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Pyriproxyfen: Effect on Morphometrics and Total Protein of Accessory Sex Glands of *Spodoptera mauritia* Boisd

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Abstract— *Spodoptera mauritia is a sporadic pest of Oryza sativa L. which usually occurs on rice from July to September, feeding on the leaves in large numbers. Accessory Sex Glands (ASGs) play a crucial role in the reproductive success of insects. Pyriproxyfen (PPN), the Juvenile hormone agonist, is an environment friendly Insect Growth Regulator (IGR) well known to interfere with insect reproductive system. In the present study, PPN treatments in different ages of this insect produced variable results. All the newly ecdysed day 0 pupae of S. mauritia, topically applied with 1 µg/µl PPN didn't survive beyond day 4 whereas 0.1 µg/µl PPN treated pupae showed only 61% mortality but the survivors showed failure of emergence. In both sexes, ASGs showed retarded development when compared to control. In the male adultoids, total ASGs proteins reduced significantly and it was evident from their electrophorogram also. The day 0 adult males when topically applied with a single high dose of PPN showed no mortality and their total ASG proteins were significantly increased and this increase was reflected in their electrophorogram.*

Keywords— Spodoptera mauritia, Pyriproxyfen, Accessory sex glands, Adultoids, Reproductive success, Pupae mortality.

I. INTRODUCTION

Numerous studies have demonstrated that Accessory Sex Glands (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 & Cruz-Landim, 2005). The development and differentiation, synthesis and release of secretions of ASGs are under the control of endocrine system in insects. Critical titer of ecdysteroids secreted by prothoracic glands and juvenile hormone (JH) secreted by corpora allata (CA) are necessary for the normal development of ASGs (Gillott & Gaines, 1992). Many authors have shown that JH regulates protein synthesis in the ASGs (Blaine & Dixon, 1973; Gillott & Freidel, 1976; Venkatesh & Gillott, 1983; Ogiso & Takabashi, 1984; Gillott & Gaines, 1992).

The discovery of compounds with hormonal and antihormonal activities have greatly facilitated studies on Insect Endocrinology. These compounds designated as Insect Growth Regulators can induce hormone deficiency or hormone excess in treated insects and have greater potential in Integrated Pest Management (IPM) programmes as insect control agents (Nair, 1993; Oetken et al., 2000). The IGRs acting as ecdysone agonists/antagonists or JH analogues/anti JH agents disrupt the endocrine and reproductive physiology of a number of insects to aid in their control. The present study deals with the effect of Pyriproxyfen (PPN), a JH agonist, on the development, differentiation and the secretory activity of ASGs of *Spodoptera Mauritia.*

II. MATERIAL AND METHODS

2.1 Rearing and Maintenance:

The larvae of *Spodoptera mauritia* were reared and maintained at room temperature, RH 90 ± 3% and 12:12 light: dark photoperiod regime in the laboratory. They were fed with fresh leaves of *Ischaemum aristatum*. The sexing of the larvae

were done in fifth instar and were segregated. In all experiments, developmentally synchronous insects were used. Pyriproxyfen (PPN), an agonist of JH, was obtained as a research sample from M/s Valent Corp., USA. For treatment procedures, measured quantities of these compounds were used with a 10 µl Hamilton microsyringe.

2.2 Treatment of PPN on Newly Ecdysed Day 0 Pupae:

PPN was dissolved and diluted in acetone to obtain two different concentrations: 0.1 µg/µl and 1 µg/µl. The different doses of PPN were applied topically on the abdominal region of newly ecdysed day 0 pupae (100 Nos.) using a 10 µl Hamilton microsyringe. Same number of pupae kept as controls received an equivalent volume of acetone only. Experimental and control pupae were kept in separate beakers covered with muslin cloth. The pupae were checked daily for mortality and morphological abnormalities. To study the morphogenetic changes, ASGs were dissected out of pupae on each post treatment day. Further, ASGs of adults and adultoids were processed for electrophoretic studies.

