CHAPTER 2

OBSERVATIONS ON THE EFFECT OF TEMPERATURE AND POPULATION DENSITY ON THE INCIDENCE OF DIAPAUSE IN TROGODERMA GRANARIUM

Diapause of the khapra larva has attracted much attention because it enhances the pest status of this species by allowing mass survival of the larvae during periods of food shortage and other unfavourable environmental conditions. In this insect diapause occurs in the larval stage. Under certain conditions, the larvae, after reaching the last stadium, instead of pupating, leave the food, cluster in crevices and remain in a dormant condition. Such dormancy may extend upto 4 years till the favourable conditions return (Burges, 1959). Diapause of T. granarium is peculiar in that the larvae resting in diapause are found to moult occasionally, and at long intervals some of them emerge from the refuge,a few feed and pupate when food is available and the rest return to the dormant phase again (Burges, 1959). Burges who studied this behaviour of the khapra larvae, characterises this dormancy as a weak facultative diapause. Extention of the larval life by dormancy and low respiration with occasional short periods of rapid respiration associated with feeding are characteristics of of diapause larvae (Burges, 1960). Diapause larvae show a very low oxygen consumption and consequently a low ebb in metabolism (Burges, 1960). The diapause could be broken by subjecting the larvae to a sudden rise in temperature for a few days and bringing back to the normal.

The studies of Burges (1963) and Stanic et al. (1963) have shown that two factors, a low temperature and high population density with the consequent excssive contamination of food with the faecal matter can induce diapause. Though considerable information has accumulated on the diapause of T. granarium, no studies were reported so far from India and it was thought desirable to examine whether information obtained on khapra larvae from India to which region it is believed to be indigenous, conforms with the data available in the literature. Therefore, the incidence of diapause was studied at various temperatures and population densities. Further, this study was a pre-requisite for the design of experiments aimed at a study of the histophysiological and biochemical changes in the diapause larvae as compared to the normal reported in the following chapters.

Observations at room temperature in the winter conditions of Baroda showed that none of the larvae entered pupation in the winter months even after attaining full larval maturity. A marked diurnal variation in temperature occurs during the major part of the winter season in Baroda. Burges (1962) reported that under a regular daily fluctuating temperature regime such as between 25 and 40°C, very few diapause larvae of T. granarium would pupate. While such a diurnal variation helps to maintain the diapause state it would be interesting to know whether it would have any effect in inducing diapause. Studies were also conducted, therefore to determine the effects of an artificial variation in temperature

embracing the unfavourable and favourable temperatures for the normal development of khapra larvae at either ends (25-35°C) on the incidence of diapause.

MATERIAL AND METHODS

The larvae were maintained on crushed wheat. In all experiments except that on the study of the influence of diurnal variation in temperature, the humidity was maintained at 70% by saturated salt solutions (Winston, 1960).

To study the effect of temperature and population density on the incidence of diapause, newly emerged larvae were placed in specimen tubes (3X1") containing various quantities of food as shown below at 20±1°C, 30±1°C, 35±1°C and 37±1°C.

1- 1 larva in 100 mg of food (10 samples)

2- 10 larvae in 300 mg of food (10 samples)

3- 10 larvae in 1 g of food (10 samples)

Two replications of these groups were employed. In group 1 and2 2 each larva had 100 mg of food, which is 10 times the approximate food requirement of a larva (Burges, 1963 has shown that a single larva would require only 9 mg of food to complete the larval development) at which density the percentage of larvae entering diapause is known to be negligible, whereas in group 3 each larva had 30 mg of food at which density though the food was not a limiting factor a large percentage (nearly 40% at 30°C) would enter diapause (c.f. Burges, 1963). Pupation

was recorded at the end of the 15th day and every five days thereafter.

