INTRODUCTION

The khapra beetle, Trogoderma granarium Everts (Dermestidae, Coleoptera) is a serious pest of stored grains, mainly on wheat. Believed to be indigenous to India it has now acquired a world-wide distribution. This insect has a tremendous potential for development on a wide variety of foods, of both animal and plant origins. One of the earliest investigations on the biology of this insect was that of Barnes and Grove (1916) in India. In recent years, Burges (1957, '59 a&b, '60 and '63) in Slough (England) and Nutting and his associates (1960, '64)in Arizona (U.S.A) have contributed much to our knowledge of the biology and ecology of Trogoderma under natural and laboratory conditions. A series of investigations by Burges covering various aspects, such as their ecology under natural conditions in malt stores, the occurrence of diapause at various conditions of temperature, humidity and food, and the feeding, growth and respiration of the diapause larva deserve special mention. A study on the influence of biological and ecological factors on khapra beetle populations in commercial grain storages in Arizona was reported by Nutting. These studies have shown that T. granarium possesses remarkable adaptations to live under varied nutritional and climatic conditions. They thrive well in hot dry regions on a wide variety of food (Noon, 1958), can withstand a wide variation in temperature and humidity and survive very long periods of starvation when food supply fails (Burges, 1959, '62, '63). The larval life is extended under unfavourable conditions such as low temperature, humidity, high population density and contamination of the food with excreta (Burges, 1963, Stanic *et al.*, 1963, Bailey, 1965).

The facultative diapause of the khapra larvae has enhanced the pest status of the species (Howe, 1962) by allowing mass survival of the larvae during periods of food shortage and other unfavourable conditions; synchronizing the adult emergence with the return of favourable conditions. The diapause larvae are photophobic as the normal ones (Rahman Khan and Sohi, 1939) and spend most of the time in an inactive state and have a very low oxygen consumption (Burges, 1960). They are comparatively more resistant to fumigants, contact insecticides and also to gamma radiation (Carney, 1962, Nair and Rahalkar, 1963). These adaptations of *Trogoderma* with added capacity for autogenous egg production have contributed much to the survival of the species.

Apart from the above studies on their biology and control measures, the literature on *T. granarium* also include some nutritional studies. Studies on the nutritional requirements of this insect have shown that a diet of simple sugars, lipids, essential amino acids and vitamins of the B complex group are essential for healthy growth (Pant, 1953, '56, Pant *et al.*, 1958, Pant and Pant, 1960, Pant and Pant, 1961). An analysis of the larvae and other developmental stages of *Trogoderma* showed that the third instar larva had

12 amino acids (Lallan Rai et al., 1962) and that the different stages of development had a qualitatively divergent pattern of free amino acids (Bhattacharya and Pant, 1965).

The physiological and metabolic adaptations of Trogoderma have received little attention in the past and the present study was undertaken to shed some light on these aspects. Emphasis was given to the function of the fat body during development and diapause.

The present study includes the following main aspects; a study of the biology under various experimental conditions with special reference to the incidence of diapause, a histological and histochemical study of the fat body during larval develoment, diapause and metamorphosis and a quantitative analysis of the levels of some metabolites during diapause and pupal-adult transformation. A cytochemical and quantitative analysis of the fat body of another beetle, Oryctes rhinoceros is also reported.

The first two chapters are devoted to a study of the biology of T. granarium. Since food and temperature had considerable influence on the biology of this insect, conditions of food and temperature for the maintainence of the laboratory culture was standardized. Incidence of diapause at various conditions of temperature, humidity and population density are reported. Studies were also made on the effect of a diurnal variation in temperature such as occurs in the winter conditions of Baroda.

