CHAPTER 2

OBSERVATIONS ON THE BIOLOGY OF *ANTHRENUS VORAX* UNDER LABORATORY CONDITIONS

Studies on the life history and biology of *Anthrenus vorax* Waterhouse were carried out by Griswold (1941), Ayappa, Cheema and Perti (1957) and Patel (1958). Ayappa *et al*.(1958) reared the larvae on hog bristles or wool with or without the addition of yeast, at different levels of temperature and humidity and gave a detailed account of the duration of the life cycle, fecundity etc. In a monograph on this beetle, Patel (1958) furnished data on their geographical distribution, voltinism and the influence of temperature, humidity and different types of food materials on the developmental stages. As the earlier literature on the biology of this species is adequately covered by Patel (1958) it is needless to repeat them here. A perusal of the available data, however, showed considerable discrepancy in the duration of the entire life cycle and of the different developmental stages as well as the fecundity. This may be attributed to the fact that the rate of growth varied in accordance with the varied food habits and the envirommental conditions. Consequently, as a pre-requisite for conducting the physiological and biochemical studies presented in this thesis, it was necessary to standardise the conditions of food and temperature, which, by far, had the greatest influence on their life-span and to study their biology under these conditions. This chapter reports the results of such

an investigation. The pertinent observations of the earlier authors are discussed and compared with the present observations.

MATERIAL AND METHODS

The insects were obtained originally from a natural infestation found during September, 1959 on some pieces of horn kept in the museum. A culture was maintained for about an year on sheep horn. In the meanwhile, it was found that dried muscle could provide a satisfactory medium for their maintainance in the laboratory and the culture was then transferred to a medium of dried pigeon breast muscle. Pigeon breast muscle was used as it was available in plenty from pigeons killed in the laboratory for routine experiments. The fresh muscle was cut to small pieces, dried in an oven at 80 to 100°C and stored. While the stock culture was maintained at room temperature, the insects used for the observations reported here were maintained at a temperature of 32 ± 1°C in an incubator.

OBSERVATIONS AND DISCUSSION

Incubation period of the egg

The data obtained on the incubation period are presented in Table I. The incubation period at 32 ± 1°C was found to be 6.78 ± 0.42 days. Ayappa *et al.* (1957) observed that the incubation period varied in relation to the temperature, humidity having no effect on it. He found that the eggs required about 13 days at 25°C (13.6 ± 0.08 days at 90% R.H. and 13.73 ± 0.07 days at 30% R.H.), about 8 days at 30°C (8.13 ± 0.04 days at 90% R.H. and 8.64 ± 0.06 days at 30% R.H.), about 6 days both at 35°C (6.69 ± 0.06 days at

90% R.H. and 6.52 ± 0.06 days at 30% R.H.) and at a temperature varying between 25 and 31°C (6.81 ± 0.07 days at 70 - 75% R.H.) and 4.46 ± 0.1 days at 37.5°C (40 - 50% R.H.) for hatching. Patel (1958) also observed a reduction in the period of incubation with increasing temperatures. His figures at room humidity were 24.1 days at 70 ± 5°F, 13.4 days at 75 ± 5 °F and 10.1 days at 81.6 ± 2°F, which should correspond to 21, 25 and 28°C respectively. The data obtained in the present investigation at 32 ± 1°C agrees well with the above observations.

TABLE I

Incubation period of the egg at 32 ± 1°C

Batch number	Total number of eggs	No. of eggs hatched on 7th day	No. of eggs hatched on 8th day	No. of eggs hatched on 9th day	Mean incubation period
1	22	nil	22	nil	
2	30	1	26	3	
3	13	1	12	nil	
4	56	4	52	nil	
5	80	14	66	nil	
6	8	nil	8	nil	
7	71	45	26	nil	
Total	280	65	212	3	6.78 ± 0.42 days