The effect of diurnal variation of temperature on development was studied by exposing the larvae to a constant temperature of 35°C for 12 hr in the day time and of 25°C for 12 hr in the night. 10 newly hatched larvae were placed in 1 g crushed wheat in a specimen tube and 10 such tubes were placed in a desiccator which was shifted at regular intervals to 35°C and 25°C maintained in the incubators. Two replications of the experiment were carried out.

RESULTS AND DISCUSSION

At 20°C and 70% R.H. no pupation occurred even at the end of 125 days under all population densities employed. The mortality was found to be 16% in the non-crowded cultures and 11% in the crowded cultures. For an animal whose optimal range of temperature lies around 35°C, these percentage mortalities should be considered quite low at such a temperature. During the 125 days, the growth of the larvae was more or less arrested. Most of them had undergone a few moults but none of them reached maturity. Diapause in khapra larva is characterized by an arrest of development after attainment of larval maturity. The larvae at 20°C in the present study, therefore, were not in a state of diapause but in a state of stunted growth. It may be mentioned here that at 25°C Burges (1963) found that the larvae reached mature size in about 40 days but none pupated in 270

days which is typical of diapause condition.

The results obtained on the effect of population density on the incidence of diapause at 30°C and 70% R.H is presented in Fig.I. Pupation was completed by the end of 35 days in individual cultures (1 larva in 100 mg). It may be noted that no diapausing larvae were encountered in this culture. However, 31.6% larvae in the crowded cultures (10 larvae in 300 mg) and 16.5% in the non-crowded cultures (10 larvae in 1 g) remained in the larval stage at the end of 40 days. These larvae are recognized as diapause larvae, since 40 days is a convenient arbitrary point between non-diapause and diapause larvae at this temperature (Burges, 1963). Of the diapause larva only 2.1% in the crowded cultures pupated during the next 65 days. Mortality was quite low at all population densities (5% in crowded and 11.6% in non-crowded cultures).

Burges (1963) has reported that under similar conditions of temperature and humidity in dense cultures (1 larva per 42 mg food) 51% of the larvae entered the diapause state. In less dense cultures (1 larva per 63 mg food) 36% diapause larvae was encountered. These and other experiments of Burges (1963) has clearly demonstrated that the percentage of diapause larvae increase with increasing population densities. The present experiments at the two population densities also confirm this observation. However, the percentage of diapause larvae (31.6%) encountered in the present study in the 'crowded cultures' (1 larva per 30 mg food) which is more or less similar to the