A comprehensive study of the cytology and cytochemistry of the fat body of normal and diapausing larvae is given in Chapter 3. The fat body of insects, as an organ of intermidiary metabolism is being increasingly realized and established in recent years. Many of the synthetic and degradative pathways in the metabolism of proteins, lipids and carbohydrates have been demonstrated in the insect fat body (Kilby and Neville, 1957, Wigglesworth, 1942, Desai and Kilby, 1958, Zebe and McShan, 1959, Clements, 1959, George and Eapen, 1959b, Candy and Kilby, 1961, George and Hegdekar, 1961, Tietz, 1961, '62, Haines and Smith, 1963, Kilby, 1963, Vardinis, 1963, Hegdekar, 1963, Nair and George, 1964, Chino and Gilbert, 1965, Wlodawer and Baranska, 1965, Hill, 1965). The present studies on the cytology and cytochemistry of the fat body have shed some light on its metabolic role. Large amounts of protein, fat and glycogen are stored in the fat body. There is a tremendous increase in the levels of fat and glycogen during the earlier part of diapause. The presence in the fat body of certain 'proteinaceous globules' which differ from the protein granules of other insects in many respects is reported. A more comprehensive study of the cytochemistry of a similar type of proteinaceous globules in the larval fat body of _Oryctes rhinoceros_ is reported in Chapter 4.

A cytological and cytochemical study of the fat body with particular reference to the proteinaceous globules during metamorphosis (Chapter 5) has shown that the proteinaceous globules are utilized to provide raw materials for the developing tissues during imagogenesis and for the formation of yolk during gonadal development. A possible similarity of the contents of the proteinaceous globules to the protein yolk of insectan egg is suggested. In addition to the proteinaceous globules a second type of much smaller protein containing granules making their appearence in the prepupal fat body and disappearing in the newly emerged adult is also reported.

From the histochemical studies it was found that the fat and glycogen contents of the fat body increase during diapause. A quantitative study of the fat, glycogen and protein contents of the normal and diapause larvae was therefore undertaken. A study of these metabolites at different intervals of diapause showed that their levels are maintained more or less steady during diapause when food was available; This was because the larvae feed occasionally if food is available (Burges, 1960). Diapausing larvae of _T. granarium_ are known to live for about 3-4 years when food is available. A study of the levels of fat and glycogen in diapause larvae denied access to food, showed that when food suply fails, the larva depends on the stored reserve materials. The increase in the levels of fat and glycogen during diapause when food was available and the subsequent utilization of the stored food when the larvae were denied food are recorded (Chapter 6).

The studies on the changes in the levels of fat and glycogen during metamorphosis, T. granarium showed that a part of the initial fat content is saved for utilization during adult life and that there exists a diversity between the sexes in the pattern of utilization of these metabolites during metamorphosis (Chapter 7).

CHAPTER 1

OBSERVATIONS ON THE BIOLOGY OF THE KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS, UNDER LABORATORY CONDITIONS

In the past few years much attention has been focussed on the khapra beetle, *Trogoderma granarium* Everts because of the bulk of damage caused by the larvae to a variety of stored grains. The biology, ecology and measures for the control of this insect were studied by many investigators throughout the world. Among these studies the reports of Barnes and Grove (1916) in India, Burges (1957, 1959 a & b, 1960, 1962, and 1963) in England and Nutting and his associates (1960 and 1964) in Arizona, U.S.A, have contributed much to our knowledge. Burges has repoted in his extensive studies on the biology and ecology of this insect, the development of the larvae at various conditions of temperature, humidity and population density and had shown the importance of the environmental factors in inducing diapause.

The food preferences of *Trogoderma* larva was studied by various workers (Burges, 1957; Hadaway, 1956; Howe, 1962 and Heller and Shulov, 1964) and it has been shown that the larvae thrive well on wheat, corn meal, barley, malt, oats, sorghum etc. Noon (1958) reported that the khapra larvae can thrive well on many products of animal origin also.

Though the literature on the biology of the khapra beetle is quite extensive, evidently there is considerable discrepancy in the duration of the entire life cycle and of the different developmental stages. This is mainly attributable to the different kinds of food on which the larvae were reared and to the varied environmental conditions.

A review of the available literature showed that the following diets were employed by various workers and cultures were maintained at temperatures shown against each: malted barley at 30°C (Hadaway, 1956), wheat feed consisting of fine bran and little endosperm at 30°C (Burges, 1957), wheat flour plus 10% yeast at 30°C (Pant and Ghai, 1959), wheat flour plus 5% yeast (Bhattacharya and Pant, 1965) and crushed wheat at 38°C (Nair and Rahalkar, 1963). It is well known that the khapra larvae thrive in a wide range of temperature (20-46°C; Burges, 1959; Howe, 1956). However, Burges had shown the optimal range of temperature for development to be between 30-40°C. He also studied the development of larvae at various humidities such as 0, 35, 70 and 90% at 30°C and found that the developmental period was the shortest at 70% R.H.