The influence of diet and temperature on larval growth

It is known that the type of food available largely determines the rate of growth and the length of life cycle of Anthrenus vorax (Ayappa et al., 1957 and Patel, 1958). Therefore in an attempt to select a suitable medium for the laboratory culture of these insects, the larvae were reared on different food stuffs such as dried pigeon breast muscle, with and without the addition of yeast, sheep horn and a mixture of casein and yeast. The temperature was maintained at 32 ± 1°C. The data obtained are presented in Table II. The larvae were capable of successful completion of development on dried muscle alone, with a larval period of 84.30 ± 23.21 days. However, there was wide individual variation in the larval period. The addition of yeast to the muscle enhanced the rate of growth so that the larvae attained their full size within 36.65 ± 4.44 days and pupated. The larvae could also be successfully reared on a mixture of casein and yeast and this had the additional advantage of having a known composition. It may be noted that the range of variation in the larval period from minimum to maximum was narrower when they were reared on horn or on media containing yeast. The wide variation in the larval period from 40 to 140 days when fed on muscle alone cannot be easily understood. It may appear that the larvae which completed their growth within a short time might have harboured some bacteria in their alimentary canal which provided some vital growth factors. The absence of such wide variations on supplanting the food with yeast powder indicates

TABLE II

Influence of diet on the larval period

Diet	Mean larval period in days				Mean larval period in days
	Batch I	Batch II	Batch III	Batch IV	
Dried pigeon breast muscle	93.88 ± 29.48 (16)	85.19 ± 13.09 (16)	60.80 ± 11.04 (15)	97.50 ± 14.87 one died as early larva (15)	84.30 ± 23.21
Muscle plus 5% yeast	38.80 ± 3.59 (15)	34.20 ± 3.49 (10)	36.00 ± 5.12 (12)	–	36.65 ± 4.44
Horn	36.60 ± 4.44 (15)	38.07 ± 5.71 (14)	–	–	37.31 ± 5.05
Casein plus 5% yeast	43.28 ± 2.95 (25)	–	–	–	43.28 ± 2.95

Figures in parentheses denote the number of individuals in the batch.

that these growth factors were provided by the yeast. It was surprising, however, to find that horn was of greater nutritional

value (in terms of rapidity of growth) than dried muscle and could even compensate the addition of yeast.

The above findings have shown that different media could be used for successful rearing of this insect in the laboratory: muscle powder providing a natural medium for sustained growth; horn a natural medium for rapid growth; muscle powder plus yeast, a semi-natural medium for rapid growth and a mixture of casein and yeast, an artificial medium having a known composition and ensuring rapid larval development. The higher temperature of 32°C employed also helped in shortening the larval period. For studies reported in later chapters the insects were maintained on a diet of dried pigeon breast muscle supplanted with 5% Brewer's yeast.

That the duration of the larval stage is affected markedly by temperature and the nature of the food was shown by the observations of Ayappa *et al.* (1957) and Patel (1958). Ayappa *et al.* reared the larvae on hog bristles. The duration of the larval period was about 330 days at a temperature of 30°C (327.9 ± 4.6 days at 30% R.H. and 333.5 ± 5.15 days at 90% R.H.), about 221 days at a constant temperature of 35°C (221.5 ± 5.62 days at 30% R.H. and 221.4 ± 5.15 days at 90% R.H.) as well as at a temperature varying between 25 and 31°C (222.6 ± 4.51 days at 70-75% R.H.). According to these authors, a temperature fluctuating between 25 and 31°C was more favourable to rapid larval development than a constant temperature of 30 or 35°C. Humidity was observed to have had no effect on the duration of larval period at the temperatures investigated. Patel (1958)

reported that given food of the same type, the duration of the larval period responded to temperature in the normal manner, in being shorter at higher temperatures.