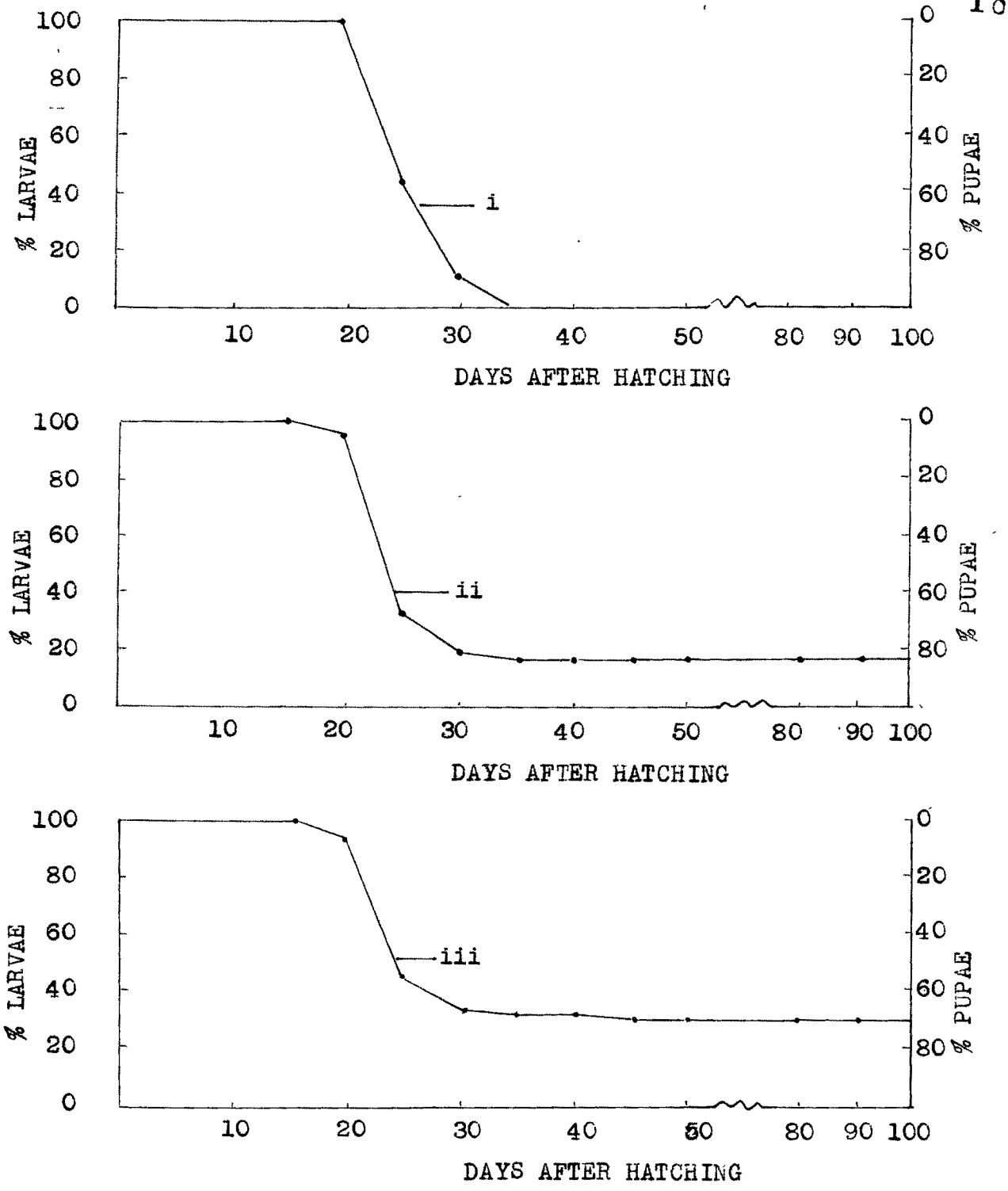

Fig. 1. Showing cumulative percentage of larvae and pupae in cultures of T. granarium at 30°C and 70% R.H.
i- 1 larva in 100 mg food, ii- 10 larvae in 1 g food, iii- 10 larvae in 300 mg food.

'dense culture' of Burges (1 larva per 42 mg food) is considerably lower than that reported (51%) by Burges. It may be pointed out that considerable variation can occur in the percentage of diapause larvae under the same conditions of temperature and population density. Thus in the data presented by Burges the following percentages of diapause were seen at the population density shown against each in different sets of experiments: 51 %- 1 larva/ 42 mg (Table I, Burges, 1963), 37.4% - 1 larva/ 33 mg (Table II, Burges, 1963) and 4.8% - 1 larva/ 66 mg (Table II, Burges, 1963). This variation can be attributed to the parentage of the hatch of larvae employed in the experiments since a higher percentage of diapause should be expected in larvae obtained from diapausing parents. Also the absolute number of larvae present in a culture rather than the amount of food available per larva may determine the diapause percentage. For example more diapause larvae may be encountered in a culture having 100 insects per 3000 mg food than in a culture having 10 insects in 300 mg of food.

The results of the experiments carried out to study the incidence of diapause at 35°C and 70% R.H and at different population densities are presented in Fig. 2. In the crowded cultures all the larvae pupated within 30 days. However, 10% of the larvae in the individual cultures and 7.2% in the non-crowded cultures remained in the larval stage at the end of 35 days which may considered an arbitrary dividing point between non-diapause and diapause larvae at this temperature.

