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Factors Influencing Population Growth of *Tribolium brevicornis* in the Laboratory

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FACTORS INFLUENCING POPULATION GROWTH OF *TRIBOLIUM BREVICORNIS* IN THE LABORATORY

by

Amin Gulla

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Entomology

Approved:

Major Professor

Head of Department

Dean of Graduate Studies

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Amin I. Gulla

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ABSTRACT

Factors Influencing Population Growth of *Tribolium Brevicornis* in the Laboratory

by

Amin Gulla, Master of Science

Utah State University, 1967

Major Professor: Dr. William P. Brindley
Department: Zoology

To determine the effect of the factors of the environment (temperature, relative humidity, and food) on the population growth of *Tribolium brevicornis*, different levels of temperatures from 17.5°C - 37.5°C were used. Effect of these levels of temperatures was tested upon both pre-embryonic and post-embryonic development. No hatching occurred at 17.5°C, but the threshold temperature (the minimum temperature for the development) was just before 17.5°C. The optimum temperature for the embryonic development was found to be between 32.5°C - 35°C.

With the use of the saturated salt solution according to Buxton (1931) and Buxton and Melanby (1934), certain humidity levels (15, 45, 77.5 and 93 percent relative humidity) were used. These levels of humidities did not seem to have a significant effect on the embryonic development.

The duration of the larval and the pupal stage was determined under three levels of temperature (21.9 ± 1, 27.11 ± 1.2 and 30°C). The shortest duration of larval and pupal stage was found to be under 30°C. The levels of the humidities tested

(15, 45, 77.5 and 93 percent relative humidity) upon the duration of larval and pupal stage did not seem to have a significant effect.

Effect of the population densities of the beetles on the cannibalism and the oviposition was tested. The total cannibalism and total oviposition were highly significantly different. The change in rates of oviposition and cannibalism under the densities used (2, 4, 8, 16, 32 and 64 beetles) were not significant, but because of the high standard deviations and coefficient of varrabilities the hypothesis (the rate of cannibalism increases with increasing densities, and the rate of oviposition decreases with decreasing densities) were not rejected.

(43 pages)

INTRODUCTION

The importance of a pest is closely related to the rate at which it increases in numbers. This depends predominantly on the speed of development which is largely governed by factors of the environment (i.e. temperature, humidity, and food). In favorable conditions we can expect that a pest with a simple life cycle and short developmental period will be the most numerous.

The potential importance of *Tribolium brevicornis* was emphasized with the recent discovery of the insect in the nests of alfalfa leaf cutter bees *Megachile rotundata*. The insect was discovered by M. J. Moradeshaghi and was sent by Dr. G. E. Bohart to the U.S.D.A. Museum where it was identified. Linsley (1934) had observed *Tribolium brevicornis* in the nest of the wild bees of the genera *Osmia* and *Xylocopa*. Mininger (1916) observed that the beetles began their feeding upon the pollen stored for larval food, but when the pollen supply ran short, they devoured the young bees. Consequently the beetles could be of potential economic importance as pest of the rather extensive traping and storing operations for commercial production of alfalfa leaf cutter bees. The current work was undertaken to further our knowledge of population regulation factors of these beetles in the laboratory.

Reports on the work done on the population regulation factors were made by many authors. Rich (1956) working with *Tribolium castaneum*, found that Cannibalism of the eggs and reduction in oviposition rate were powerful factors in the decrease in the population of these beetles. Sonleitner (1961) proved experimentally the hypothesis of Rich (1956) that the appetite of the beetles is not satiated even when tremendous amount of the eggs are present in the media of the beetles. In some laboratory populations of *Lucilia cuprina* (Wiedman) populations were limited by competition for larval and adult food as the population increased. The increase in the population also resulted in decrease in natality (Nicholson, 1954a, 1954b). Laboratory population of *Callosobruchus chimensis* (Tinn) were found to limit themselves by direct crowding effects. Increased number of adults caused an increased mortality of eggs by trampling and also a decrease in number of eggs laid (Utida, 1943). Reductions in populations of the insects due to increasing egg cannibalism with increasing population density has been demonstrated with cultures of *Tribolium* castaneum by (Rich, 1956), with the culture of *Tribolium confusun* by Chapman (1928), Chapman and Bird (1934), and Park (1932).

REVIEW OF LITERATURE

One method of studying population growth of an insect is to determine the response of immature stages to factors of the environment. Temperature and humidity are two factors which might influence insect development. Both pre-embryonic and post-embryonic developments are taken into account when we speak about development.

