STUDIES ON THE ROLE AND REGULATION OF THE MALE ACCESSORY REPRODUCTIVE GLAND SECRETIONS OF THE COTTON STAINER,
Dysdercus fasciatus SIGNORET (HETEROPTERA : PYRRHOCORIDAE)

A Thesis submitted in part fulfilment for the degree of Master of Science (Entomology) in the University of Nairobi

1986.
DECLARATION

This thesis is my original work and it has not been presented for a degree in any other University.

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

It has been established that in most insects, reproduction is under the influence of hormones produced by the neuroendocrine 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 eighth 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/l μl doses had no effect on the activity of the CA or ARG as indicated by the morphometric changes. Doses above 5 μg/l 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/l μl led to atrophy of the CA, retarded ARG development and prevented sperm descent. Concentrations of 25 μg/l 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.
ACKNOWLEDGEMENTS

I am greatly indebted to my supervisors, Dr. G.R. Karuhize and Dr. L.R.S. Awiti for their supervision and constructive criticisms on the subject of this thesis. I wish to thank them for their encouragement and learned comments throughout this study.

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The immense support received from the technician in charge of the Departmental Insectary Mr. C. Apat is greatly appreciated. The services of Mr. J. Gitau of the Department of Animal Production at Kabete Campus who typed this thesis are greatly appreciated.
I wish to thank the Federal Republic of Germany through their agency the "Deutscher Akademischer Austauschdienst" (DAAD) and the Deans Committee of Makerere University Kampala (Uganda) for providing me with the scholarship which enabled me to undertake this study.
1.1 Occurrence 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 occurring 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 superstitionis* (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 membraneous 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 disengaging 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 \textit{D. fasciatus} is remarkably longer than that of a related bug \textit{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 \textit{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 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 Johnson, 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 existence 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), Hylemya 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 consensus 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 reproductive 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 \textit{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 \textit{Melanoplus sanguinipes}, stimulates egg laying. In contrast, studies by Leahy (1973) with \textit{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 \textit{L. migratoria marilensis}, males deprived of their testes and 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 \textit{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 transferred 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 investigations 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 stercoralia* (Foster, 1967), *O. fasciatus* (Economopoulos and Gordon, 1971). In female insects, the endocrine control of oocyte development applies strictly to vitellogenesis 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. maderaee* (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 the females.
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 occurred 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 up to 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 $29 \pm 1^\circ C$ 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 transferred 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 transferred 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:

\[ C = \text{Cotton wool moistened to supply moisture.} \]
\[ \text{TW} = \text{Twig used as a moulting site} \]
\[ F = \text{Food in petri dish} \]
\[ N = \text{Netting material for ventilation} \]
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 choosing 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 MSA 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 oesophagus.

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 surrounding 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:

\[ \text{Volume} = \frac{2}{3} \pi a \times b^2 \]
Where \( \pi = 3.14 \)

\[
\begin{align*}
a &= \text{Mean length of the gland} \\
b &= \text{Mean width of the gland}
\end{align*}
\]

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 largely 100% ethanol (2-4 hours). Following dehydration, the specimens 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 transferred into a 1:1 mixture of benzene and paraplast at 60°C for 30 minutes followed by infiltration with two changes of pure molten paraplast wax at 60°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 transferred 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°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, transferred 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 $4.60 \pm 0.20 \times 10^{-3} \text{ mm}^3$. During the first three days following adult emergence, its volume increased to $7.52 \pm 0.28 \times 10^{-3} \text{ mm}^3$. 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 $6.55 \pm 0.51 \times 10^{-3}$ mm$^3$ was attained. Unlike $P_1$, $P_2$ was short-lived staying on only up to 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
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 ($P_1$) 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 ($P_2$) on the 14th day. This lasts until the 15th day when the volume falls off again. Note that value $P_2$ is smaller than $P_1$. 

