CHAPTER 1
GENERAL INTRODUCTION
Abounding almost everywhere and feeding on plants, many insect pests transform millions of tons of valuable plant matter into animal matter and wastes. One important reason for the domination of insect pests in this biosphere has been attributed to their remarkable ability for successful insemination and subsequent ovipositional activities that are primarily controlled by accessory sex glands (ASGs). ASGs occur as secondary structures associated with the primary reproductive organs to ensure the reproductive success of the species either by facilitating the transfer or by protecting the gametes.

1.1 Insect Reproductive System

The male reproductive organs typically consist of a pair of testes connected to a pair of seminal vesicles and a median ejaculatory duct. In some Lepidoptera, the two testes are fused completely to form a median structure. The testes are composed of a number of follicles. Each testis follicle is connected to tubular vas deferens by means of a short vas efferens. The vas deferens runs backwards to lead into the distal end of the ejaculatory duct. In many insects, the seminal vesicles in which the sperms are stored before transfer to the female are dilations of the vasa deferentia. The ejaculatory duct extend inwards as an unpaired duct. This is called simplex. Its anterior end known as duplex is dilated and bifurcated. This is connected with the ASGs
and vasa deferentia. In Lepidoptera sperms are temporally stored in the expanded regions of the vasa deferentia and then are transferred to duplex.

Among the various insect orders, the external shape and morphology of organs in the female genital tract are variable. In female insects the reproductive system consists of a pair of ovaries. Each ovary is composed of several ovarioles that lie loose in the body cavity. The ovarioles are joined at their distal end by an apical filament that is connected to the body wall, and proximally by the lateral oviducts. The number of ovarioles per ovary is species specific. Each ovariole has a germarium at its distal tip where oogonia undergo meiosis to produce oocytes. Each oocyte becomes surrounded by a single layer of follicle cells that in the early stages of oocyte growth controls the incorporation of vitellogenin, and later secretes the egg shell around the mature egg. The lateral oviducts merge to form a median oviduct opening posteriorly into a genital chamber. Sometimes the genital chamber forms a tube, the vagina and this is often developed to form a bursa copulatrix for reception of aedeagus from the males. Associated with the median oviduct a single spermatheca with spermathecal gland and paired ASGs are seen.

1.2 Reproductive structures involved in secretory processes

Within the class Insecta the form and relative function of various glands that participate in the reproductive process are almost as diverse as the insects themselves. In many species of insects the structures associated with
the reproductive system are involved in different kinds of secretory processes. They include:

1. Gonadal glands comprising glands of testes and glands of ovaries.
2. Ductal glands including glands of the vasa deferentia, ejaculatory duct, lateral oviduct, common oviduct and vagina including bursa copulatrix and spermatophoral receptacle.
3. Seminal glands
4. Spermathecal glands
5. Collateral glands

1.3 Reproductive structures involved in secretion in male insects

1.3.1 Gonadal glands

The principal function of the glandular cells in the testes is to supply nutrients to the differentiating germ cells. In the testes of many insects including Lepidopterans a large cell (Verson’s cell) in the apex of germarium assumed to have a nutritive function has been found. In *Dielphia euphorbiae* at the time of maturation of spermatids in the late larval and pupal period a maximum number of granules and droplets are observed in the cells of the inner lining of testicular sheaths (Buder, 1917). Nutritive function for Verson’s cell of testes is reported in *Bombyx mori* (Omura, 1938). Nurse cells
of testes are involved in the secretion of a mucoprotein which forms the caps in the sperm heads (Szollosi, 1974). Testes are also found to secrete chemicals during insemination.

