$ mm^3, n = 10)	
	Experimental[a] Precocene treated	Control[b] Acetone treated
1	7.19 ± 0.78	8.79 ± 0.61
2	8.35 ± 0.48	9.82 ± 0.82
3	8.66 ± 0.70	13.50 ± 0.91
4	14.77 ± 1.54	20.61 ± 1.51
5	18.09 ± 1.42	25.63 ± 1.51
6	20.78 ± 1.45	27.16 ± 2.07
7	16.46 ± 2.20	18.51 ± 1.58
8	24.73 ± 1.59	28.89 ± 1.81

(a) $\bar{X} = 14.87$ S.D. = 6.38   S.E. =.2.26 $P \leq 0.05$

(b) $\bar{X} = 19.11$ S.D. = 7.84   S.E. = 2.78   (not significant)

APPENDIX XV :   The effect of various doses of precocene II
                 on sperm descent in the male D. fasciatus

Age (days)	% Estimate of insects which show sperm descent				
	Precocene concentration (µg/1 µl)				
	1 ug	5 ug	10 ug	15 ug	20 ug
0	0	0	0	0	0
1	0	0	0	0	0
2	10	0	0	0	0
3	40	35	0	0	0
4	40	50	0	0	0
5	45	50	0	0	0
6	65	80	0	0	0