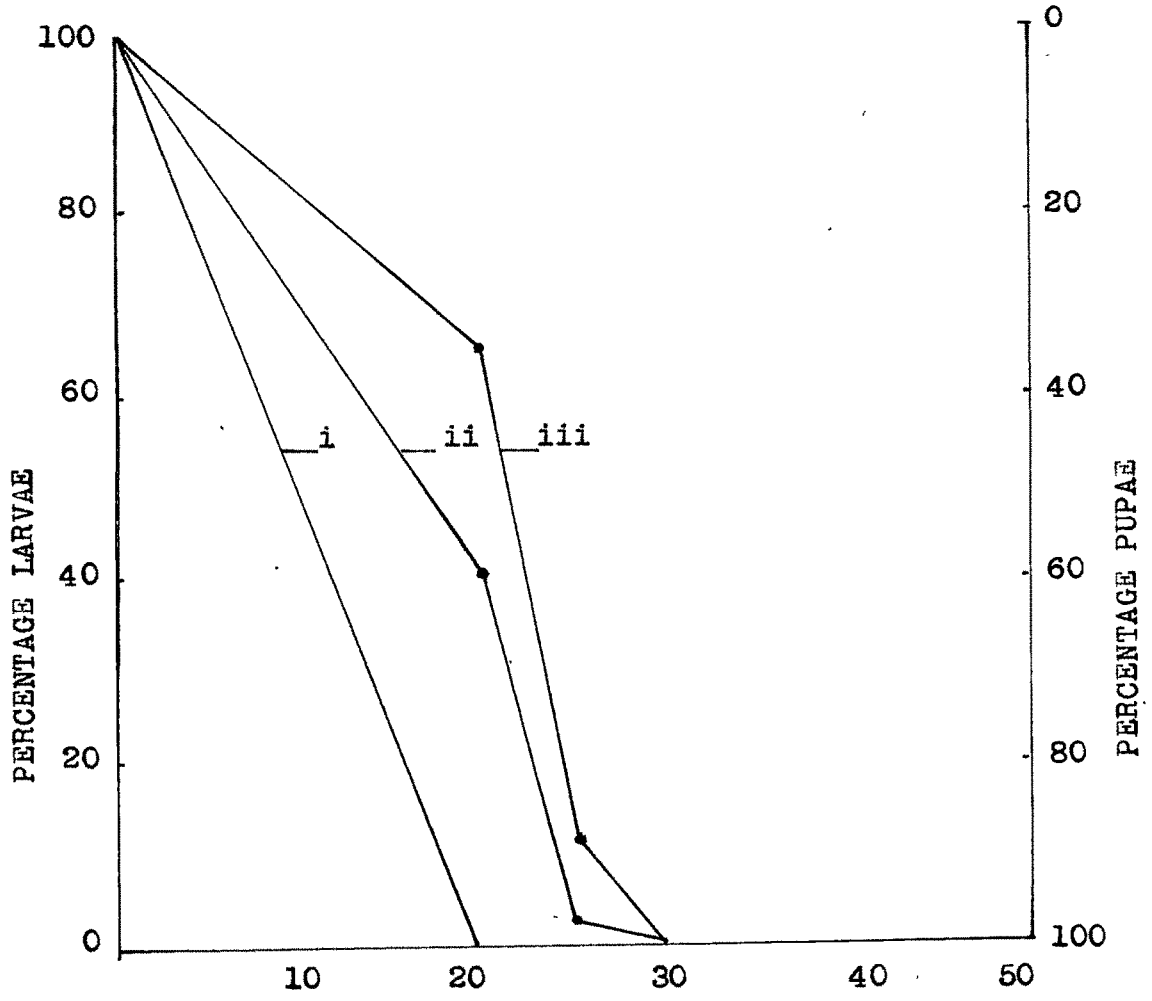

Fig. 2. Showing cumulative percentage of larvae and pupae in cultures of T. granarium at 35°C and 70% R.H.
i- 1 larva in 100 mg food, ii- 10 larvae in 300 mg food, iii- 10 larvae in 1 g food.