1.3.2 Ductal glands

They include glands of vasa deferentia and ejaculatory duct. They vary from primitive secretory epithelial cells to compact glands in nature. Secretory cells of the ejaculatory duct may have either ectodermal or mesodermal origin. Ectodermal origin of ejaculatory duct is reported from *Locusta migratoria* (Gregory, 1965); *Drosophila melanogaster* (Bairati, 1968); *Melolontha melolontha* (Landa, 1960) and *Chironomus plumosus* (Wensler and Rempel, 1962). When the ejaculatory duct includes a mesodermal component, the epithelial cells of this component are almost always secretory. Species with mesodermal secretory cells are found in the ejaculatory duct of *Diadromus pulchellus* (Rojas Rousse, 1972), *Plecia nearctica* (Trimble, 1974) and in all Lepidoptera (Norris, 1932; Musgrave, 1937; Omura, 1938; Callahan and Cascio, 1963; Reimann and Thorson, 1976, 1979; Lai-Fook, 1982). In Lepidoptera the mesodermal ejaculatory duct component includes the paired ‘duplexes’ and a median noncuticular simplex. The simplex is divisible into distinct regions on the basis of the secretions it contains and the histology of the cells. In ejaculatorius duplex of *Spodoptera litura* apocrine mode of secretion with
occasional merocrine or holocrine secretion is observed (Amaldose, 1987). Leopold (1970) concludes that the nature of ejaculatory duct secretions in *Musca domestica* is proteinaceous and contain a high proportion of dibasic aminoacids. Thibout (1971) demonstrated the presence of proteins, mucopolysacharide and lipid glycoproteins in *Acreolepia assectella*.

Various functions have been ascribed to the ejaculatory duct secretions of insects. Wensler and Rempel (1962) have reported that in insects like *Chironomus* where the collateral glands are absent, the ejaculatory duct secretes the components of seminal fluid. Sheehan *et al.*, (1979) and Stein *et al.*, (1984) reports the presence of carboxylesterase enzyme in the anterior ejaculatory duct of *D. melanogaster* which appears to be involved in the sperm motility perhaps by facilitating metabolism of lipids in the ejaculate (Gilbert, 1981).

In Lepidoptera secretions of the noncuticular simplex contribute along with those from collateral glands for the formation of the spermatophore. Several authors have shown that the non-cuticular simplex of Lepidoptera secretes a sperm activator (Omura, 1938; Shepherd, 1974; Herman and Peng, 1976). The ejaculatory duct of *Musca domestica* produces a secretion which inhibits receptivity in females (Reimann *et al.*, 1967).

Secretory cells of vas deferens vary considerably in shape, size and stainability among species or in different parts of the duct in the same species.
The biochemical nature of secretory product of the vas deferens is not well understood. Cantacuzene (1968, 1971) and Rojas-Rousse (1972) have identified the secretion as a mucopolysaccharide. Gerber et al., (1971) on the other hand state that the secretion is a carbohydrate-protein complex, but does not include glycogen or chitin.

Landa (1959) have observed that in *Melolontha melolontha* the secretion of vas deferens is used for growth of the cyst cells which are later transferred to the female ducts along with the spermatophore. Bouix (1966), Rojas–Rousse (1972) and Gerber et al., (1978) speculate that the secretion of vas deferens is used to nourish sperm while they are being retained in the male genital tract. Involvement of the vas deferens in spermatophore production has been clearly demonstrated in some Trichoptera (Khalifa, 1949) and in *Lytta nuttali* (Gerber et al., 1971). In Tettigonoids (Orthoptera) the material which binds sperm together as a spermatodesma is secreted by cells of the intratesticular region of the vas deferens.

### 1.3.3 Seminal Glands

Seminal glands are glandular structures occurring in the sperm storage organs of male insects called the seminal vesicles. They are absent in Trichoptera. The seminal vesicles are simply dilations of the vas deferens in Thysanura, Ephemeroptera, most Hemiptera, Neuroptera and in some Hymenoptera. While in other Hymenoptera and nematocerous Diptera, they
are dilations of the ejaculatory duct. Seminal glands are not secretory in all species. In most species they are of mesodermal origin. Ectodermal origin of seminal glands is reported in nematocerous Diptera. In Lepidoptera, there are two sites of storage. With in each vas deferens is a swollen region which is normally referred to as the true seminal vesicles. In *Anagasta kuhniella*, the upper part of each branch of the ductus ejaculatorius duplex act as seminal vesicles (Reimann and Thorson, 1976).