*Figure 1:*
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 $28.24 \pm 1.60 \times 10^{-1} \text{ mm}^3$ ($P_1$) 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 $33.07 \pm 3.06 \times 10^{-1} \text{ mm}^3$ ($P_{II}$) 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 $P_1$ 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 up to the end of the second copulatory cycle, which comes after the 18th day. In
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 ($P_1$) 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.
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 continuous 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 occurring 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 $12.65 \pm 0.67 \times 10^{-1}\text{mm}^3$ (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 ($P < 0.05$) 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 entwined 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 have a 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 mesadinal 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 suggests 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 difficult 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

$S = \text{Secretion}$
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).
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 considerable shrinkage in the size of the CA, which had a characteristic 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 < 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.

<table>
<thead>
<tr>
<th>Precocene dose</th>
<th>Mean volume of the CA $mm^3 \times 10^{-3}$</th>
<th>% difference in mean volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu g/ul$</td>
<td>Acetone controls</td>
<td>Precocene treated</td>
</tr>
<tr>
<td>1</td>
<td>5.69</td>
<td>5.31</td>
</tr>
<tr>
<td>5</td>
<td>5.68</td>
<td>3.78</td>
</tr>
<tr>
<td>10</td>
<td>5.47</td>
<td>3.52</td>
</tr>
<tr>
<td>15</td>
<td>5.31</td>
<td>2.75</td>
</tr>
<tr>
<td>20</td>
<td>5.59</td>
<td>2.86</td>
</tr>
</tbody>
</table>

*: 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 doses 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 antiallactotropic 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

<table>
<thead>
<tr>
<th>Precocene dose (µgs/1 µl)</th>
<th>Mean Volumes of the ARGs mm$^3 \times 10^{-3}$</th>
<th>Differences in volume</th>
<th>% Differences in mean volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetone controls</td>
<td>Precocene treated</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21.15</td>
<td>18.80</td>
<td>- 2.34</td>
</tr>
<tr>
<td>5</td>
<td>20.90</td>
<td>17.30</td>
<td>- 3.60</td>
</tr>
<tr>
<td>10</td>
<td>19.39</td>
<td>15.87</td>
<td>- 3.52</td>
</tr>
<tr>
<td>15</td>
<td>19.05</td>
<td>14.51</td>
<td>- 4.54</td>
</tr>
<tr>
<td>20</td>
<td>19.11</td>
<td>14.87</td>
<td>- 4.24</td>
</tr>
</tbody>
</table>
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.

\[ \text{Corpus allatum volume} \times 10^3 \text{ mm}^3 \]

\[ \text{Accessory reproductive gland volume} \times 10^7 \text{ mm}^3 \]

- ▲ 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/l 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/l 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. 

\[ \text{Precocene treated insects} \]

\[ \text{Acetone treated control insects} \]

Vertical bars: SEM

Figure 5b. 

\[ \text{Precocene treated insects} \]

\[ \text{Acetone treated control insects} \]

Vertical bars: SEM
Figure 6a

Graph showing changes in the volume of the CA in the male _D. fasciatus_ treated with 10 μg/l 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/l 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

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/l 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/l 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.

\[ \text{Corpus allatum volume } \left[ x10^{-3} \text{mm}^3 \right] \]

\[ \text{Days since adult emergence} \]

\[ \text{Accessory reproductive gland volume } \left[ x10^{-3} \text{mm}^3 \right] \]

\[ \text{Days since adult emergence} \]

Vertical bars: SEM.

\[ \triangle \text{ Precocene treated insects} \]

\[ \bullet \text{ Acetone control insects} \]

\[ \circ \text{ Precocene treated insects} \]

\[ \cdot \text{ Acetone control insects} \]
Figure 8a

Graph showing changes in the volume of the CA in the male *D. fasciatus* treated with 20 μg/l 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/l μl precocene II. A retarded pattern of development is shown in the precocene treated insects.
Figure 8a.