Continued observation, however, showed that all the remaining insects died before 55 days in the larval stage itself. It is debatable therefore, whether they could be regarded as diapause larvae. Burges (1963) observed that even at 35°C and 40°C, in dense cultures, a very small percentage (2 and 3% respectively) of the larvae were stragglers which had pupated by the 78th day as against most of the larvae which had pupated by the 32nd day. He observed that Trogoderma stragglers are best regarded as diapause insects. In the present study since all the stragglers died eventually they are better regarded as larvae whose normal metabolism was impaired. A prolonged larval stage with eventual mortality due to impairment of the metabolic processes is not uncommon in insects. Such an effect was observed in the related dermestid beetle, Anthrenus vorax as a result of exposure to gamma radiation (Nair and George, 1965).

Fig. 3 presents the data obtained at 37°C, In both the non-crowded and crowded cultures all the larvae had pupated by the end of the 30th day, and most of them did so before the 25th day. The mortality was 20% in the crowded cultures and 11% in the non-crowded cultures.

It may be concluded from the above studies that diapause in khapra larvae do not occur at or above 35°C in the crowded or the non-crowded conditions employed in the present study and that the stock of T. granarium used in this study is similar in their diapause characteristics to thise studied by other investigators.

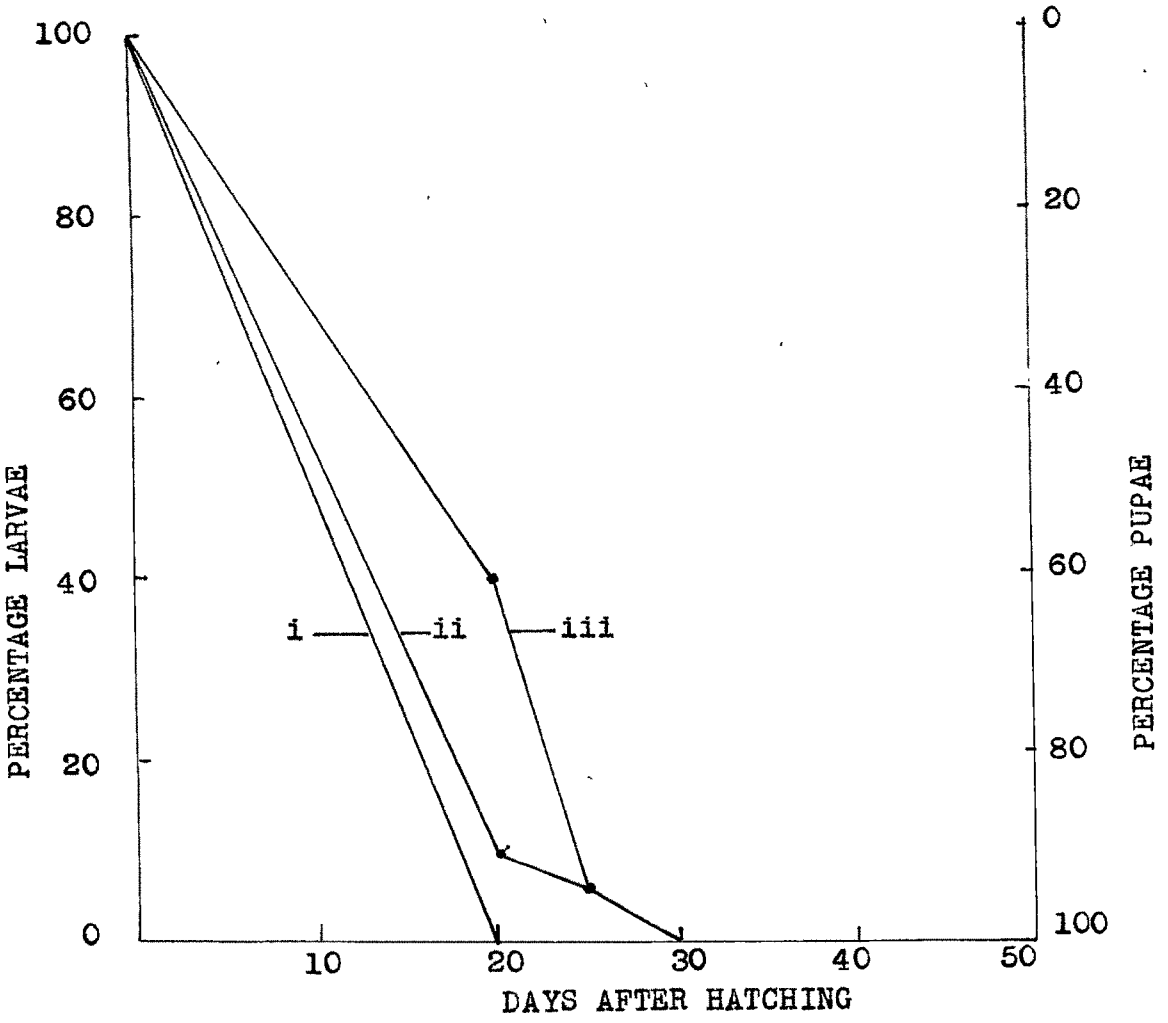