The histology of the seminal vesicles is basically similar to that of other parts of efferent duct. In almost all species examined to date the epithelium of the seminal vesicles apparently functions as a store for sperm. In most species however the epithelium is secretory, at least temporarily and is therefore columnar, though when the seminal vesicles replete with sperms cells often take a more flattened appearance. The nature of seminal vesicle secretion in *Schistocerca gregaria*, studied by Cantacuzene (1967) showed that it primarily contains proteinaceous granules which are later replaced by acidic mucopolysacharides. The seminal plasma of *Periplaneta americana* contains much glycogen and phospholipids, other unidentified PAS positive substances and a small amount of proteins (Vijayalakshmi and Adiyodi, 1973).
1.3.4 Collateral glands

Collateral glands are paired glandular structures which in most species release their product into the common genital tract at its anterior end or at some point along its length. They include ASGs, prostate glands, the mushroom shaped glands and the congoblate glands of cockroach.

1.4 Reproductive structures involved in secretion in female insects

1.4.1 Gonadal Glands

Glandular cells involved in secretory process include nurse cells and follicular epithelium of ovaries. They help in the nourishment of the developing gametes, in the production of yolk components, secretion of chorion and in the formation of vitelline membrane. The follicular cells secrete, at different times throughout oocyte development, a variety of material. In *Nepidae* the follicular epithelium secretes a cementing substance for gluing the eggs to the substratum (Hinton, 1961).

1.4.2 Ductal glands

It includes glands of the lateral oviduct, common oviduct and vagina. The epithelial cells of lateral and common oviduct have a secretory function. Lateral oviduct secretions have several functions including ootheca formation, lubrication of eggs passing through the genital tract, cementing the eggs to each other and to the substrate as observed in *Lytta* (Sweeny *et al.*, 1968;
Gerber et al., 1971). In Acrididae the common oviduct secretion forms an extra chorion around the egg (Hartley, 1961). Although the vagina in most species is nonsecretory, the diverticulum at the anterior end of vagina called ‘Bursa copulatrix’ has a secretory function. Spermatophore is digested and absorbed in the bursa in Melolontha melolontha (Landa, 1960) and in Lytta nuttali (Gerber et al., 1971). Khalifa (1949) observed that a bursal gland might provide nourishment for the sperm in the absence of spermatophore.

1.4.3 Spermathecal Glands

In many insects, the spermatheca serves both as a sperm storage structure and as a secretory organ. In many species, storage and glandular functions are physically separated through the development of one or more spermathecal glands. Secretion of spermatheca provides nutrients for the sperm.

1.4.4 Collateral glands

Collateral glands in female insects include ASGs, colleterial (cement) glands of cockroaches and Milk glands of tsetse flies.

1.5 Accessory sex glands: An Overview

The classification of ASGs in insects is essentially based on anatomical and ontogenic relationships. In different groups of insects these glands vary considerably in size, shape, number, anatomical placements and
embryological origin (Blain and Dixon, 1973; Ramalingam, 1974; Adiyodi and Adiyodi, 1975; Leopold, 1976; Happ, 1984; Couche and Gillott, 1990; Chapman, 1998; Ferreira et al., 2004). They may occur as heterogeneous, unpaired structures as in Dictyoptera, as multiple paired structures as in the Thysanoptera (Shaaya, 1933) and Coleoptera (Escherich, 1894) or just as paired structures which is most common. ASGs are primitively absent in Thysanura, Ephemeroptera, Plecoptera, Dermaptera and in most Odonata but in many higher Diptera they are secondarily lost.

ASGs vary from a simple tube, identical to other conductive channels of the reproductive tract to histologically complex tubes with regional differentiation as occurs in most lepidopterans (Riemann and Thorson, 1979; Lai-Fook, 1982). Anatomically ASGs of most male insects possess a single glandular epithelium surrounding a lumen filled with secretion. Outer to the epithelium either a single or a double layer of muscle layer is seen (Adiyodi and Adiyodi, 1974; Lai-Fook, 1982; Couche and Gillott, 1990; Fernandez and Cruz-Landim, 2005; Cruz-Landim and Dallacqua, 2005).