Figure 8b.

\[ \text{Corpus allatum volume} \times 10^{-3} \text{mm}^3 \]

Days since adult emergence

\[ \text{Accessory reproductive gland volume} \times 10^{-3} \text{mm}^3 \]

Days since adult emergence

\[ \Delta \rightarrow \Delta \text{ Precocene treated insects} \]

\[ \bullet \rightarrow \bullet \text{ Acetone control insects} \]

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 antiallactotropin 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 hardly 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/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).
CA = Corpus allatum

Cl = Clumped cytoplasmic contents
3.3.4 The effect of Precocene II on the cellular structure of the ARGs

The histology of the ARGs following the application of various doses of precocene II corroborated 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 showing 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 smaller 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 13:

A photomicrograph of a longitudinal section of the mesadenes of a four day old male *D. fasciatus* treated topically with 5 μg/l 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/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).
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 entered 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>
<thead>
<tr>
<th>Days after adult-emergence</th>
<th>Percentage of experimental bugs in copulation</th>
<th>Percentage of control bugs in copulation</th>
</tr>
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<tbody>
<tr>
<td>0</td>
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</table>
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 Corpora allata (CA) in the male D. fasciatus 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 Corpora allata (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. Despite 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 D. fasciatus 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 other hand, in the male insects, no cyclical changes in the size of the CA have been observed (Johansson, 1958; Scharre 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 gonadotrophic 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 development 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 D. 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 occurring 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 widespread 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 ≤ 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 allatomection 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 occurred 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 \( \mu g \) doses of precocene, normal descent of sperm was observed. Doses of 5 \( \mu g \) and above delayed sperm descent by three to four days before it finally started to come in trickles. Higher doses (above 10 \( \mu g \)), 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 contrary 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 need 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*


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

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Mean volume $\pm$ S.E. ($x 10^{-3} \text{ mm}^3$, $n = 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.60 $\pm$ 0.20</td>
</tr>
<tr>
<td>1</td>
<td>6.20 $\pm$ 0.20</td>
</tr>
<tr>
<td>2</td>
<td>6.64 $\pm$ 0.30</td>
</tr>
<tr>
<td>3</td>
<td>7.52 $\pm$ 0.28</td>
</tr>
<tr>
<td>4</td>
<td>7.53 $\pm$ 0.28</td>
</tr>
<tr>
<td>5</td>
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</tr>
<tr>
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<td>7.25 $\pm$ 0.43</td>
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<tr>
<td>9</td>
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</tr>
<tr>
<td>10</td>
<td>4.61 $\pm$ 0.30</td>
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<tr>
<td>11</td>
<td>4.30 $\pm$ 0.36</td>
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<tr>
<td>12</td>
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</tr>
<tr>
<td>20</td>
<td>4.83 $\pm$ 0.24</td>
</tr>
</tbody>
</table>

$X = 6.14$  
S.D. = 0.98  
S.E. = 0.21
APPENDIX II: Changes in size of the ARGs in the unpaired male
\textit{D. fasciatus} during sexual maturation

<table>
<thead>
<tr>
<th>Age (days)</th>
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<td>17.54 ± 1.34</td>
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<td>3</td>
<td>19.90 ± 1.09</td>
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<td>4</td>
<td>19.27 ± 1.02</td>
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<td>25.63 ± 3.29</td>
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<td>28.24 ± 1.60</td>
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<td>27.92 ± 2.25</td>
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<tr>
<td>20</td>
<td>30.08 ± 3.15</td>
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</table>

$\bar{x} = 24.39$ \hspace{1cm} S.D. = 6.16 \hspace{1cm} S.E. = 1.34
APPENDIX III: Changes in size of the CA in paired male *D. fasciatus* during the 1st copulatory cycle

<table>
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<tr>
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<td>$5.36 + 0.21$</td>
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<td>8</td>
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<td>$5.36 + 0.25$</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>$5.29 + 0.36$</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>$5.00 + 0.35$</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>$3.81 + 0.38$</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>$4.98 + 0.31$</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>$2.50 + 0.19$</td>
</tr>
</tbody>
</table>

$\bar{x} = 4.80$  
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

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

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

- = Period after the insects have disengaged.
Changes in size of the CA in the male *D. fasciatus* treated topically with 1 μg/μl Precocene II at adult emergence.