Good (1936) reported complete mortality of the eggs of Tribolium confusum at 35° C in the presence of an uncontrolled humidity. Neither Khalifa and Badawy (1955) nor Lin et al. (1954) obtained any hatching of *Tribolium confusum* eggs at 16° C. Lin et al. (1954) attributed the failure of embryonic development below 17° C. to the exhaustion of food reserves in the eggs. At 17° C, only 21 percent of the eggs hatched. Hatching of the eggs at 17° C was also recorded by (Chapman and Bird, 1934). Butler (1949) working with *Tribolium confusum* reported 15° C to be the minimum temperature for eggs hatching (a threshold of development). The term threshold, which is considered by Lin et al. (1954) to be a poorly defined term, is considered by Peairs (1927) to indicate the temperature at which, on the descending scale, development definitely ceases, and on the ascending scale development is again initiated. Johnson (1940), working with the bed bug, *Cimex lactularius*, observed that the lowest constant temperature at which complete development from oviposition to the conclusion of the hatching process occurred was 13° C. He also observed a slight amount of development below 13° C without hatching. He therefore proposed the term developmental hatching threshold for the first (lower) temperature, and developmental threshold for the second (higher) temperature. Probably one of the most useful definitions is that which is given by Allec et al. (1949), that the developmental threshold is the lowest temperature at which a given physiological process, or development through a given stage in the life history can be carried to completion. The lethal effect of high temperatures upon pre-embryonic as well as post-embryonic development are known to vary with different species of insects. Thomas and Shepard (1940) found that eggs of the saw-toothed grain beetle are incapable of developing at 40° C, while Howe (1956) obtained 86 percent hatch with the eggs of *Tribolium castaneum*. Clark (1935), working with the bug, *Rhodnius prolixus*, found that the upper temperature limit beyond which no hatch could be obtained was 34° C. However, at temperatures above 34° C (35 and 36° C) they observed complete embryonic development within the Chorion without hatching of the eggs. They concluded that the upper temperature limit, above which no hatching occurs, is determined by some physical effect of heat upon the Chorion rather than upon the development of the embryo. The Chorion appeared to be hard, so that the cap could not be forced off by the embryo.

A report by Howe (1960) indicated that relative humidity had no effect on either the percentage of hatch, or the period between oviposition and hatching of the eggs of *Triboluim confusum*. Working with *Tribolium madens* the same author (1962) found that humidity had no effect on the duration of egg stage. Holdaway (1932) found that *Tribolium confusum* eggs were very little affected by changed humidity conditions except at a very high relative humidity when development was prevented by the growth of fungi. At 75 percent R.H. there was a higher survival than at 50 percent relative humidity. Viability of the eggs was highest at low humidities (1 percent and 25 percent R.H.), the maximum survival being at the lowest humidity (1 percent R.H.). However, the result of Headlee (1918) with regard to Bruchus obtectus Say., indicate a different conclusion. When the eggs of Bruchus obtectus Say. were placed on beans in a 1 percent relative humidity no progeny were obtained. Headlee concluded that the egg stage was the most susceptible to low humidity conditions.

Lin and Ritehard (1952) observed that an abnormal hatching behavior occurred at 15° C, he found that many of the mature embryos could force open the egg shell but not free themselves from the shell. Some could emerge but some appendages stuck to the embryonic cuticle. Onchopeltus eggs held at 5° C and 10° C developed only to the blastoderm stage. Eggs held at 13° C for 15-18 days developed germ bands and segmentation began. According to Butt (1949) longer exposures of the eggs of Onchopeltus fasciatus to 14° C resulted in normal development by some embryos almost to maturity, but they lacked complete coloration.

The period required for the complete development of an insect from the laying of the eggs to the emergence of the adult is not only dependent on the temperature or the humidity in which it was exposed to, but also to the food conditions available for the species. Nutritional requirements of Tribolium confusum with regards to vitamins have been studied by Sweetman and Palmer (1928) who found that vitamin A was not essential for Tribolium confusum fed on a diet containing a purified protein fraction, salts, dextrin and a small portion of yeast.

Howe (1960) found that larvae of Tribolium confusum failed to develop to pupae at 17.5° C and 37.5° C. Larvae could not complete their growth in some of the more extreme conditions in which eggs hatch. Thus at 37.5° C, larvae placed at

10 percent R.H. all died and the mortality at 30 percent R.H. was heavy. The shortest larvae period was recorded by Mickel and Standish (1947) to be 14.6 days at 35° C and 80 percent R.H. on whole wheat flour with 5 percent yeast. Sweetman and Palmer (1928) recorded 14.6 days for ground husked barley at 31-32° C and 75 percent R.H.

A most important paper with regard to population growth is that of Chapman (1928) in which he demonstrated that the growth of a population of Tribolium confusum is dependent, at any temperature, upon the size of the environment, and upon the initial concentration of the beetles. The final concentration is independent of either the initial concentration or the size of the environment. He hypothesized that the balance of numbers of organisms in nature is maintained by the tendency of the organism to produce large numbers of offspring and for the environment to take a large toll from the numbers produced. He tested this hypothesis by a series of experiments under controlled conditions in which flour beetles, Tribolium confusum, were maintained in environments with a controlled volume of food. He found that the number of individuals increased until there were 43.97 per gram of flour. Even though thousands of eggs were laid, the number of individuals remained constant because cannibalism of immature stages by the adult beetle was in a rate proportional to the concentration of the beetles per gram of flour. Increased cannibalism with increased densities of the beetles has been also shown by Chapman (1933), Chapman and Bird (1934), Chapman and Whang (1934) and Boyce (1946).