2.3 Treatment of PPN on Day 0 Adult Males:

Ten newly emerged (day 0) adult males of *S. mauritia* were treated topically with a single dose of 20 µg/µl of PPN. Same number of adult insects kept as controls were treated with an equivalent volume of acetone. Experimental and control insects were kept in separate beakers covered with muslin cloth. ASGs were dissected out from PPN treated insects on day 1, their total protein content was estimated and were further subjected to electrophoretic studies

III. RESULTS AND DISCUSSION

3.1 Effect of PPN on Pupal Development and Survival:

Pupae treated with 1 µg/µl PPN showed 100% mortality by day 4. When they were dissected open, ASGs were not developed at all. In 0.1 µg/µl PPN treated pupae, 61% mortality was observed by day 4. The survivors did not emerge as adults. These pupae when dissected open on day 8 were found to contain adultoids with unstretched wings inside the pupal cases. The control male insects emerged normally on day 8 and females emerged on day 7, showing normal imaginal differentiation.

3.2 Effect on Male Accessory Sex Glands:

In 0.1 µg/µl PPN treated male insects, ASGs appeared as small buds on day 4. They didn't elongate much on subsequent pupal days (Table 1) and they were seen as a fused pair of blind tubes without any regional differentiation (Figure 1b). In control pupae, ASGs showed normal development as seen in normal insects.

TABLE 1
LENGTH OF MALE ASGS IN TREATED AND CONTROL INSECTS (MM)

Day	Treated	Control
1	-	9.03 ± 0.209
2	-	15.1 ± 0.1
3	-	20.03 ± 0.057
4	1 ± 0.082	33.1 ± 0.1
5	2.5 ± 0.217	35.93 ± 0.115
6	3 ± 0.18	50.13 ± 0.058
7	4 ± 0.163	57.73 ± 0.152
8	4 ± 0.245	77 ± 0.812

3.3 Effect on Female Accessory Sex Glands:

In females, ASGs were developed only in pupae treated with 0.1 µg/µl PPN. The mortality rate was similar to that of males in both concentrations. In 0.1 µg/µl PPN treated insects, ASGs appeared as small buds on day 3. In females, ASGs showed an inhibition of growth under the effect of PPN as in males (Table 2). Reservoirs were hardly distinguishable from the glands (Figure 1d). They did not emerge as adults. These pupae when dissected open on day 7 were found to contain adultoids (Table 2).

TABLE 2
LENGTH OF FEMALE ASGS IN TREATED AND CONTROL INSECTS (MM)

Day	Treated	Control
1	-	2 ± 0.849
2	-	3.1 ± 0.1
3	1 ± 0.0816	7.13 ± 0.152
4	2 ± 0.05	10 ± 0.1
5	2 ± 0.141	12.03 ± 0.577
6	3 ± 0.812	12.13 ± 0.115
7	4 ± 0.216	13.26 ± 0.723

3.4 Effect of PPN on Adult Males:

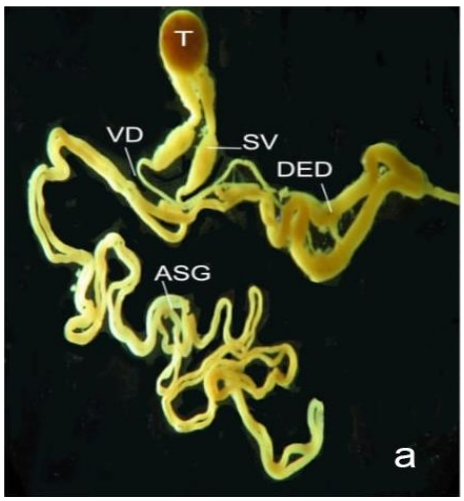
A single high dose of PPN (20 µg/µl) was applied on the adult insects. They did not show any mortality and were quite healthy and active once recovered from a short period of anesthetic effect. When they were dissected on day 1, ASGs looked slightly swollen, evidently due to the accumulation of secretion.

Amount of protein in ASGs of day 0 control insects was found to be 9.28 ± 0.876 µg/mg of tissue, whereas the amount of protein in the ASGs of adultoids was considerably decreased to 1.9 ± 0.141 µg/mg of tissue. At 0.05 levels, these values are significantly different.

Amount of protein in ASGs of day 1 control insects was found to be 13.63 ± 0.410 µg/mg of tissue, whereas the amount of protein in the ASGs of day 1 males treated with 20 µg PPN immediately after eclosion on day 0 had increased to 42.75 ± 1.343 µg/mg of tissue. At 0.05 levels, these values are significantly different.