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. (x 10⁻³ mm³, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental Precocene treated</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.35 ± 0.35</td>
</tr>
<tr>
<td>2</td>
<td>5.76 ± 0.32</td>
</tr>
<tr>
<td>3</td>
<td>5.43 ± 0.35</td>
</tr>
<tr>
<td>4</td>
<td>5.11 ± 0.42</td>
</tr>
<tr>
<td>5</td>
<td>6.05 ± 0.39</td>
</tr>
<tr>
<td>6</td>
<td>5.65 ± 0.36</td>
</tr>
<tr>
<td>7</td>
<td>5.38 ± 0.26</td>
</tr>
<tr>
<td>8</td>
<td>5.41 ± 0.47</td>
</tr>
<tr>
<td>9</td>
<td>4.85 ± 0.29</td>
</tr>
<tr>
<td>10</td>
<td>5.14 ± 0.27</td>
</tr>
</tbody>
</table>

(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 *D. fasciatus* treated topically with 1 μg/1 μl Precocene II at adult emergence.

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume + S.E. (x 10⁻¹ mm³, n = 10)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Precocene treated</td>
<td>Acetone treated</td>
</tr>
<tr>
<td>1</td>
<td>7.65 ± 0.47</td>
<td>8.80 ± 0.76</td>
</tr>
<tr>
<td>2</td>
<td>13.41 ± 1.83</td>
<td>13.32 ± 0.90</td>
</tr>
<tr>
<td>3</td>
<td>15.01 ± 0.95</td>
<td>18.35 ± 1.11</td>
</tr>
<tr>
<td>4</td>
<td>15.71 ± 1.23</td>
<td>18.13 ± 1.20</td>
</tr>
<tr>
<td>5</td>
<td>20.73 ± 1.54</td>
<td>22.27 ± 2.02</td>
</tr>
<tr>
<td>6</td>
<td>23.61 ± 1.04</td>
<td>25.65 ± 1.08</td>
</tr>
<tr>
<td>7</td>
<td>23.28 ± 2.23</td>
<td>28.24 ± 1.60</td>
</tr>
<tr>
<td>8</td>
<td>22.27 ± 2.03</td>
<td>24.04 ± 0.76</td>
</tr>
<tr>
<td>9</td>
<td>22.87 ± 1.80</td>
<td>25.45 ± 1.55</td>
</tr>
<tr>
<td>10</td>
<td>23.64 ± 2.44</td>
<td>27.28 ± 2.27</td>
</tr>
</tbody>
</table>

(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 *D. fasciatus* treated topically with 5 μg/μl Precocene II at adult emergence

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

(a) $\bar{X} = 3.78$  \hspace{0.5cm} S.D. = 0.58  \hspace{0.5cm} S.E. = 0.18
(b) $\bar{X} = 5.68$  \hspace{0.5cm} S.D. = 0.79  \hspace{0.5cm} S.E. = 0.25  \hspace{0.5cm} P \leq 0.05 (significant)
APPENDIX VIII: Changes in size of the ARG in the male D. fasciatus treated topically with 5 μg/1 μl Precocene II at adult emergence