The studies of McLagan (1932), Park (1933), Crombie (1944) and Boyce (1946) have firmly established the fact that an increase in density of the population is accompanied, within certain limits, by a direct decrease of progeny per beetle per day. Pearl (1925) experimented with cultures of Drosophila populations in environments of known size, and concluded that populations of organisms usually follow rather definite trends and tend to approach the point of saturation for their environment. Thus, it seems that whatever the potential rate of development of an organism may be, its environment offers sufficient resistance to multiplication to cause its numbers to tend to remain relatively constant.

A survey of work on egg cannibalism shows clearly that the number of eggs destroyed is dependent upon the number of eggs present and the number of imagoes.

Crombie (1942) showed that crowding had a much more important effect than cannibalism on the rate of growth of egg populations. This point was also emphasized by McLagan (1932) who was working with *Tribolium confusum*. He concluded that above a certain optimum density for egg production, crowding adversly affected the rate of reproduction. Boyce (1946) concluded that while cannibalism is present, it plays a lesser role than the reduction of fecundity by crowding in the decline of the growth rate. Reduction in fecundity as the population density increases has also been shown by McLagan and Dum (1936) with *Calamdra grauaria*. This view is opposed to that of Chapman and Whang (1934), who ascribe the major role to egg cannibalism. Park (1933) observed that copulation stimulated fecundity up to a point after which reproduction was not stimulated. Birch et al. (1951) considered both fecundity and cannibalism under conditions of crowding of females by imagoes of their own species and by imagoes of another species. They concluded that there is a difference in the reduction of fecundity depending upon which sex contributes most to the crowded condition. Crowding by females directly affects the fecundity while crowding by males can be accounted for by cannibalism.

Under a given set of environmental conditions, the average apparent fecundity (net number of eggs present at the end of 24 hours) was shown by Crombie (1942) to be very materially reduced with increased densities of adult *Tribolium confusum*. He also showed, that with increasing adult densities and constant egg densities, there was a tendency to reach a maximum for egg consumption at a population of eight beetles per culture.

MATERIALS AND METHODS

Effect of Temperature on the Eggs

Eggs of Tribolium [illegible] were obtained by sifting ~~the~~ fine enriched flour through a 35 mesh sieve ~~in which the beetles had been~~ incubated for 24 ~~hours~~. ~~Eggs were easily separated by a 35 mesh seive.~~ The eggs were then transferred to fresh media in Petri dishes 5 cm in diameter for experiments on the effect of temperature and humidity on hatchability [illegible] and growth of the insects. The humidity was kept constant at 77.5 percent with the use of ~~a saturated~~ salt solution of ammonium chloride (Buxton, 1931). Under this humidity ~~the~~ effect of temperature on the hatchability of the eggs was determined within a range of 17.5° C to 30° C with steps of 2.5° C. Humidity in the remperature ranges from 32.5 to 37.5° C was maintained at 75 percent by salt solutions of ~~potassium~~ tartarate (Buxton and Melanby, 1934).

Humidities were controlled with the salt solutions (Buxton 1931) and checked [illegible] hygrometer

Effect of Humidity on the Eggs

Eggs obtained by the same method as described above were used. The temperature was kept constant at 32.5° C and different levels of relative humidity were made available to determine their effect on the hatchability of the eggs. The saturated salt solutions used to give these different levels of relative humidities were lithium chloride, potassium nitrite, ammonium chloride and ammonium phosphate which according to Buxton (1931) give relative humidities of 15 percent, 45 percent, 79 percent and 93 percent respectively. In all these cases relative humidities were checked by an A.C. wheat stone bridge. The measured values were not more than

1 to 3 percent different than the reported values (Buxton, 1931 and Buxton and Melanby, 1934).

Under these experimental conditions the eggs collected from the flour media of the beetles incubated for 24 hours were placed in Petri dishes measuring 5 x 1.5 cm. The dishes were held on a suspended wooden stand page (11) which was found to serve the purpose better than an ordinary wooden stand (Figure 1), because in the latter case the metal of the wooden stand touches the salt solutions in the container and will rust and probably change humidity. The quart jar containers (Figure 1) were then kept under various temperatures and humidities. The experimental jars were checked daily to obtain data on the egg hatching rate.

Larvae and pupae were placed in groups of 20 in 5 x 1.5 mm Petri dishes with 2 gm pollen (9% safflower and alfalfa pollen; 91% sweet and red clover pollen)(Nye, 1967) or enriched flour. The Petri dishes were placed in a wooden stand and the stand was kept in a quart jar with appropriate salt solution for humidity regulation.