It may be mentioned here that the khapra is mainly found attacking wheat in India, though occasionally they are also found in stocks of rice and other food grains. From preliminary experiments, crushed wheat was found to be an ideal medium for maintaining the laboratory cultures. The cultures were therefore maintained on this medium at 35°C and 70% R.H.

The present study was undertaken to establish standard conditions for maintainence of laboratory cultures of this insect and to record their biology under these conditions to serve as a basis for the histophysiological and biochemical studies reported in the present thesis.

MATERIAL AND METHODS

The stock larvae of Trogoderma granarium Everts were obtined from the Atomic Energy Establishment, Bombay. For observations on the moults and duration of different stadia, 25 newly hatched larvae were placed individually in specimen tubes (3X1") containing enough crushed wheat to fill the bottom of the tube and were maintained at 35±1°C and 70% R.H. in incubator. Humidity was controlled by placing the culture tubes in a desiccator over saturated $NaNO_3$ solution (Winston, 1960). The larvae were observed for moults daily or at intervals depending upon the next moult in each case.

RESULTS AND DISCUSSION

The results obtained under the present laboratory conditions on the number and duration of the developmental stages are presented in Table I.

It is well documented that the dermestids, the number of larval instars and the duration of life cycle vary greatly depending upon factors such as food, temperature, humidity and population density. Data obtained by other investigators on the life cycle of T. granarium at different conditions of temperature, humidity and food are reviewed by Burges (1957). However, no data are available for the same conditions of temperature, humidity and food as employed

Table. I. Showing the number and duration of the different instars of _T. granarium_ maintained on crushed wheat at 35±1°C and 70% R.H.

Instar	MALE			FEMALE		
	Mean duration in days	Range in days	No. of insects out of 11 showing the particular instar	Mean duration	Range in days	No. of insects out of 11 showing the particular instar
Larva I	5.2	4-6	11	4.8	4-5	8
" I2	3.8	3-4	11	3.9	3-4	8
" 3	3.9	3-4	11	3.9	3-4	8
" 4	3.8	3-4	11	4.3	3-5	8
" 5	4.6	3-5	11	4.9	3-4	8
" 6	3	3	2	3.9	3-4	8
Pupa	2.9	2-3	11	3	3	8
Pre-em. adult	2	2	11	2.3	2-3	8

(Six insects which died in the larval stages are excluded) in the present study. From observations under conditions in the wheat stores in Punjab, Barnes and Grove (1916) reported that the total larval period shows considerable variation (from 20 to 230 days) depending upon the season. In larvae maintained on malt at a constant temperature of 30°C and 73% R.H, the male took 26 days and females 30.6 days to complete development (Hadaway, 1956). When larvae reared on wheat and wheat feed at 30°C (Burges, 1957) males took 32.8 days and females 37.4 days to complete the life cycle. The results of the present study are in agreement with those of the previous investigators. The developmental period was found to be 26.2 days and 30.9 days for male and female respectively. The difference between the male and female is largely due to the difference in the number of instars. The female had six instars whereas the males had only five. However, two males out of eleven individuals showed an additional instar (sixth). Instances of such inconsistency in the number of larval instars under constant environmental conditions were also reported by Burges (1957), Voekel (1924) and Hadaway (1956).

Burges (1957) reported that the duration of the larval instars varied from 5 to 6 days at 30°C. In the present study at 35°C it was found that generally the duration of the larval instar ranged from 3 to 4 days, with a few larvae showing a duration of 5 or 6 days in some instars.

The duration ofthe pupal period showed no significant difference between the sexes and was found to be 2 to 3 days. After the pupal-adult moult the fully formed adult remained quiescent for a few days. This 'pre-emergent adult' period lasts for about 2 days in both sexes.

The adults soon after emergence mate and lay eggs. Most of the eggs are laid during the first two days. Females were found to lay approximately 45 eggs. Adult beetles do not feed and live for comparatively short periods (4to 5 days). However, females not allowed to mate and lay eggs and males prevented from mating were found to live about 12 and 7 days respectively. The egg production in *T. granarium* is autogenous.