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume + S.E. (x 10^{-1} mm^3, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Experimental</strong>^a</td>
</tr>
<tr>
<td></td>
<td>Precocene treated</td>
</tr>
<tr>
<td>1</td>
<td>8.97 ± 1.14</td>
</tr>
<tr>
<td>2</td>
<td>10.19 ± 0.80</td>
</tr>
<tr>
<td>3</td>
<td>11.19 ± 0.93</td>
</tr>
<tr>
<td>4</td>
<td>14.33 ± 0.60</td>
</tr>
<tr>
<td>5</td>
<td>18.59 ± 2.05</td>
</tr>
<tr>
<td>6</td>
<td>21.87 ± 2.77</td>
</tr>
<tr>
<td>7</td>
<td>21.86 ± 3.15</td>
</tr>
<tr>
<td>8</td>
<td>23.70 ± 2.55</td>
</tr>
<tr>
<td>9</td>
<td>19.13 ± 1.98</td>
</tr>
<tr>
<td>10</td>
<td>23.23 ± 2.71</td>
</tr>
</tbody>
</table>

(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 ≤ 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

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. (x 10^{-3} mm^3, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental (^a)</td>
</tr>
<tr>
<td></td>
<td>Precocene treated</td>
</tr>
<tr>
<td>1</td>
<td>4.32 ± 0.18</td>
</tr>
<tr>
<td>2</td>
<td>4.08 ± 0.34</td>
</tr>
<tr>
<td>3</td>
<td>3.17 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>2.03 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>2.95 ± 0.22</td>
</tr>
<tr>
<td>6</td>
<td>3.52 ± 0.21</td>
</tr>
<tr>
<td>7</td>
<td>3.41 ± 0.28</td>
</tr>
<tr>
<td>8</td>
<td>3.70 ± 0.22</td>
</tr>
<tr>
<td>9</td>
<td>3.76 ± 0.19</td>
</tr>
<tr>
<td>10</td>
<td>3.69 ± 0.20</td>
</tr>
</tbody>
</table>

(a) \(\bar{X} = 3.52\) \hspace{1cm} S.D. = 0.64 \hspace{1cm} S.E. = 0.20 \hspace{1cm} P < 0.05 \hspace{1cm} (significant)

(b) \(\bar{X} = 5.47\) \hspace{1cm} S.D. = 0.42 \hspace{1cm} S.E. = 0.13
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

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. ( \times 10^{-1} \text{ mm}^3 ), ( n = 10 )</th>
<th>Experimental Precocene treated</th>
<th>Control Acetone treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1 )</td>
<td>9.39 ± 0.54</td>
<td>10.29 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>( 2 )</td>
<td>12.09 ± 0.47</td>
<td>14.03 ± 1.06</td>
<td></td>
</tr>
<tr>
<td>( 3 )</td>
<td>12.65 ± 1.21</td>
<td>14.58 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>( 4 )</td>
<td>12.12 ± 0.96</td>
<td>14.57 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>( 5 )</td>
<td>16.38 ± 1.70</td>
<td>25.33 ± 1.31</td>
<td></td>
</tr>
<tr>
<td>( 6 )</td>
<td>15.47 ± 1.51</td>
<td>24.59 ± 1.61</td>
<td></td>
</tr>
<tr>
<td>( 7 )</td>
<td>20.55 ± 1.94</td>
<td>20.24 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>( 8 )</td>
<td>19.94 ± 2.12</td>
<td>21.46 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>( 9 )</td>
<td>21.12 ± 2.06</td>
<td>24.03 ± 1.37 ( \cdots )</td>
<td></td>
</tr>
<tr>
<td>( 10 )</td>
<td>19.01 ± 1.23</td>
<td>24.87 ± 1.12</td>
<td></td>
</tr>
</tbody>
</table>

(a) \( \bar{X} = 15.87 \) \hspace{1cm} S.D. = 4.17 \hspace{1cm} S.E. = 1.32 \hspace{1cm} P < 0.05 (not significant)

(b) \( \bar{X} = 19.39 \) \hspace{1cm} S.D. = 5.54 \hspace{1cm} S.E. = 1.75
APPENDIX XI: Changes in size of the CA in the male *D. fasciatus* treated topically with 15 μg/μl Precocene II at adult emergence