Fig. 3. Showing cumulative percentage of larvae and pupae in cultures of T. granarium at 37°C and 70% R.H. i- 1 larva in 100 mg food, ii- 10 larvae in 300 mg food, iii- 10 larvae in 1 g food.

The larval development was profoundly affected by the diurnal variation of temperature. The results are presented in Fig. 4 and 5. A high percentage of mortality occurred in these cultures. The observations were made for 110 days. Most of the mortality occured during the first half of this period. The mortality figures at the end of 110 days were 75% for the crowded cultures, 40% for the non-crowded cultures and 50% for the individual cultures, of which 72%, 38% and 50% respectively had occurred before 55 days. Of the total initial number of larvae, 3% of the crowded cultures, 15% of the non-crowded cultures and 20% of the individual cultures pupated by the end of 110 days. Most of the pupation occurred during the first 55 days. The remaining larvae were in different stages of growth, a few of them apparently full grown and most in the earlier stages.

Though the present study on the effect of diurnal variations does not lead to any conclusions, certain points stand out clearly. Firstly, some of the larvae were able to develop and pupate successfully even at such severe temperature conditions though the resulting individuals were comparatively smaller than the normal adults. This shows the extent of tolerence and adaptation of the khapra beetle to extreme conditions of existence. Secondly, most of the mortality and pupation had occurred during the first half of the experimental period and for the remaining half they neither died nor pupated. But since all the remaining larvae were not full grown it is