Studying the development of the larva on wool impregnated with different substances like cow-dung extract (which was supposed to contain a growth factor, possibly vitamin B 12), glucose-albumin solution and commercial yeast, Ayappa et al. (1957) observed that the development of the larva was quickest (about 50 days on the average) at 25-31°C on woolen fabrics treated with 10 to 20% yeast. Only 28% of the larvae developed successfully on untreated woolen fabrics and the larval period varied between 287 and 406 days with an average of 339 days at a temperature of 25-31°C. On untreated hog bristles, however, 46% of the larvae survived with the larval period varying between 158 and 283 days, at the same temperature. The larvae failed to grow on silk and cotton fabrics even when impregnated with yeast. Patel (1958) also observed a considerable reduction in the larval period on the addition of veterinary yeast to the food. The larvae when fed on wool alone managed to remain alive but apparently without any sign of development. The addition of yeast, however, enabled them to develop on wool, the larval period lasting between 43 and 56 days at 81 ± 2°F (approximately 28°C) and 26% R.H. On a diet of wool plus bone and meat meal or wool plus blood meal, the average duration of the larval period was 155.8 days at at/temperature of about 28°C and room humidity. The addition of yeast to the above food reduced the larval period

to an average of 110.3 days. Given bone plus meat meal, the larval life lasted 57-70 days at about 28°C and 37% R.H.

This brief resume of the earlier studies shows that under various conditions of food and temperature the larval period varied between the range of 43 to 406 days. The present study has shown that by employing a mixture of dried muscle and yeast or of casein and yeast the larval period could be brought down to the minimum with very little individual variations.

Duration of the pupal period

Observations on the duration of the pupal period are presented in Table III. All pupae were obtained from larvae maintained on a diet of dried muscle.

TABLE III

Duration of the pupal period at 32 ± 1°C

Actual pupal period				Quiescent adult period					
No. of insects examined and sex	No. of insects which showed an actual pupal period of		Mean actual pupal period in days	No. of insects examined and sex	No. of insects which showed a quiescent adult period of				Mean quiescent adult period in days
	6 days	7 days			3 days	4days	5days	6days	
100 males	54	46	6.46 ± 0.25	255 males & females mixed	32	124	96	3	4.27 ± 0.69
100 females	39	61	6.61 ± 0.24						

Unlike the larval period, the pupal period did not show considerably high individual variations. In general, the

pre-emergent stage was reached on the 7th or 8th day of pupation and the adults emerged on the 4th or 5th day of becoming the pre-emergent adult. According to Ayappa et al. (1957) the actual pupal period (the period from the day of pupation to becoming the quiescent or the pre-emergent adult) lasted for about 13 days at 25°C, about 8.5 days at 30°C and about 7 days at 35°C. The quiescent adult period was about 8 days at 25°C, 6 days at 30°C and between 4 and 6 days at 35°C. Humidity had no appreciable influence on the duration of either the actual pupal period or the quiescent adult period. The duration of these stages was nearly the same for both the sexes. According to Griswold (1941), however, the actual pupal period and the quiescent adult period were shorter in males than in females. Patel (1958) also reported that the females have a longer pupal period than the males. The present study showed only a very slight difference between male and female in their pupal period.

The figures obtained by Patel (1958) as regards the actual pupal period as well as the quiescent adult period show wide individual variations. The actual pupal period, for example, was found to vary between 8 and 21 days with an average of 12.8 days at about 28°C. However, such wide variations between individuals were not observed in the present study. The figures of Ayappa et al. (1957) also do not show a mean deviation above ± 0.10 day to ± 0.39 day.

The duration of adult life

Under natural conditions, the adults are reported to frequent flowers and feed on pollen and nectar (Hinton, 1945).