3.5 Electrophoretic Analysis:

In the electrophorogram (Figure 1e), Lane 2 shows the electrophoretic profile of ASG proteins of day 1 adult males treated with 20 µg/µl of PPN immediately after eclosion on day 0. In this, neither bands were found to disappear nor were new bands appeared. The staining intensities of most of the peptides of treated males were considerably more when compared to that of control. Lane 3 shows the electrophoretic profile of ASG proteins of adultoids. The staining intensities of most of the bands were considerably less and some bands have disappeared when compared to that of control (Lane 4).



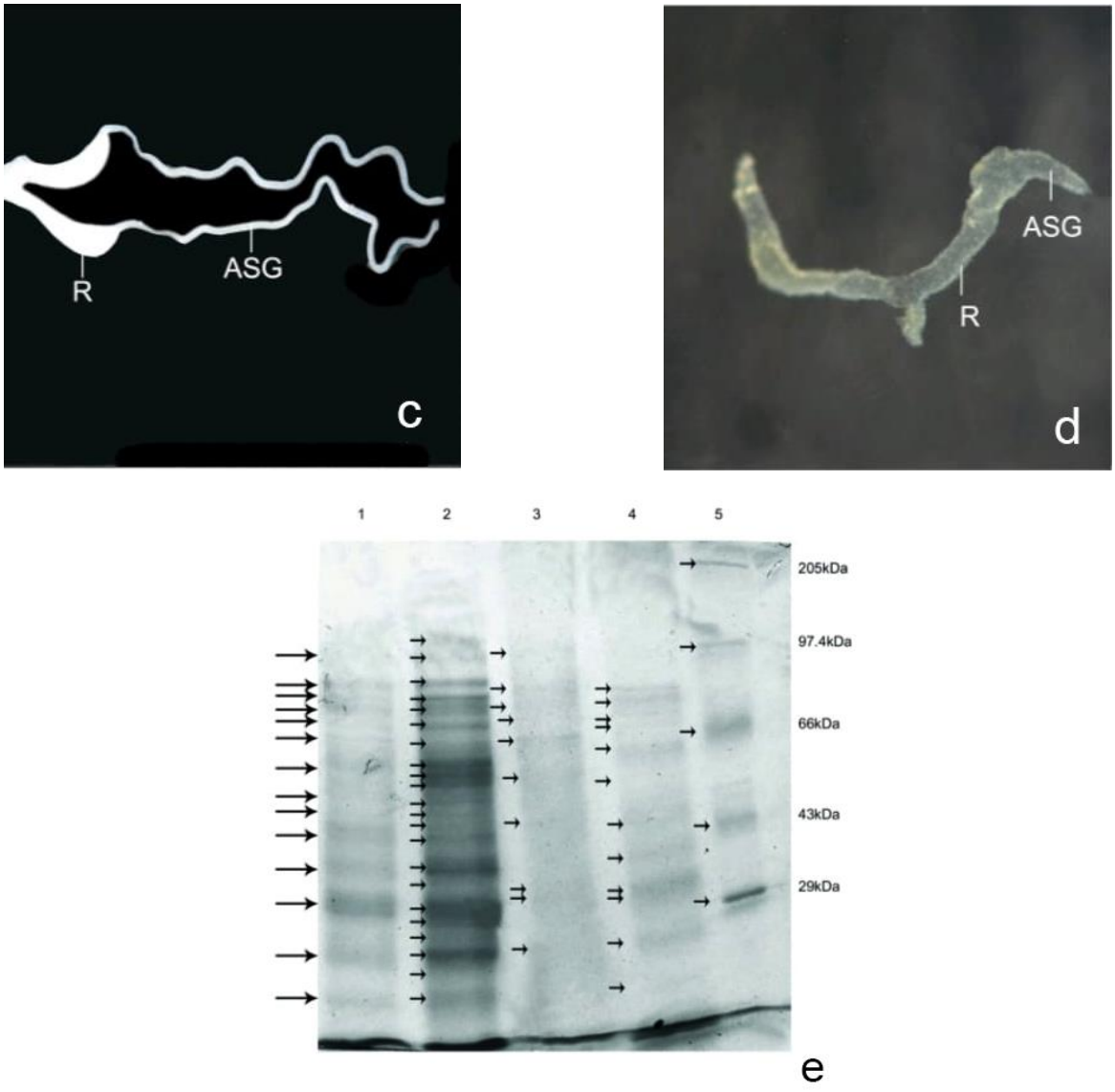


FIGURE 1

a. Reproductive system of male kept as control showing Testis (T), Vas deferens (VD), Seminal vesicles (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)
b. Male Reproductive system of male adultoid showing Accessory sex glands (ASG)
c. Accessory sex glands (ASG) of female kept as control showing Reservoir (R)
d. Accessory sex glands (ASG) of female adultoids showing Reservoir (R)
e. SDS-PAGE profile of ASG proteins in PPN treated and control male insects

Lane 1: ASG protein profile of day 1 adult controls
Lane 2: ASG protein profile of day 1 adults treated with 20 µg PPN immediately after eclosion on day 0
Lane 3: ASG protein profile of adultoids
Lane 4: ASG protein profile of day 0 adult controls
Lane 5: Molecular weight markers

3.6 Discussion:

An inhibitory action of JH analogues on the development and differentiation of ASGs has been reported by several authors (Landa & Metwally, 1974; Ramalingam & Craig, 1977; Gelbic & Metwally, 1981; Roychoudhury & Chakravorty, 1987). In the present study also, a significant amount of retardation is noted in the development and differentiation of ASGs. Happ (1984) reported that usually the ASG development takes place in pupal period in holometabolous insects. Studies show that

there is a decrease in JH titre in pupal period in insects. In *Locusta migratoria*, the committance for the terminal differentiation of ASGs is triggered during the critical period when there is a decrease in the JH titre (Baehr et al., 1979).

In the present study, in PPN treated males, ASGs were fused as seen in the early pupal stage of normal/control insects. Similar observations were reported by other workers (Cantacuzene, 1968; Szollosi, 1975). In the present study, degenerative, irregular and inhibitory effects on the development of ASGs might be due to the high titre of JH circulating in the haemolymph due to treatment. In *S. mauritia*, the inhibitory effect of PPN is more conspicuous as this JH agonist was applied to the pupae when the endogenous titre of JH was minimum or absent. Studies of Mariamma (1989) in *Oryctes rhinoceros* show a dose dependent effect of JHA with regard to differentiation of ASGs.