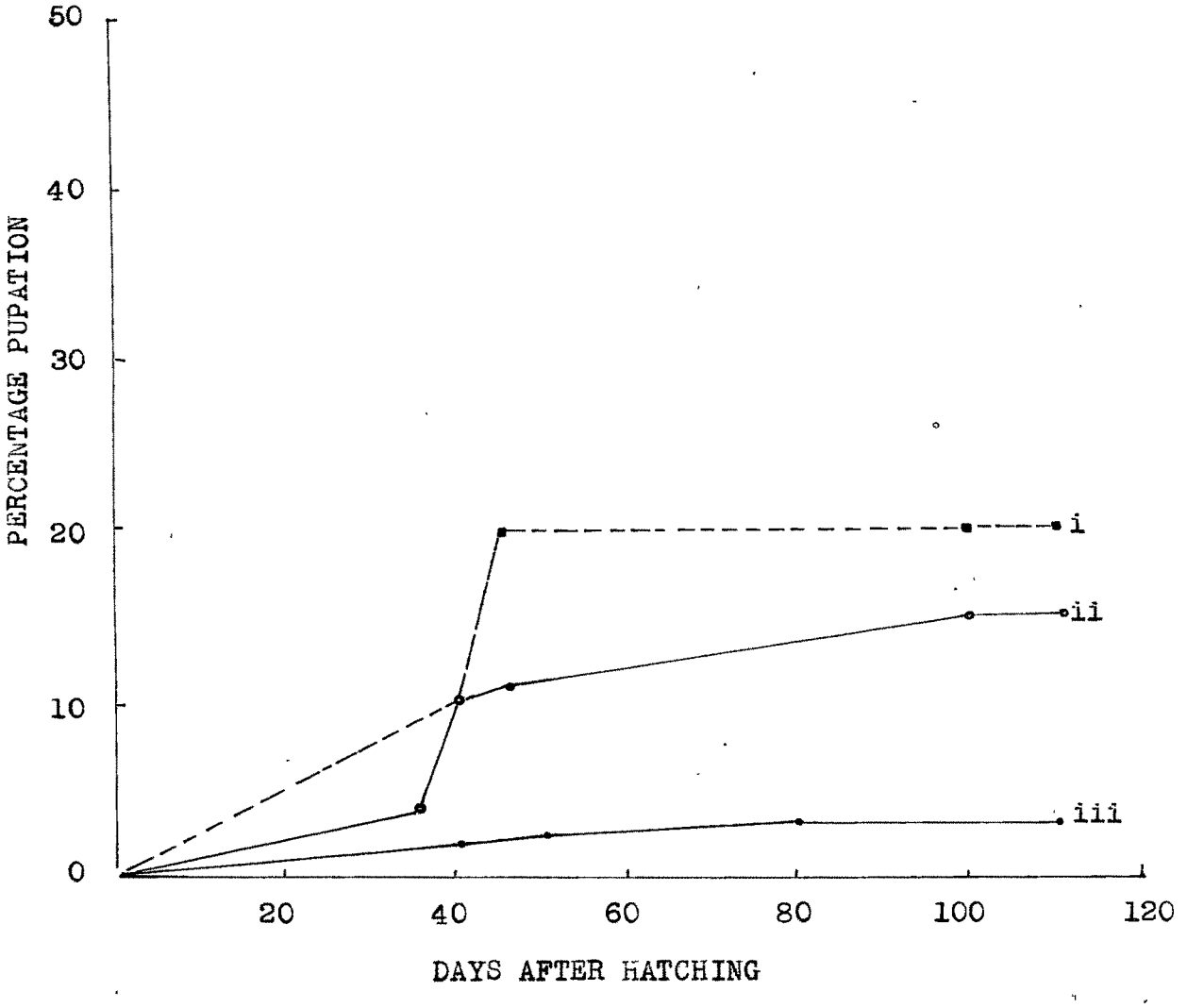

Fig. 4. Showing cumulative percentage of pupation in cultures of T. granarium exposed to a diurnal temperature change of 25- 35°C. i- Culture containing 1 larva in 100 mg food, ii- culture containing 10 larvae in 1 g food, iii- culture containing 10 larvae in 300 mg food.