Effect of Temperature on Larval and Pupal Growth

The developmental rates of the larvae and the pupae were determined at three levels of approximately constant temperature (21 ± 1.6° C, 27 ± 1.5° C and 30° C) by temperature controls in a rearing room and a laboratory oven. The humidities in all these cases were kept at 32 percent by the use of saturated salt solution of calcium chloride (Buxton, 1931). The hatching larvae were placed in groups of 20 into 5 x 1.5 cm dishes (Figure 1). Each dish contained 2 gms of pollen (9 percent safflower and alfalfa pollen; remainder sweet and red clover pollen) (Nye, 1967) which was changed weekly during the larval stage. The dishes were held in a wooden stand (Figure 1) which were put into quart jar containers with enough of the appropriate salt solution to regulate the humidity. The quart jar containers were checked daily to record the dates of pupation and emergence to adults. The duration of larbal and pupal stages was recorded. The same procedure was adopted in other experiments in which food provided for the larvae was enriched flour.

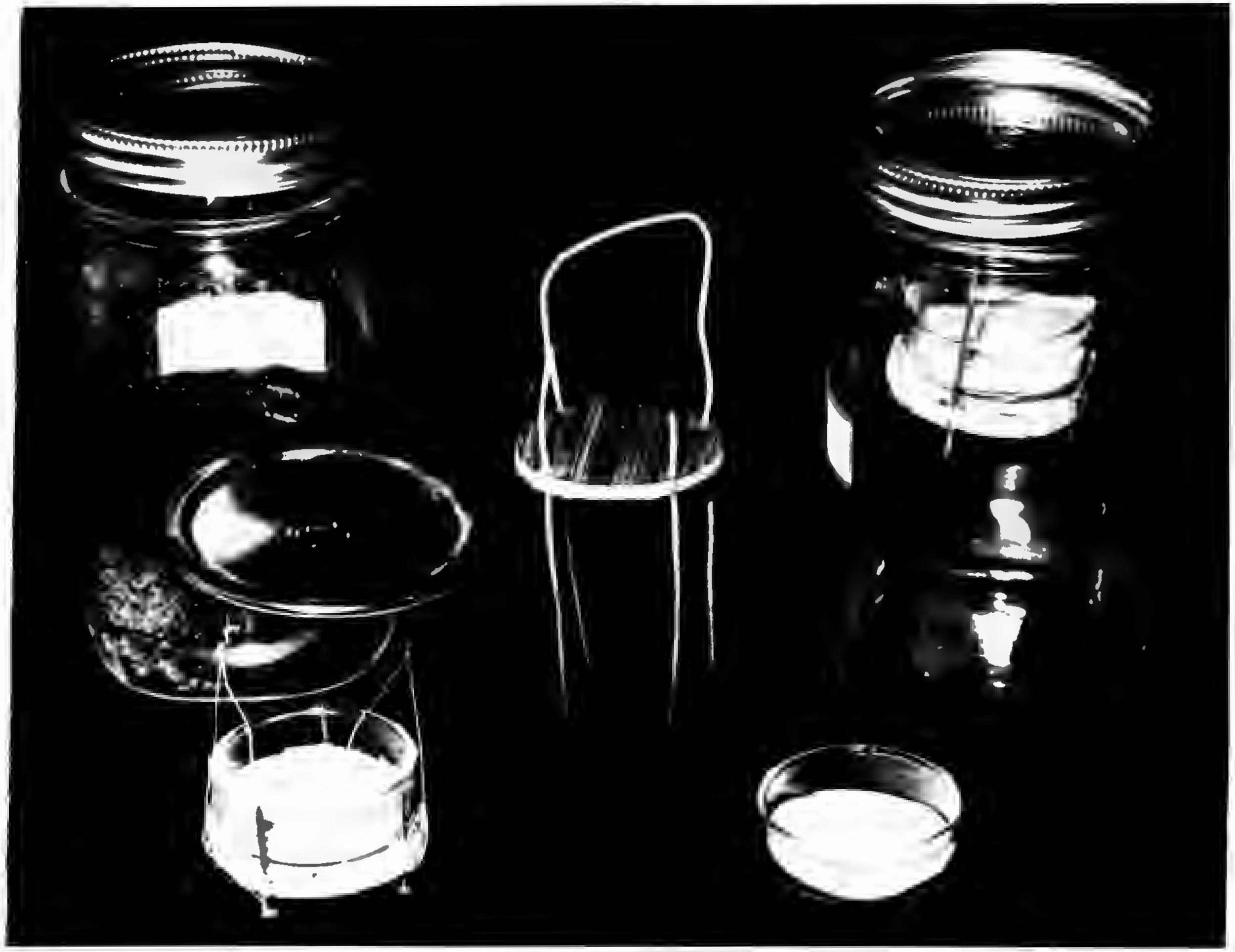


Figure 1. Rearing equipment for Tribolium brevicornis

Effect of Relative Humidities on the Larval and Pupal Stage

The temperature was kept constant at 30° C by the use of a laboratory oven. Levels of 15 percent, 45 percent, 77.5 percent and 93 percent R.H., were obtained in the same way described earlier. Under these humidities the larvae were reared in groups of 20 in each petri dish with 2 gms of pollen, and in other experiments 2 gms of enriched flour was used. The experimental dishes were held on a wooden stand in the quart jar containers.