Sohi (1951) reported that the adults visit flowers especially roses to feed on pollen. No information is available on the longevity under these conditions. Studies under laboratory conditions indicate that food and temperature influence their longevity (Ayappa et al., 1957; Patel, 1958). The relevant observations of various authors together with the data obtained in the present investigation are summarised in Table IV. That temperature had a marked effect on the longevity of the adult beetles is clear from the data given in the table. They tended to live longer at lower ~~et lower~~ temperatures. Griswold (1941) and Patel (1958) noted that females lived longer than males. According to Ayappa et al. (1957) the females lived longer than the males at 25°C, but at 30°C or at 35°C they did not show much difference. In the present investigation, however, at 32 ± 1°C the males lived longer than the females, without food, the figures being 22.75 ± 2.75 and 18.25 ± 0.50 days respectively.

Ayappa et al. (1957) observed that the nature of the food influenced the longevity of the adult. There was, however, no difference in the life span of females when released on hog bristles alone (apparently the adults do not feed on hog bristles) or on hog bristles smeared with glucose-albumin, but the males on the other hand, lived longer on the latter. Patel (1958) also fed the adults on different diets for observations on fecundity but did not record the duration of life under these nutritional conditions.

TABLE IV

LONGEVITY OF THE ADULT

Author	Temperature and humidity	Adult diet	Other conditions	Longevity in days Male	Longevity in days Female
Ayappa, Cheema and Perti (1957)	25 C 50-60% R.H.	Hog bristles smeared with glucose-albumen	-	36.3 ± 0.8	40.6 ± 2.7
	30°C 30% R.H.	"	-	16.4 ± 0.4	17.4 ± 0.9
	30°C 90% R.H.	"	-	16.1 ± 1.8	16.3 ± 1.0
	35°C 30%R.H.	"	-	14.7 ± 1.1	14.3 ± 0.6
	35°C 90%R.H.	"	-	18.1 ± 1.2	16.4 ± 0.8
		Hog bristles plus glucose-albumen	-	23.3 ± 1.6	25.5 ± 1.9
	25-31°C 70-75% R.H.	Hog bristles smeared with cow-dung extract	-	18.6 ± 1.2	23.0 ± 1.3
		Hog bristles alone	-	18.1 ± 1.8	26.3 ± 1.7
Patel (1958)	81.6 ± 2°F Room humidity	No food	Unmated	33.5 (9-62)*	45.3 (28-79)
	70-85°F Room humidity	No food	Unmated	29.0 (16-38)	56.4 (37-70)

Author	Temperature and humidity	Adult diet	Other conditions	Longevity in days	
				Male	Female
Griswold (1941)	Room temperature	-	Unmated	(30-200)	(45-237)
	77-80°F	-	Unmated	(46-197)	(78-253)
Present author	32 ± 1°C Room humidity	No food	4 pairs kept separately on wool fibres	22.75 ± 2.75	18.25 ± 0.50
	32 ± 1°C Room humidity	No food	114 adults in mixed ~~mixed~~ batches on wool fibres	18.05 ± 3.61 (14-30)	

* Figures within brackets indicate the range.

An extensive study on the duration of adult life, fecundity and the oviposition cycle as affected by the diet of the adult has been conducted by Blake (1961) on a closely related species, Anthrenus verbasci - the Varied carpet beetle. The natural diet of this species, so far as is known, is restricted to pollen, nectar and possibly rain water and dew (Blake, 1961). In her studies, the natural diet was closely simulated by feeding them with a combination of glucose, sucrose and fructose to represent 'nectar', and pollen was obtained from michaelmas daisy. There were no significant differences between the life span of beetles deprived of food and water and those given water, water with pollen or water with albumen. This was true for both male

and female and there were no differences noted between the sexes. When sugar solution was given, there was a significant increase in the life span in both the sexes and when albumen was given in addition to sugar solution, there was a further increase in both the sexes. There was a sex difference in the effect of pollen; the males lived longer than when given sugar solution alone or sugar solution with albumen, but the females did not live significantly longer than when given sugar solution alone.

Oviposition

Patel (1958) reported that the pre-oviposition period varied from 3 to 30 days but that for the majority of insects it ranged from 3 to 8 days. Ayappa et al. (1957) observed that it varied from 4 to 7 days at 30°C . In the present observations it ranged from 2 to 3 days when a proper substratum was provided for egg laying.