ASGs show remarkable uniformity in terms of both their embryonic origin and their general cytology. In males ASGs are of mesodermal in origin and are described as mesadenia. Specifically they arise from the terminal ampullae of the vasa deferentia which themselves are derived from the coelomic cavities of the ninth or tenth abdominal segment. Throughout the
larval period the mesadenial anlagen remains in an embryonic condition i.e.,
small hollow vesicles attached to the mesodermal cords which later become
the vasa deferentia. Ectadenia which opens into the ejaculatory duct are found
in Orthoptera and in many other insects. In some species of Heteroptera and
Coleoptera, both ectadenia and mesadenia are present. In endopterygotes
organogenesis takes place during the pupal stage. In *Bombyx mori* ASGs are
fully differentiated during eclosion whereas in *Tenebrio molitor*
differentiation is not completed until several days after adult emergence
(Gillott and Gaines, 1992).

Numerous studies have demonstrated that ASGs play an essential role
in reproduction. In most insects ASGs become functional in adults. Several
functions have been attributed to the secretion produced by ASGs. The
functions of ASGs can be classified as structural, biochemical, behavioural
and physiological (Fernandez and Cruz-Landim, 2005).

Early histochemical studies showed that the ASG secretion is a
complex mixture of proteins, often conjugated with lipid or carbohydrate
moieties, free lipids, carbohydrates, prostaglandins, amines and cGMP, uric
acid aminopeptidases, free amino acids and hydrolytic enzymes like esterases,
amidases etc (Roth, 1967; Cmelik *et al.*, 1969; Leopold, 1981; Federer and
Chen, 1982; Judd *et al.*, 1983; Sevala and Davey, 1991; Muse and Balogun
The ASGs produce secretions with a variety of functions, including contribution to the seminal fluid and activation of the spermatozoa (Davey, 1985; Chen, 1984). The primary function of the secretion produced by the male ASGs is spermatophore formation. ASGs are involved in the building of spermatophore for sperm transfer to the female (Viscuso, et al., 2001). Spermatophore of Lepidoptera is formed wholly within the female ducts after the start of copulation. The secretion from ASGs form the outer matrix of spermatophore and form the spermatophragma which blocks the duct to the female’s bursa copulatrix (Osanai et al., 1987; Fanger and Naumann, 1993).

Male insects often transfer a number of auxiliary substances to females during copulation. Male *Drosophila* transfer seminal fluids which, among other things, stimulate egg laying (Kubli, 1996). Males may also transfer nutrients to females which are subsequently incorporated into somatic maintenance or reproductive output (ova) (e.g. Simmons, 1995), and for some Diptera it has also been suggested that spermatozoa additionally function as nutrient provisioning (Pitnick and Markow, 1994). Importantly however, many theoretical and empirical studies indicate that it is the interactions between male and female characteristics which determine the outcome of many reproductive processes (Knowlton and Greenwell, 1984; Rice, 1996; Zeh, 1997; Otronen et al., 1997; Wilson et al., 1997; Holland and Rice, 1998; Hosken and Stockley, 1998). The secretion changes the female reproductive behaviour and physiology after copulation. (Chen, 1984; Happ, 1984; Gillott,
1996; Herndon et al., 1997; Smid, 1997; Wolfner, 1997; Chen et al., 1998; Heifetz et al., 2001). Fecundity enhancing and receptivity-inhibiting substances have also been reported in the secretion of ASGs (Gillott, 2003).

In females, ASGs are found in Thysanura, Odonata, many Orthopteroid insects, Thysanoptera, Homoptera and most endopterygotes. Unlike male insects they are absent in Orthoptera, Psocoptera, Heteroptera and in most Coleoptera (Mustuda, 1976). In most insects they are paired structures and join the common genital tract at a point behind the opening of spermathecal duct. The glands normally originate from an invagination on the ninth abdominal sternum. The anlagen remain small throughout most of the larval period with organogenesis beginning during the last juvenile stadium.

