CHAPTER IV

EFFECTS OF GROWTH REGULATORS ON METABOLITE LEVELS DURING DEVELOPMENT
IV.1. EFFECTS OF PRECOCENE-I ON H. SERRATA

INTRODUCTION

Holotrichia serrata (Coleoptera: Melolonthinae) is a monovoltine insect found in various agro-ecological zones (Srivastava and Mathur, 1979). There are three larval instars and the fully grown grub is creamy white in colour. The head is dark brown with strong mandibles. The depth at which the grubs generally occur depends upon the type of soil, temperature, host plant and the species involved (Srivastava and Mathur, 1979). The grubs thrive well not only in sandy and sandy loam soils, but also equally well establish in black cotton soil, clay and the laterite soils of heavy rainfall area (Veeresh, 1977b). In Kerala, they are seen in rubber plantations attacking the root system of the seedlings in the nurseries. Grub-infested plants grow weak and eventually perish. The white grubs have attained the status of major devastating pest of a variety of rainy season crops in India and in endemic areas, the quantum of losses to various crops due to these white grubs has been estimated to be 40-80 per cent (Srivastava and Mathur, 1979).

Generally, the larval period of H. serrata extends from August to October and pupation occurs during November. Pupae remain in earthen cells constructed by the third instar grubs. The pupal period ranges from 10-27 days depending upon the type of soil and other ecological conditions (Srivastava and Mathur, 1979). The freshly formed beetle is cream coloured with soft white elytra. Later, the colour changes to brown and the elytra gets hardened. The pre-emergent adults remain within the earthen cell for 4-5 months and emerge during
April-June with the onset of summer rains. It has been determined that once the gonads are fully developed, the adults will not stay for a longer period inside the soil and in case the rains do not occur in that period, the beetles will die within the cell. The adults of the root grubs feed on the foliage of plants and for distribution and abundance of this pest, food for adults is found to be the chief environmental factor (Srivastava and Mathur, 1979).

This study is undertaken to determine the quantity of various metabolites in third instar grubs and the subsequent levels during the long period of pre-emergent adult stage. The influence of precocene-I on protein levels in the insect was also studied.

MATERIALS AND METHODS

Third instar grubs and pre-emergent adults of *H. serrata* were obtained from the Indian Rubber Board nurseries, Central rubber nursery (Karikatoor) and Regional Nursery (Neriamangalam). A record on the weight of individual insect was kept.

Haemolymph samples (10-20 µl) were collected from the insects with a micropipette. Fat body (grubs) and flight muscle (pre-emergent adults) were dissected out under Ringer's solution.

Protein concentration was determined by the method of Folin-Ciocalteau (Lowry *et al.*, 1951). Glycogen and lipid estimations were made in the manner described by Karnavar and Nair (1969). Trehalose estimation was carried out following the method of Wyatt and Kalf (1957).

In the pre-emergent adults, haemolymph and flight muscles were subjected to protein analyses after exogenous application (100 µg/insect) of precocene-I; 7-methoxy-2, 2-dimethylchromene (Sigma Chemicals Company, USA). Control insects were treated with acetone. Estimations were made 48 and 72 hours after the treatment.
RESULTS

Third instar grubs

Body weight (gm) of the third instar grub was found to be $3.640 \pm 0.37$. The haemolymph protein concentration of the grub was $5.4684 \pm 0.47$ mg/ml and the trehalose content was $5.8208 \pm 0.64$ mg/ml. The fat body of the grub showed a protein and glycogen content of $10.9658 \pm 1.51$ mg/gm and $0.7626 \pm 0.04$ mg/gm of tissue respectively while the percentage of lipid in this tissue was $6.34 \pm 1.46$.

Pre-emergent adults

The weight of the pre-emergent adults was comparatively low during the quiescent stage which lasted from December (body weight : $2.397 \pm 0.31$ gm) to the earlier half of April (body weight : $1.997 \pm 0.56$ gm). During this period, the protein content (mg/ml) of the haemolymph was found to be $3.7579 \pm 0.44$ (Dec), $2.7404 \pm 0.68$ (Jan), $4.2842 \pm 0.57$ (Mar) and $7.0526 \pm 1.41$ (Apr) while the trehalose level (mg/ml) was $6.3098 \pm 0.49$ (Dec) and $4.2060 \pm 0.71$ (Jan). In the flight muscle, the concentration of protein (mg/gm) during the months was found to be : $17.2842 \pm 1.29$ (Dec), $22.3368 \pm 0.36$ (Jan), $14.063 \pm 1.48$ (Mar) and $12.399 \pm 2.68$ (Apr). The glycogen content (mg/gm) during the corresponding period was $0.2061 \pm 2.25$, $0.2886 \pm 0.06$, $0.1953 \pm 0.11$ and $0.1256 \pm 0.06$. The percentage of lipid in the tissue was $5.484 \pm 1.41$ (Jan), $4.4 \pm 1.20$ (Mar) and $4.772 \pm 0.57$ (Apr).