Irregular pieces of a mat-work made up of larval hairs, which incorporated the larval exuviae, the pupal coverings and concretions of faecal matter, were found in the culture medium. Microscopical examination showed that all types of hairs of the larval body are incorporated into this mat-work of hairs. The hairs were not woven in any definite pattern and appear to be simply gathered and glued together. Most of the eggs were found laid on this substratum and were attached to it at one end of the egg.

It was observed that both the larvae and the adults are capable of constructing this 'mat of hairs'. When a number

of larvae were separated out into a petri-dish, a small piece of this mat-work was found after a few days. The larvae have a profuse clothing of hairs which is easily detachable, and it appears that the detached hairs and hairs of the exuvia are used for constructing this structure. Similarly, when a number of adults were separated out into a petri-dish and the moulted larval and pupal coverings were provided, the so-called 'bed of hairs' was formed a few days after, which at this instance, was constituted of the different types of hairs of the larval exuvia with pupal coverings incorporated into it.

The exact purpose of such a bed is not clearly understood. It appears primarily to provide a suitable substratum for the deposition of eggs. However, the ability of the larvae to construct it suggests other possibilities as well. When the larvae were liberated on the surface of this mat-work separated out into a petri-dish, they could be seen sheltering beneath it within a short time. While feeding into the horn, the insects would eat into the interior and the surface could be seen covered by the mat. These observations tempt one to believe that it helps the larvae in protecting themselves from light. Another function could be protection from predators like wall lizards which have often been found to feed on these insects.

It is significant that egg laying was hampered in the absence of some sort of an egg-laying substratum. A number of adults were collected at random and kept in clean petri-dishes in four groups, of 12, 10, 25 and 20 individuals respectively.

No eggs were laid till the 4th day. The first two batches were then supplied with woolen threads, the third batch with a piece of black paper and the fourth kept without any substratum. A number of eggs were laid on the next day and on the successive days in the first two batches. From the third batch, two eggs were obtained on the 15th day, 13 eggs on the 17th day and 14 eggs on the 19th day. After this no egg was obtained and the insects died out one by one. In the fourth batch, 2 eggs were laid on the 13th day, 3 eggs on the 16th day and 2 eggs on the 17th day. The number of eggs laid by the third and the fourth batches of insects were extremely low compared to their natural fecundity. It may also be pointed out that oviposition was belated in these insects. These observations point to the fact that a suitable egg laying substratum is necessary for proper oviposition and fecundity.

Table V shows the frequency of oviposition and the number of eggs laid in four pairs of insects studied separately, with wool fibres as the egg laying substratum. It can be noted that the eggs were laid in batches with a few days interval. Three distinct peaks of egg laying could be noticed, after which the eggs were laid rather irregularly. In Anthrenus verbasci, Blake (1961) reported an oviposition cycle with three clearly defined peaks of oviposition occurring on about the 6th, 12th and 17th days. The existence of a similar oviposition cycle in Anthrenus vorax is indicated by the present observations.

TABLE V

Fecundity and frequency of oviposition

Day of egg laying	Number of eggs laid			
	Pair I	Pair II	Pair III	Pair IV
1st day	-	-	-	-
2nd day	-	-	-	-
3rd day	-	-	-	15
4th day	19	6	32	9
5th day	2	26	-	-
6th day	-	-	-	-
7th day	-	7	24	24
8th day	18	23	-	-
9th day	-	-	-	-
10th day	14	16	-	20
11th day	-	-	-	-
12th day	1	4	16	-
13th day	4	-	-	12
14th day	-	-	-	-
15th day	-	-	-	-
16th day	-	-	-	4
Total no. of eggs laid	58	82	72	84