Though the ASGs differ in form among female insects, their histology is quite uniform and includes from inside to outside, a chitinous intima, one or two layers of cells, and a basement membrane. A layer of muscle outside the basement membrane may or may not be present. The presence of chitinous intima explains the ectodermal origin of ASGs as reported earlier (Gillott, 1988; Kaulenas, 1992). Though the morphology of ASGs vary among different insect orders, and also within a limited group of insect species, their internal structure depends upon the function of the organs (Brunet 1952; Gillott 1988; Kaulenas 1992). Synthesis of proteins, lipids and glycogenous polysaccharides are reported from the ASGs of female lepidopterans
(Salkeld and Potter, 1953; Beament and Lal, 1957; Grayson and Berry, 1974; Geetha, 2003).

The function of ASGs of female insects varies (Davey, 1985). ASGs produce oviposition pheromone, secretions which coat and fasten eggs to laying substrates, silk to form egg cocoon, provide lubrication, egg protection, dissolve spermatophores and provide nutrition for the young larvae. In *Musca domestica* ASG secretions are moved with spermatozoa to the fertilization chamber where they aid micropyle cap removal, allowing fertilization to take place (Leopold and Degrugillier, 1973; Leopold *et al*., 1978). Furthermore, female ASG secretions trigger the acrosome reaction when present with micropylar cap substance and, in higher concentrations, cause degradation of spermatozoa (Degrugillier, 1985). However in some insects female ASGs are typically adhesive-producing (Lococo and Huebner, 1980). The other functions of the secretions are relatively unknown, especially when compared with male ASGs and their secretions (Chapman *et al*., 1995; Fernandez and Klowden, 1995; Kubli, 1996; Rice, 1996; Soller *et al*., 1997; Tram and Wolfner, 1998). Callahan and Cascio (1963) suggest that secretions of female ASGs in noctuid moths act as a lubricant to aid the movement of the sperm from the spermathecal duct.
1.6 Role of hormones in the development and differentiation of ASGs

Previous studies show that in most insects including lepidopterans the post-embryonic development and differentiation of ASGs are regulated by the interplay of two major insect hormones; juvenile hormone (JH) and ecdysteroids, the former inhibiting and the latter promoting these processes. Growth and protein synthesis in the ASGs are regulated by both ecdysteroids and juvenile hormone (JH), with development and differentiation being under the control of ecdysteroids and protein secretion being regulated by JH in Lepidoptera (Herman, 1973; Herman and Bennett, 1975; Herman and Dallmann, 1981). In males of Bombyx mori and Tenebrio molitor ecdysteroids were found stimulating the development of ASGs during the pupal period but acting antagonistically during the adult stage (Shinbo and Happ, 1989; Yaginuma and Happ, 1989). In the Lepidopteran Heliothis virescens (F), the differentiation of ASGs from the genital imaginal discs requires the presence of both a sufficient titer of ecdysteroids and testis sheath factors (Loeb, 1991).

In contrast to this, post-eclosion activity (i.e. production of secretion) of ASGs for most species is regulated by JH. In male moths of Ephesia cautella ecdysteroid titres are relatively low throughout their adult life (Shaaya et al., 1991). The allatectomy inhibit post-eclosion growth of ASGs in Danaus Plexippus L. (Herman, 1975, 1975). The differences in JH titres affect reproductive output (Trumbo and Robinson, 2004). A rapid increase in
the JH titre in the newly eclosed adults is reported in *Drosophila melanogaster* and is a probable key feature in the maturation of gametes and testes (Bownes and Rembold, 1986).

Many authors have reported hormonal regulation of ASG secretory activity in female insects (Ejeze and Davey, 1974, 1976, 1977; Koepp *et al.*, 1985; Davey, 1985). Specifically JH inhibits and ecdysteroids promote differentiation of ASGs (Bodenstein and Sprague, 1959). With regard to the endocrine control of collateral gland secretion in Lepidoptera. Several authors have reported that allatectomy or head/neck ligation prevented normal development of the female glands (Herman, 1975; Herman and Bennet, 1975; Herman and Dallmann (1981) and Lessman *et al.*, (1982). Herman and Barker (1976) reported that a single large dose of ecdysterone stimulated gland development in monarch butterflies.