Previous studies show that development and differentiation of ASGs involve cell multiplication, increases in cell size and acquisition of competence to make adult specific proteins accompanying rapid synthesis of secretory proteins. According to Highnam & Hill (1969), in insects, cell division and differentiation are intrinsic properties of the cell, residing in the genes and that the particular expression at any stage is controlled by JH. In *T. molitor*, development and differentiation of ASGs occurring in pupal stage are characterized by two bouts of mitosis (Grimes & Happ, 1980; Happ & Happ, 1982; Happ et al., 1985). The first bout of mitosis is not ecdysteroid dependent while the second required the addition of physiological amounts of ecdysterone. The second bout of mitosis coincides with the peak of ecdysteroid in the pupal stage (Delbecque et al., 1978). Yaginuma et al. (1988) describe that ecdysteroids promote the flow of cells from the G2 into the G1 and S phases of cell cycle.

An excess of JH or its analogues in the haemolymph might inhibit the ASG development in two different ways. It might directly block the cell division and differentiation of ASG cells. It is assumed that in *S. mauritia*, the cells of ASGs might have undergone the first bout of mitosis as evidenced by the presence of a proliferated mass of cells. At a later stage, cell proliferation got arrested possibly due to the high titre of JH in the haemolymph. Alternatively, the second bout of mitosis might not have occurred possibly due to a failure of an ecdysteroid peak or a decrease in the ecdysteroid titer as explained by earlier workers (Zufelato et al., 2000). Many authors have opined that degradation of JH mimics takes place rapidly (Gilbert et al., 2000; Kamita et al., 2003) but Edwards et al. (1993) suggest that the action of PPN has a delay of approximately 24 h. Mona (2001) showed that in *S. mauritia*, the ecdysteroid peak reaches maximum in day 2 pupae and then diminishes in subsequent days. This means that even though PPN is applied on day 0 pupae, it may induce a delayed effect to block the ecdysteroids peak. A disruption in the differentiation of the cells might have affected the maturation of the cells, which explains why secretory material is not observed in the lumen of ASG of treated insects.