Treatment of pre-emergent adults with precocene-I

Haemolymph (mg/ml) and flight muscle (mg/gm) protein
concentration in the pre-emergent adults was found to be 3.799 ± 0.27 and 7.389 ± 1.69, 48 hour after precocene-I treatment while in the controls it was 4.316 ± 0.50 and 7.910 ± 0.68. The haemolymph protein level was significantly different (P < 0.05) from the controls. 72 hour after treatment, the level of protein in the haemolymph (mg/ml) and flight muscle (mg/gm) was 4.0737 ± 0.48 and 6.847 ± 2.22 respectively and 4.347 ± 0.61 and 8.042 ± 0.69 in the controls. The flight muscle protein content showed significant difference (P< 0.05) while the haemolymph protein was not significantly different (P> 0.05) from the controls, 72 hours after treatment.

DISCUSSION

Haemolymph proteins are synthesized and released by the fat body (Hill, 1962; Coles, 1964; Hill, 1965). Third instar grubs of H. serrata show a protein content of 5.4684 ± 0.47 mg/ml in the haemolymph while the newly formed pre-emergent adult had a relatively lower haemolymph protein content(3.7579 ± 0.44mg/ml). The grubs are active feeders, and hence maintain their haemolymph metabolites at a homeostatic level based upon feed back relationships between crop emptying, haemolymph osmotic pressure, metabolite utilization and feeding as in other insects (Gelperin, 1971). The pre-emergent adults undergo a quiescent stage in earthen cells in the soil that lasted from December to the early half of April. During this period, their body weight was greatly reduced [from 2.397 ± 0.31 gm (Dec) to 1.776 ± 0.40 (Mar)]. The level of protein
in the haemolymph during this dormant period was more or less steadily maintained. However, protein registers a high level towards the end of the dormant period. Adult emergence is followed by a short active feeding and maturation of oocytes and the high level of haemolymph protein indicates the activation of yolk protein synthesis. In contrast to the pre-emergent adults, the grub maintains a steady nutrient input to keep the circulating haemolymph metabolites at a constant level. The fat body content of pre-emergent adults is very low and the reserved materials are used for adult development.

Haemolymph components are maintained at their maximum directly by the products of digestion (Keeley, 1978). In the grubs the haemolymph trehalose content was $5.8208 \pm 0.64$ mg/ml while the freshly developed pre-emergent adults showed trehalose levels of $6.3048 \pm 0.49$. In the subsequent dormant period the level of blood trehalose was $4.2060 \pm 0.71$ mg/ml. Metabolite reserves from the haemolymph are called upon only in an emergency when nutrient intake is restricted or when metabolic demands exceed the capacity of digestion to supply the metabolites (Keeley, 1978). High levels of trehalose formed from a dietary source permit conservation of the glycogen stores. This would be possible only in the grubs. The pre-emergent adults are non-feeding, therefore depletion of trehalose precursors take place and the haemolymph trehalose level is maintained by degrading fat body glycogen. Neurohormones stimulate the production of trehalose and constitute a means to increase and maintain haemolymph trehalose levels.
(Keeley, 1978). In *Locusta migratoria*, metabolic stress induced by flight not only has a profound effect on metabolism of the flight muscles but also markedly affects glycogenolysis in the fat body (Steele, 1983). Pre-emergent adults of *H. serrata* are incapable of flight. Their flight muscle metabolites showed a low level.

The haemolymph plays an important role in the maintenance of homeostasis since it serves as a medium high in amino acid and protein nitrogen and shares an intimate relationship with the fat body (Mullins and Cochran, 1983). Haemolymph proteins are produced primarily by the fat body, but other tissues may also participate in their production (Chen, 1978). It has been postulated that juvenile hormone enhances flight performance, resulting in the utilization of lipid reserves (Beenakkers, 1983). Protein metabolism in the flight muscle of *H. serrata* is presumably regulated by juvenile hormone since in precocene treated pre-emergent adults protein content of the flight muscle was significantly reduced in 72 hours. Precocene treated male *Locusta* show low level of protein synthesis in both the fat body (source of haemolymph protein) and accessory reproductive gland (Lange *et al.*, 1983). In *H. serrata* the haemolymph protein level was significantly lower in the pre-emergent adults 48 hours after precocene-I treatment while 72 hour after treatment the haemolymph protein composition was comparable to that of the controls. Jungreis (1980) pointed out that because of its relative constancy in composition, haemolymph
has a static rather than a dynamic nature. He also ascertained that the turnover times for many compounds in the haemolymph are rapid and that they have relatively brief half-lives. This view supports the present findings and in *H. serrata* the observed effects can be generalized to be the result of reduced juvenile hormone titers brought about by precocene treatments.