The experimental containers (Figure 1) were checked daily to record the dates of pupations and emergence to adults. The duration of larval and pupal stages was obtained by calculating the means of the values obtained throughout the experiments. The same procedure was adopted in other experiments in which food provided for the beetles was enriched flour.

Oviposition and Egg Cannibalism

Oviposition and egg cannibalism as related to population density was studied. An egg-marking

A method of marking eggs for rapid identification was used (Rich, 1956). ~~This~~ method (Rich, 1956) made it possible to distinguish ~~the~~ egg which had been introduced at the beginning of the incubation period from those which were laid during the incubation period. At the time of ociposition the egg is covered with a moist sticky substance. Particles of the media um (pollen which was made fine by grinding and sifting through a 35 mesh seive) adhere to the surface of the eggs as the sticky substance coating [illegible] dries. The medium was colored pink by addition of about 1 gm of neutral red (powder) to about 100 gms of pollen or flour. The eggs became, by ~~then~~ their pink coating, quite readily observed when transferred to rresh uncolored medium. The introduced eggs could thus be distinguished from freshly laid eggs.

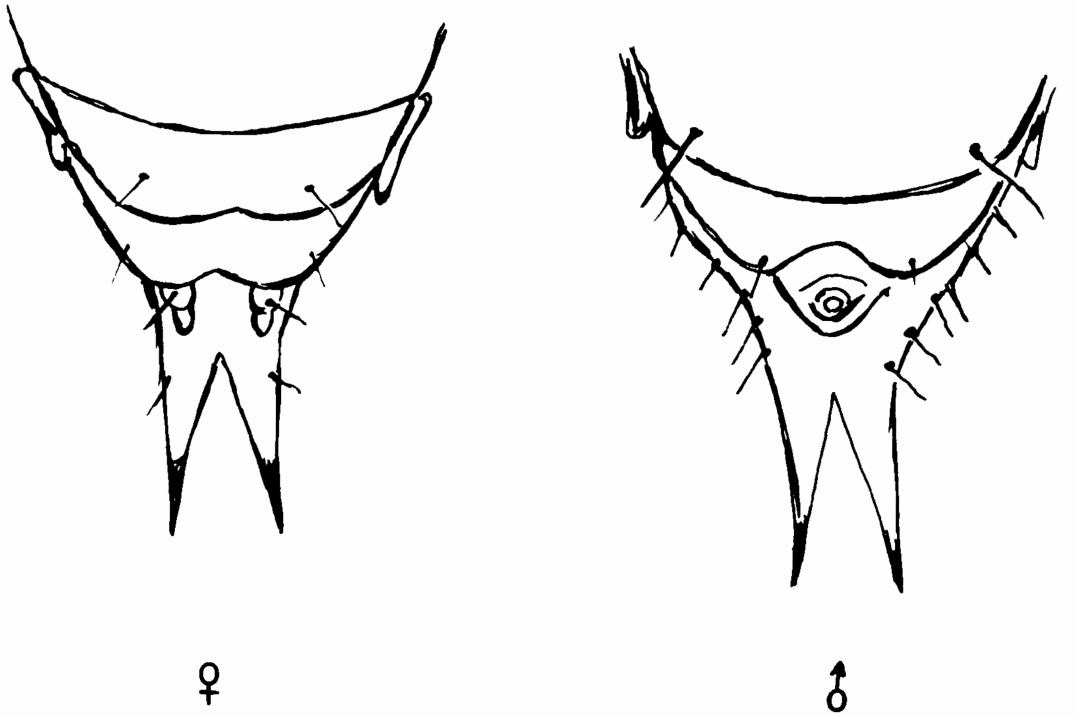
The beetles could be sexed as pupae by the characteristics of the genitalia (Figure 2). Female pupae have a pair of segmented plates on the ventral surface of the genitalia which are absent in male pupae.

The emerging mole adults were painted on their thorax with green house paint. The female were then mixed with the males and incubated for a period of a week to provide ample time for copulation to take place. As the beetles are difficult to separate by sex as adults, the pupal sexing procedure was found necessary.

A completely randomized block design (one way analysis of variance) was used in which six densities of beetles (2, 4, 8, 16 and 64 beetles) were replicated six times. The sex ratio of the beetles was unity. At the beginning of the experiment each dish 5 x 1.5 cm was supplied with 100 marked eggs. The marked eggs were mixed thoroughly with 2 gms of pollen in the dishes (5 x 1.5 cm). Various numbers of the beetles were then transferred to the dishes into which the marked eggs had been added. The colonies were then incubated for 24 hours, at approximately 27 ± 1° C and R.H. 30 percent for a period of 48 hours, after which the eggs were separated and counted.