### 1.7 Insect Growth Regulators

Insect growth regulators (IGRs) are insecticides that mimic the action of hormones on the growth and development of insects. The influence of hormones in an insect’s life cycle and reproduction is the centre point in the development of IGRs which act as hormone agonists or antagonists. These compounds induce a disruption of the normal growth and reproduction of insects. IGRs with their reduced toxicity to the environment and target specificity are highly advantageous when compared to conventional
insecticides in integrated pest control strategies. They have a good margin of safety to man and domestic animals and to other most non target biota including invertebrates, fish, birds and other wild life. The effectiveness and selectivity of IGRs provide new tools in Integrated Pest Management (IPM) Programmes (Oberlander and Silhacek, 1998).

There are three categories of IGRs

1) Compounds which directly or indirectly influence the hormones which regulate post embryonic development, metamorphosis and reproduction of insects e.g. Juvenile hormone (JH) analogues and anti-JH agents

2) Compounds which inhibit cuticle formation through an effect on cuticle synthesis e.g. benzoyl phenyl ureas

3) Compounds with miscellaneous modes of action e.g. azadirachtin.

IGRs based on insect hormones have great significance as pesticides of the future and also as excellent chemical probes to elucidate the role of hormones in the basic physiological processes of insects.

1.8 IGRs based on hormones

There are three major categories of insect hormones: neurohormones secreted by the neurosecretory cells of brain and segmental ganglia, Juvenile hormone (JH) secreted by corpora allata (CA) and ecdysteroids secreted by
the prothoracic glands and other tissues. Ecdysteroids and JH regulate many physiological events throughout the insect life cycle including moulting, metamorphosis, ecdysis, diapause, reproduction and behaviour (Gelman et al., 2007). A critical titre of hormones in body fluids is a prime requirement in different physiological processes of insects. Any interference in the biosynthesis and degradation of hormones will disrupt the hormone dependent physiological processes of insects. Further the regulation of secretion, transportation from the secretory to the target site, binding to the membrane receptors, degradation, excretion and feed back control are all biochemical steps vulnerable to manipulation for insect control purposes. Such manipulation of the hormonal levels in the haemolymph will cause a derangement of hormone dependent processes of morphogenesis and reproduction. Based on this concept many hormone analogues and antihormones have already been developed.

(i) IGRs based on neurohormones

Peptide hormones produced and released from the neurons play diverse functional roles in insects as chemical messengers controlling growth and development in insects. A number of neuropeptides are synthesized in the median and lateral neurosecretory cells of brain. IGRs based on neuropeptides for insect pest control is not well advanced since many neurohormones in insects have not been fully characterized. The diversity and complexity of
neurohormones however offer a lot of possibilities for design and development of neurohormone analogues.

(ii) IGRs based on ecdysteroids

Ecdysteroids are the steroid hormones of insects. Ecdysteroids control insect development, being known primarily as regulators of moulting and metamorphosis, but they have also been implicated in the control of many other physiological and developmental processes e.g. reproduction and embryogenesis (Koolman, 1989). Ecdysone mimics or ecdysoids are compounds which are structurally similar to ecdysteroids and possess moulting hormone activity in insects. They are classified into four groups. Zooecdysteroids, phytoecdysoids (extracted from plants), synthetic ecdysoids (steroids with moulting hormone activity) and nonsteroidal agonists. There have been a number of studies on the effect of ecdysone analogues/agonists on the reproduction of important pest species (Carpenter and Chandler, 1994; Smagghe and Deghlee, 1994; Biddinger and Hull, 1999; Knight, 2000). The ecdysone analogues/agonists are highly specific to lepidopteran larvae and their effectiveness against many economically important horticultural, agronomic and forest pests have been reported (Chandler et al., 1992; Charmillot et al., 1994; Retnakaran et al., 1997; Trisyono and Chippendale, 1997, 1998). Wing (1988) has suggested that the ecdysteroid analogues/agonists would interact with the ecdysteroid receptor complex and thereby
induce their effects. The first bisacylhydrazine ecdysteroid agonist was discovered by Rom and Hass Company in 1983. Subsequent chemical modification of this compound led soon to the discovery of a slightly more potent analogue, RH-5849 (Wing, 1988). Treatment of insects with minute doses of RH-5849 interferes with normal feeding activity in larval lepidopterans and insects belonging to other orders, by forcing a lethal, premature moult (Wing et al., 1988; Sakunthala and Nair, 1995). Later another non-steroidal ecdysone mimic RH-5992 (tebufenozide) was discovered and this compound was more potent than RH-5849 in lepidopteran larvae.