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. (x 10^{-3} mm^3, n = 10)</th>
<th>( X )</th>
<th>S.D.</th>
<th>S.E.</th>
<th>P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental(^a)</td>
<td>Control(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Precocene treated</td>
<td>Acetone treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.00 ± 0.22</td>
<td>3.59 ± 0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.97 ± 0.17</td>
<td>4.99 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.77 ± 0.10</td>
<td>4.79 ± 0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.59 ± 0.07</td>
<td>4.40 ± 0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.58 ± 0.06</td>
<td>5.91 ± 0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.57 ± 0.14</td>
<td>5.66 ± 0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.97 ± 0.42</td>
<td>6.62 ± 0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.59 ± 0.23</td>
<td>6.57 ± 0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) \( \bar{X} = 2.755 \)  
(b) \( \bar{X} = 5.31 \)
APPENDIX XII: Changes in size of the ARGs in the male D. fasciatus treated topically with 15 μg/1 μl Precocene II at adult emergence

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. ( \times 10^{-1} \text{ mm}^3 ), ( n = 10 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental a</td>
</tr>
<tr>
<td></td>
<td>Precocene treatment</td>
</tr>
<tr>
<td>1</td>
<td>7.57 ± 0.50</td>
</tr>
<tr>
<td>2</td>
<td>7.77 ± 0.62</td>
</tr>
<tr>
<td>3</td>
<td>9.44 ± 0.63</td>
</tr>
<tr>
<td>4</td>
<td>11.05 ± 1.02</td>
</tr>
<tr>
<td>5</td>
<td>17.48 ± 1.01</td>
</tr>
<tr>
<td>6</td>
<td>21.75 ± 1.90</td>
</tr>
<tr>
<td>7</td>
<td>21.01 ± 2.98</td>
</tr>
<tr>
<td>8</td>
<td>20.08 ± 0.93</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume ± S.E. ($x 10^{-3}$ mm$^3$, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental Precocene treated</td>
</tr>
<tr>
<td>1</td>
<td>3.18 ± 0.26</td>
</tr>
<tr>
<td>2</td>
<td>3.09 ± 0.29</td>
</tr>
<tr>
<td>3</td>
<td>2.27 ± 0.34</td>
</tr>
<tr>
<td>4</td>
<td>2.45 ± 0.36</td>
</tr>
<tr>
<td>5</td>
<td>2.61 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>2.80 ± 0.22</td>
</tr>
<tr>
<td>7</td>
<td>3.35 ± 0.28</td>
</tr>
<tr>
<td>8</td>
<td>3.13 ± 0.24</td>
</tr>
</tbody>
</table>

(a) $\bar{X} = 2.86$  
S.D. = 0.38  
S.E. = 0.13  
P $\leq 0.05$

(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

<table>
<thead>
<tr>
<th>Period after treatment (days)</th>
<th>Mean volume + S.E. (x 10^{-1} mm^3, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental(^a)</td>
</tr>
<tr>
<td></td>
<td>Precocene treated</td>
</tr>
<tr>
<td>1</td>
<td>7.19 + 0.78</td>
</tr>
<tr>
<td>2</td>
<td>8.35 + 0.48</td>
</tr>
<tr>
<td>3</td>
<td>8.66 + 0.70</td>
</tr>
<tr>
<td>4</td>
<td>14.77 + 1.54</td>
</tr>
<tr>
<td>5</td>
<td>18.09 + 1.42</td>
</tr>
<tr>
<td>6</td>
<td>20.78 + 1.45</td>
</tr>
<tr>
<td>7</td>
<td>16.46 + 2.20</td>
</tr>
<tr>
<td>8</td>
<td>24.73 + 1.59</td>
</tr>
</tbody>
</table>

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

(b) $\overline{X} = 19.11$  
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*

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>% Estimate of insects which show sperm descent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precocene concentration (μg/1 μl)</td>
</tr>
<tr>
<td></td>
<td>1 ug</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
</tr>
</tbody>
</table>