Method of Calculating the Rate of Cannibalism and Oviposition

A deterministic method introduced by Rich (1956) and adopted by Sonleitner (1961) was used for this experiment to determine the rate of cannibalism and the rate of oviposition from the marked and unmarked eggs recovered from the experimental dishes. According to Sonleitner (1961), the general equation for the model



Genitalia of pupa of *Tribolium brevicornis*

Figure 2. Characters for sexing *Tribolium brevicornis*

$$Nt = Noe^{-bct} + \frac{E}{C} (1 - e^{-bct}),$$

Where Nt = number of eggs recovered

No = initial number of eggs

C = Cannibalism rate (the fraction of eggs eaten per beetle per day)

b = number of beetles

t = time in days.

Because the oviposition rate of marked eggs is equal to zero, the cannibalism rate C and the real fecundity (oviposition rate) E may be calculated using the first and the second terms respectively, on the right-hand side of the equation which, after rearanging, take the following forms:

$$C = \frac{1}{bt} \log_e \frac{M}{Mt}$$

and

$$E = Nt\ C \frac{e^{bct}}{e^{bct}-1} \cdot \frac{b}{f}$$

where Mo = the initial number of the marked eggs introduced,

Mt = the number of unmarked eggs recovered.

The factor b/f (where f = the numbers of females) has been added to the equation for E so that real fecundity will be in terms of eggs per female per day.

The simplicity of the model as Sonleitner (1961) pointed out, stems from the assumption that C and E are constant. The hypothesis of a constant cannibalism rate assumes that every egg has an equal probability of being destroyed. Some of the conditions implicit under this assumption, mentioned by Rich (1956), include non-random searching, no "satiation," and equal mortality of marked and unmarked eggs.

"Random Searching" is defined by Sonleitner (1961) to mean that each subvolume of the medium used (flour) has the same probability of being traversed by a beetle. In a homogeneous medium the assumption is considered to be satisfied.

It is conceivable that the application of the dye (neutral red) might alter the palatability of the eggs to the beetles. The following experiment was designed to check this point. Two groups of eggs (marked and unmarked eggs) were used. The first group (marked eggs) were placed in groups of one hundred in ~~the experimental~~ vials 5 x 1.5 cm and were mixed with the pollen thoroughly. ~~Ten vials (repl~~icates) were used for the marked eggs and so for the unmarked eggs, i.e. a total of a thousand marked eggs and a thousand unmarked eggs were introduced into the experiment. Eight beetles (4 males and 4 females) were introduced into each ~~experimental~~ dish. The ~~experimental~~ dishes were then incubated under temperature of 30° C with an unadjusted humidity. ~~The experimental dishes were checked after 48 hours.~~ ~~The~~ recovered eggs from ~~Treatment~~ I (the treatment in which marked eggs were introduced at the beginning of the experimental period) were counted and recorded with no regard to their colors. The same was done for Treatment II (the treatment in which unmarked eggs were intorduced at the beginning of the experimental period.

RESULTS AND DISCUSSION

By recording the number of eggs hatching each 24 hours within the range of the temperatures used (from 17.5 to 37.5° C) with a step of 2.5° C and constant R.H., 77.5 percent the rate of development and the viability of the eggs were obtained. In Figure 3 ~~the~~ The reciprocals of the duration of the egg period ~~data of~~ stage (Table 1) with [illegible] are plotted in Fig 3 ~~against~~ temperature. (Fig. 3). The deleterious effect of high temperature is shown by the arching of the curve to an optimum of 35° C. A straight line can reasonably be fitted to the region between 32.5 and 30° C, the extension of which clearly would fall at the middle of the region between 27.5 and 20° C. Butler (1949) and Howe (1960) plotted a straight line through some of the published data and by extrapolating the former predicted a developmental threshold of 15° C and the latter estimated that the developmental minimum would be just below 17° C. The curvature observed in Table 1 would lead to a lower developmental minimum whereas the observed developmental threshold is higher, just above 17.5° C. Lin et al. (1954) attributed the failure of embryonic development below 17° C to the exhaustion of the food reserves in the egg. However, curves relating the rate of development (i.e. the reciprocal of the developmental period) to temperature and humidity may be useful for the effects of the environmental conditions because the developmental rate is directly related to the intrinsic rate of increase of the insects (Howe, 1960).