Methoxyfenozide (RH-2485) belongs to the novel class of IGRs, (bisacylhydrazine ecdysteroid agonists) mimicking natural ecdysteroids. They have same mode of action as the endogenous 20-hydroxyecdysone (20-H) but the effects are long lasting (Retnakaran et al., 1995). Dhadialla et al., (1998) have reported that RH-2485 has a selective action on lepidopteran insects. The other important ecdysteroid agonists or analogues are RH-5849, Tebufenozide (RH-5992) and Halofenozide (RH-0345). N- tert-Butyl, N, N’ dibenzoylhydrazine and its analogues are nonsteroidal ecdysone agonists that exhibit insect moulting hormonal and larvicidal activities (Minackuchi et al., 2003).
(iii) Anti ecdysteroid Agents

Since ecdysteroids play a critical role in insect development, reproduction and embryogenesis, anti ecdysteroid agents which alter ecdysteroid titre have great potential as insecticides. The normal growth and development of Manduca sexta larvae can be inhibited by two vertebrate hypocholesterolaemic agents, triparanol and 22, 25 di-azacholesterol, by blocking the conversion of β-sitosterol to cholesterol which is a precursor of ecdysone synthesis (Svoboda et al., 1972).

(iv) IGRS based on JH

The major role of JH in insects is to modify the action of ecdysteroids and prevent the switch in the commitment of epidermal cells from larval to imaginal type. In the presence of JH, ecdysteroids are unable to promote the current program of gene expression. JH promotes sexual maturation and behaviour in mature insects. Williams (1967) was the first to suggest that this hormone or its analogues could be used as specific insect control agents. This led to the discovery of JH analogues or juvenoids with great potential in IPM programmes. Juvenoids functionally resembles JH but may or may not be similar in structure. Synthetic JH and JH analogues/ agonists (JHAs) have been shown to have sterilizing and toxic activities against many insects (White and Lamb, 1968; Lim and Yap, 1996; Parkman and Frank, 1998;
Liu and Chen, 2001; Rajapakse et al., 2002; Abo-Elghar et al., 2004; Lim and Leu, 2005; Ouchi, 2005; Liu and Trumble, 2005; Darriet and Corbel, 2006).

The well known juvenoids include Epofenonane (Hangartner et al., 1976) Methoprene, Hydroprene, Kinoprene (Henrick et al., 1976) Phenoxy phenoxy carbamate (Peleg, 1982) Fenoxycarb and Pyriproxyfen (PPN). They are highly effective IGRs that cause a wide range of developmental derangements in susceptible insect species affecting embryogenesis, larval development, metamorphosis and reproduction.

PPN (2-[1-methyl -2-{4-phenoxy phenoxy} ethoxyl] pyridine) is a potent JH agonist that is active in a wide range of arthropods including ants (Vail and Williams, 1995; Vail et al., 1996); fleas (Bull and Meola, 1993); white flies (Ishaaya et al., 1994; Ishaaya and Horowitz, 1995), scale insects (Peleg, 1988); cockroaches (Koehler and Patterson, 1991); and lepidopterans (Smagghe and Deghlee, 1994). It is a relatively stable JHA with low mammalian toxicity (Yokoyama and Miller, 1991; Higbee et al., 1995; Abdallah et al., 2000). It was first registered in Japan in 1991 for controlling public health pests (Miyamoto et al., 1993). As seen with other JH agonists multiple effects were induced in a single species. The compound interferes with embryogenesis, oocyte production, emergence, metamorphic moult and causes morphological deformities ((Miller, 1989; Hatakoshi, 1992; Bull and Meola, 1993; Miller and Miller, 1994; Vennard et al., 1998). It has limited
bioaccumulative ability (Sahaefer et al., 1988; Sahaefer and Murba, 1990) and at present PPN is among the most frequently used pesticides.