In *S. mauritia*, PPN exerts different effects in the protein synthesis of ASGs when applied in different phases of life cycles. Amount of protein decreases significantly in ASGs when PPN is applied on newly ecdysed pupae, whereas the amount of protein significantly increases in the ASGs when PPN is applied on newly eclosed adults. These results show dual effects of PPN in two different phases of the life cycle of *S. mauritia*. These antagonistic effects of PPN could be explained based on the physiological role of JH in natural conditions. JH is a unique developmental hormone in several aspects. During metamorphosis, JH blocks the expression of subset of genes that specifies the imaginal phenotype and during adult reproductive stage, it activates the expression of subset of genes that are necessary for reproduction (Jones et al., 1993).

In insects, the titre of JH is usually controlled by JH-specific esterases and some other mechanisms (Gade et al., 1997; Gilbert et al., 2000; Kamita et al., 2003). However, treatments of PPN may disrupt the regulatory mechanisms resulting in the presence of detrimentally high JH titres. This will disrupt not only the endocrine physiology but also might cause pharmacological effects (Webb et al., 1999; Wilson, 2004). In *Locusta migratoria*, PPN repressed the synthesis of two proteins and stimulated the synthesis of another high molecular weight protein (de Kort & Koopmanschap, 1991). It is assumed that PPN has impaired protein synthesis in the ASGs of 0.1 µg treated insects. In *Aedes aegypti*, JHA impaired the capacity of fat bodies of pupae to synthesize proteins, resulting in a lowered concentration of fat body proteins (Gordon & Burford, 1984).

There are evidences that JH affects the post transcriptional processing or translation processes. The ability of JH to prevent translation of the transcripts for the basic hexameric proteins of *Trichoplusia* after it no longer affected their gene transcripts supports the possible role at this level (Jones et al., 1993). Another possibility is that PPN might have hindered the synthesis of protein products by inhibiting the transcription of many genes or by promoting the degradation of RNAs.

Previous studies show that the secretory function of ASGs in adults (reproductive stage) is under the influence of JH. In many insect species including *Drosophila melanogaster*, JH has been shown to influence post-ecdysial maturation of male

ASGs. A rapid increase in the JH titer in the newly eclosed adults is reported in *D. melanogaster* and is a probable key feature in the maturation of gametes and testes (Bownes & Rembold, 1986). In male moths of *Ephestia cautella*, ecdysteroid titres are relatively low throughout their adult life (Shaaya et al., 1991). Studies of Gillott & Friedel (1976) show that treatment of synthetic JH in adult *Melanoplus sanguinipes* results in an increase in the protein content of ASGs. They further suggest that synthesis of proteins takes place in fat body under the stimulatory effect of the JH analogue and these proteins are incorporated into the secretions of ASGs. In *S. mauritia* also, the increase in the staining intensity of the peptides is thought to be correlated with the stimulatory effect of PPN on the enhancement of ASG proteins in adult insects.

IV. CONCLUSION

The newly ecdysed day 0 pupae, topically applied with 1 µg/µl PPN showed hundred percent mortality on day 4. In 0.1 µg/µl PPN treated pupae, only 61% mortality was observed but the development of ASGs were highly reduced in both sexes when compared to control. The total ASG proteins of male adultoids were significantly reduced than that of emerged control male insects. Their electrophoretic profile showed less staining intensities in most peptide bands as well as disappearance of some bands when compared to that of control. Day 0 adult males showed no mortality when applied with a single high dose of PPN. Their total protein of ASGs significantly increased than that of the control insects and the staining intensities of most of the peptides were considerably more when compared to that of control. This study demonstrates the dual, stage-dependent effect of Pyriproxyfen on the reproductive physiology of *Spodoptera mauritia*—inhibitory during pupal development and stimulatory in adults—highlighting its potential as an effective IGR for pest management strategies

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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