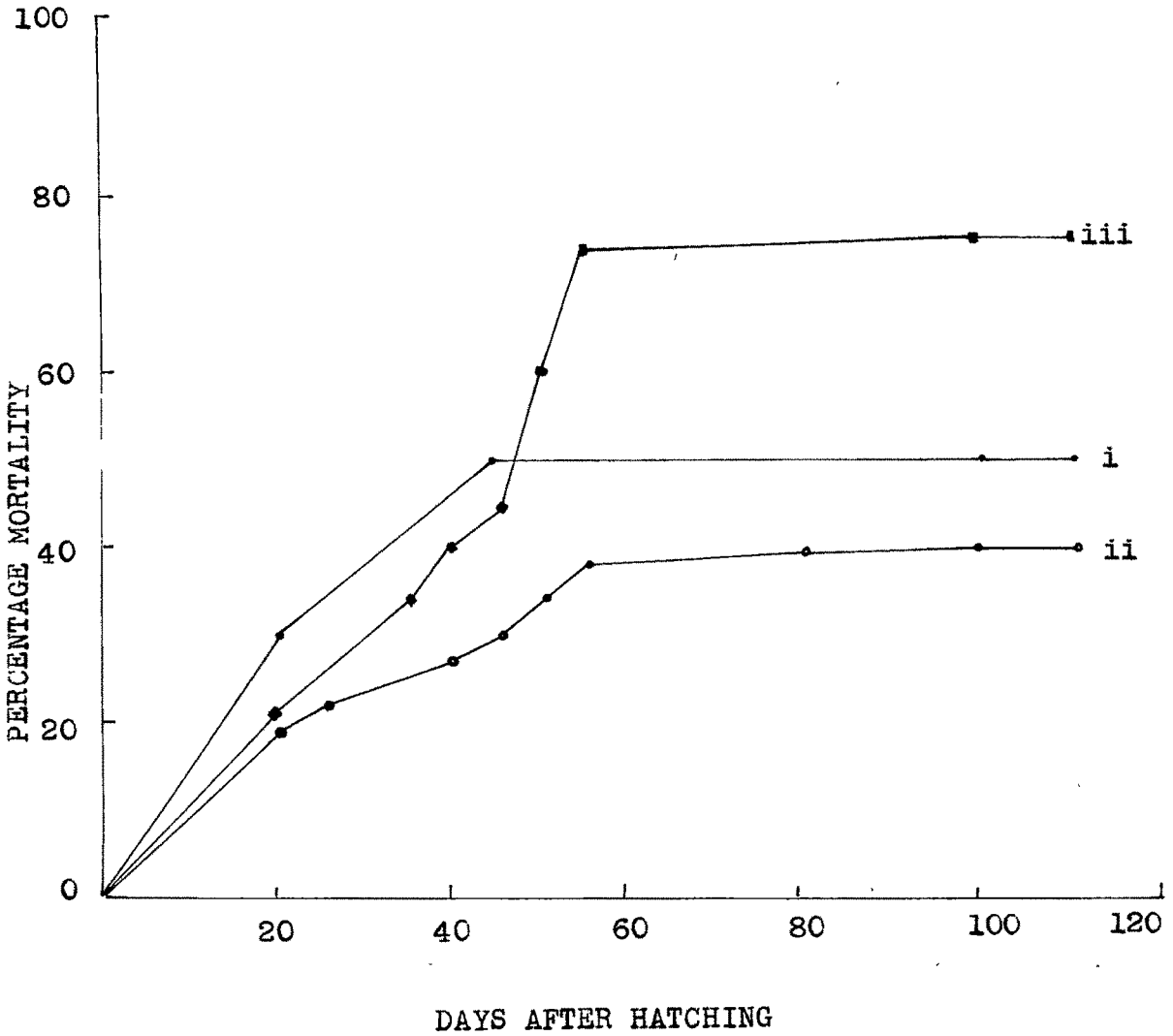

Fig. 5. Showing the cumulative percentage of mortality of T. granarium exposed to a diurnal temperature change of 25-35°C. i- Percentage mortality in culture containing 1 larva in 100 mg food, ii- 10 larvae in 1 g food, iii- 10 larvae in 300 mg food.

debatable whether they were in a diapause state, unless ofcourse the khapra larvae are capable of diapausing even in the earlier instars. It may be noted that at the lowest temperature obtained in the present study the larvae are known to reach maturity in about 40 days if maintianed continously (Burges, 1963). And yet, when the temperature fluctuated between this (25°C) and 35°C at which the larval maturity is reached earlier, mast of the larvae did not reach the mature size even after 110 days. This can be attributed to the extreme daily alteration in temperature. It would be interesting to study the development of khapra larvae under conditions which are less critical ie. under experimental conditions which simulate the natural diurnal fluctuations of temperature with out an abrupt change from the minimum to the maximum and vice versa. This would show whether the diurnal variation in temperature has any effect in retarding development and in inducing diapause.