Mean fecundity = 74 $\pm$ 11.89 eggs per female

Fecundity

Back and Cotton (1938) reported that the female lays as many as 37 to 96 eggs and according to Hocking (1943) the number of eggs laid ranges from 40 to 100. Sohi (1951) observed that 11 to 52 eggs were laid by the female. But the observations of Patel (1958) showed that a female without food laid only 23 eggs on the average, the average for the four different generations studied being 29, 19, 21 and 23 respectively. He observed that foods containing carbohydrates and protein raised the number of eggs to a maximum of 36 per female. Ayappa et al. (1957) reported 13 ± 0.6 eggs per female when pairs of insects were liberated on hog bristles; it varied between 19 ± 2.4 and 36 ± 5.3 when observed at different temperatures and humidities with insects liberated on hog bristles smeared with glucose-albumen. Patel (1958) observed that the maximum number of eggs laid was 36 and argued that since the ovaries consist of six ovarioles with three eggs in each, the maximum number of eggs could not be more than 36 and that there was no evidence to indicate the development of any additional number of eggs. However, in the present observations on groups of males and females mixed at random, a considerably large number of eggs were noticed, suggesting the possibility of more than 36 eggs being laid by the female. With the four pairs studied separately the number of eggs laid was observed to be 58, 82, 72 and 84, amounting to an average of 74 eggs per female (Table V). This is essentially in agreement with the observations of Back and Cotton (1936) and Hocking (1943).

The lesser number of eggs recorded by Patel (1958) and Ayappa et al. (1957) might perhaps be attributed to the effect of larval nutrition. It is known that larval nutrition has some effect on the fecundity of the adult in many species of insects (Wigglesworth, 1953). Ayappa et al. seem to have used hog bristles as food for the larva. It is not clear what food was provided in Patel's observations. It should be mentioned here that a significant correlation between the weight of the females on emergence and their fecundity have been reported in Anthrenus verbasci. (Blake, 1961).

Ayappa et al. (1957) and Patel (1958) studied the influence of different diets of the adults on the fecundity and reported an increase in the number of eggs laid when provided with carbohydrates and protein. Without food the average number of eggs was 24, with plain water 24.6, with sugar syrup 30, with honey 30.5 and with milk 33. It may be seen, however, that the increase was well within limits; about 72% of the maximum number of eggs being laid even without feeding. In Anthrenus verbasci, though the fecundity was significantly increased with a diet of sugar plus protein, 74% of the maximum number recorded with these diets were laid by those deprived of food and water (Blake, 1961). Such deprivation of food and water did not reduce the viability of the eggs.

"The adults of many dermestid genera normally live outdoors, usually frequenting flowers where they draw their sustainance from pollen and nectar. The adults of Trogoderma

versicolor require neither food nor water in order to attain their full fecundity and longevity. The adults of Attagenus partake only to a slight extent, if at all, of the larval type of food" (Patel, 1958). In the light of the above observations, it may be concluded that the adults of dermestids, as a group, are capable of laying the majority of eggs without feeding- in other words egg production is autogenous. In Anthrenus vorax, there is a large reserve of stored fat at emergence which can be mobilized for the production of eggs (Chapter 5). Under laboratory conditions, therefore, the reserves built up during larval life seems to be a limiting factor for the longevity and fecundity of the adults. These insects are well equipped to continue the generations even if they do not fly out to feed. However, under natural conditions, they might fly to the flowers, feed on pollen and nectar, whereby their life span is increased and they could eventually fly to suitable sites for oviposition such as the nests of birds and could also lay more eggs. This offers them an opportunity for wider distribution. The observation of Blake (1961) that the adults of Anthrenus verbasci are attracted by the odour of bird nest material is interesting in this connection. She assumes that in nature, the olfactory responses assist in guiding females to both flowers and oviposition sites. The present author has observed the larvae and adults of Anthrenus vorax in a nest of house swifts, Apus affinis. The swift's nest which consist of feathers and a considerable amount of mucoid material (Naik and Naik, 1963) appears to offer a suitable medium for oviposition as well as the sustainance of the larva.