~~The~~ ~~data~~ in Table 2 indicate ~~that~~ Relative humidity ~~used~~ had was of little effect on the duration of the egg stage (Table 1) but temperature was important (Table 2). The work of Howe (1960) with Tribolium madens conform to this result. However, Holdaway (1932) showed a very slight lengthening of egg

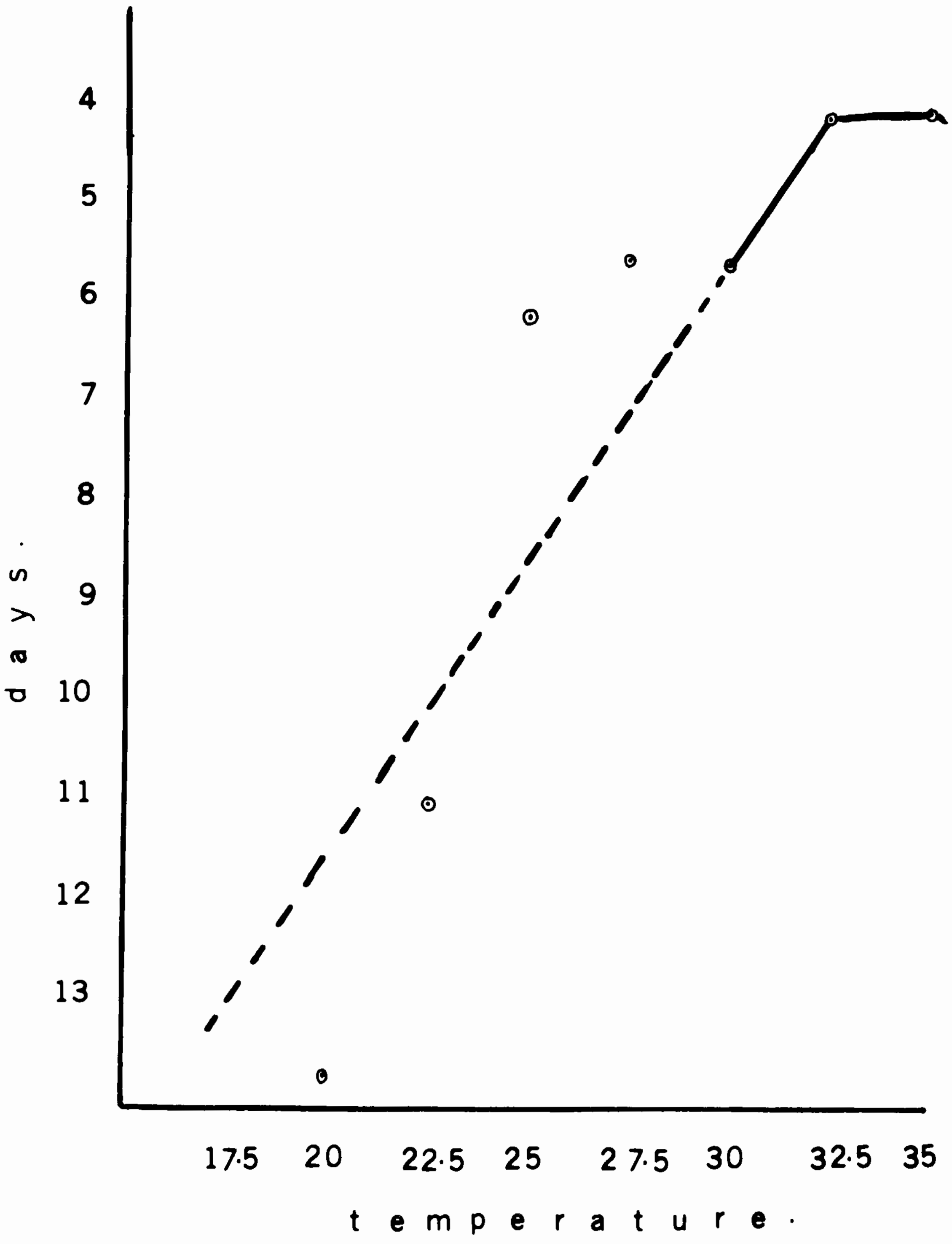


Figure 3. Reciprocal curve of developmental periods of eggs of Tribolium brevicornis

Table 1. Hatchability of eggs at 77.5 percent relative humidity with different temperatures

Temp. ° C	No. of eggs incubated	No. hatched	% hatched	Av. duration of egg stage
17.5	228	-	0	-
20	300	192	64	13.7
22.5	264	144	54.5	11
25	198	178	88.9	6.15
27.5	310	284	78.8	5.44
30	329	267	81.15	5.48
32.5	380	339	89.21	4.04
35	300	18	6	4.00
37.5	300	-	-	-

Table 2. Hatchability of eggs at 32.5° C with different relative humidity

R.H. %	No. of eggs incubated	No. hatched	% hatched	Av. duration of egg stage
93	330	267	80.2	4.63
75	329	267	88	4.48
45	300	234	78	4.15
15	252	230	90.9	4.01

period at 27° C from 6.15 days at 25 percent R.H. to 6.55 days at 1 percent R.H.

Figure 4 shows that the highest percentage of hatching was under 32.5° C; under 35° C the developmental rate was the highest but the mortality of the eggs was also very high. This indicates that the optimum temperature is between 32.5 to 35° C.

Figure 5 shows that the percentage hatch was highest in the lower humidity (15 percent R.H.), but the developmental period under the four humidities tested were almost the same. This actually indicates that the humidities tested have no effect on duration of the egg stage.