**(iv) Antijuvenile hormone agents**

The limited scope of JHAs as insect control agents necessitated the discovery of compounds with anti JH activity. Anti JH agents disrupts the normal development of early larval instars and inhibits JH dependent reproductive activities (Sam Mathai and Nair, 1984a; Santha and Nair, 1986, 1988, 1991; Santha et al., 1987; Nair, 1993). Some well known examples of anti JH agents are Precocenes, Fluromevalonolactone (FMev, ETB, EMD, Compactin, Piperonyl butoxide, Allylic alcohols, Bisthiolcarbamate etc. Anti JH agent, precocene causes JH deficiency in treated insects by selectively destroying the parenchymal cells of corpora allata (Unnithan et al., 1977).

**1.9 Objectives of the investigation**

From the foregoing review it is evident that ASGs play a crucial and critical role in the reproductive biology of insects. The development and differentiation of ASGs takes place in the pupal-adult metamorphosis under hormonal regulation. ASGs have been subjected to extensive investigations in many insect orders. However studies of ASGs of lepidopteran insects are comparatively few. Hence it was thought worthwhile to have an extensive analysis of ASGs of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) at morphological, histological and ultrastructural levels. Further studies on the
development and differentiation of ASGs during pupal-adult metamorphosis of *S. mauritia* have been carried out. Also a few studies on the influence of mating on the secretory activity of ASGs have been looked into. In addition an elaborate study on the effects of two IGRs with hormonal activity on the development, differentiation and secretory activity of ASGs has also been analyzed.

*S. mauritia* is a pest of paddy in Kerala. This species is chosen for the present study due to the availability of a sizable background data from this laboratory on the effects of insect growth regulators with hormonal and antihormonal activity on larval development, metamorphosis and reproduction of this insect (Nair, 1981, 1993; Sam Mathai and Nair, 1983, 1984a,b; Santha and Nair, 1986, 1987, 1988; Santha *et al*., 1987; Nair and Rajaleksmi, 1989; Pradeep and Nair, 1989; Balamani and Nair, 1989a,b, 1991, 1992; Jagannadh and Nair, 1992, 1993; Sakunthala and Nair, 1995; Venugopalan *et al*., 1994; Benny and Nair, 1999; Safarulla *et al*., 2003; Sindhu and Nair, 2004; Pradeep and Nair, 2005).

The effects of treatments of IGRs on the histomorphogenesis of ASGs, development and differentiation of ASGs and the secretory activity of ASGs of adult male *S. mauritia* are dealt with the present study. It is hoped that the results of this investigation will lead to a better understanding of endocrine regulation of development and differentiation of ASGs as well as will provide
valuable information concerning the potential of IGRs in pest control strategies.

Chapter 1 deals with a detailed review of ASGs, their classification, structure, function and hormonal regulation.

Chapter 2 provides basic information on the pest status and a detailed account of the rearing and maintenance of *S. mauritia* Boisd. (Lepidoptera: Noctuidae) under laboratory conditions.

Chapter 3 has given emphasis on the structural details of ASGs of adult male and female *S. mauritia* utilizing histological and ultrastructural techniques.

Chapter 4 deals with the development and differentiation of ASGs during pupal-adult metamorphosis and preliminary studies on how mating influences the secretory activity of male ASGs utilizing biochemical procedures.

Chapter 5 examines the effect of two IGRs on the histomorphogenesis, ultrastructure and the secretory activity of *S. mauritia*. 