The effect of temperature on the duration of the larval and the pupal stages is shown in Table 3. The life stages of Tribolium brevicornis are illustrated in Figures 6 and 7. When larval food was pollen, and the rearing was under 30° C, the larval and pupal periods were shorter than at 27.11 ± 1.6° C. This indicates that under favorable food conditions and favorable temperature, the larval and pupal development rates increase, and consequently the population growth increases also. When enriched flour was the larval food the larvae did not survive. At 21.9 ± 1.6° C the development of the larvae did not pass the prepupal stage. This demonstrates the role of temperature on the physiological activities of insects. Harvey (1962) considered temperature to be a signal of the environment which is transduced by receptors to become nerve impulse which, in turn, are transduced by neurosecretory cells to become hormonal messages regulating development. The pollen used for the larval food seems to be a sufficient diet for larval developments.

When larvae were bred at 30° C (Table 4), the duration of larval and pupal stages did not differ significantly with relative humidities of 15 percent, 45 percent,

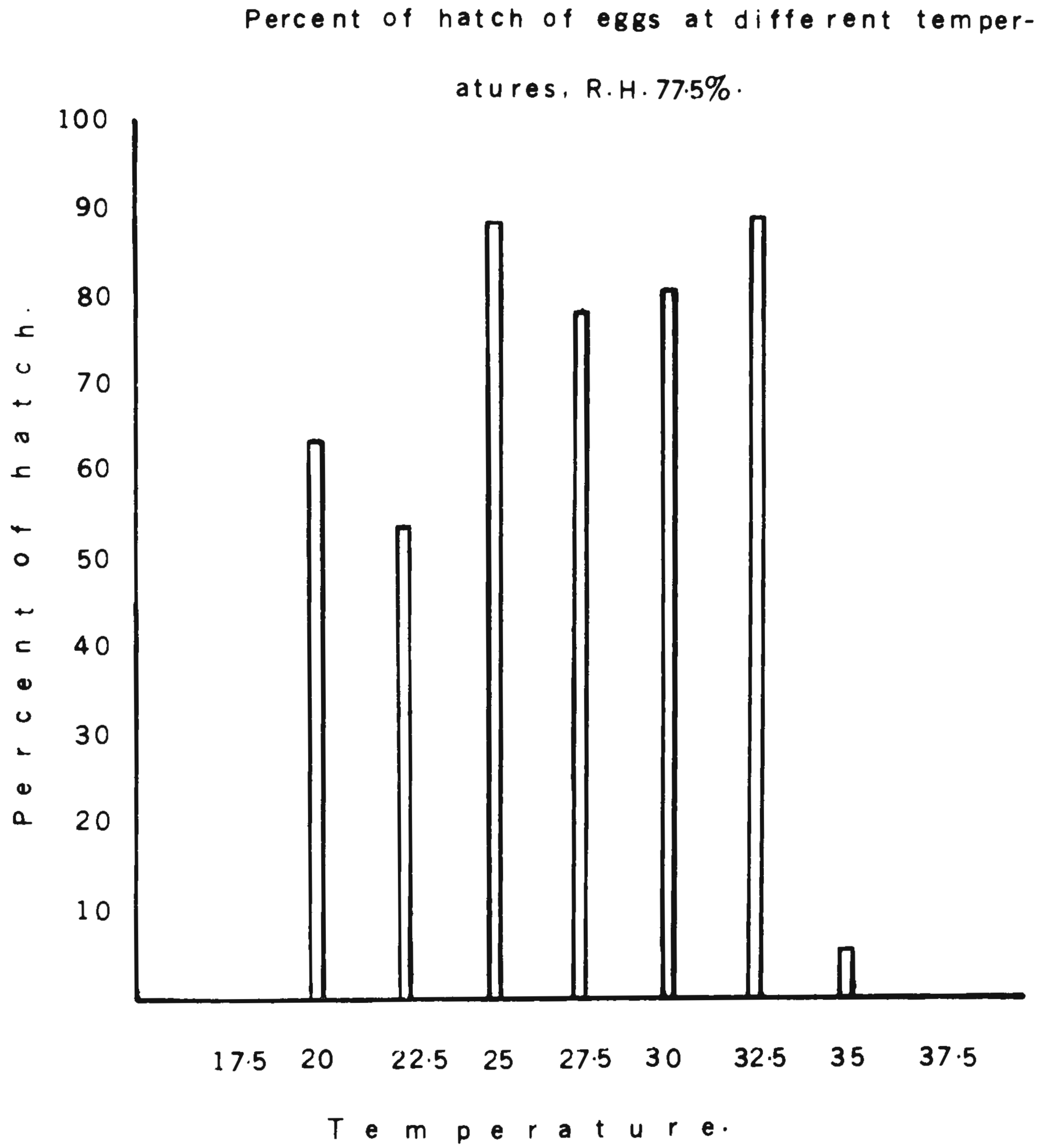


Figure 4. Hatchability of the eggs under different temperature and 77.5 percent relative humidity

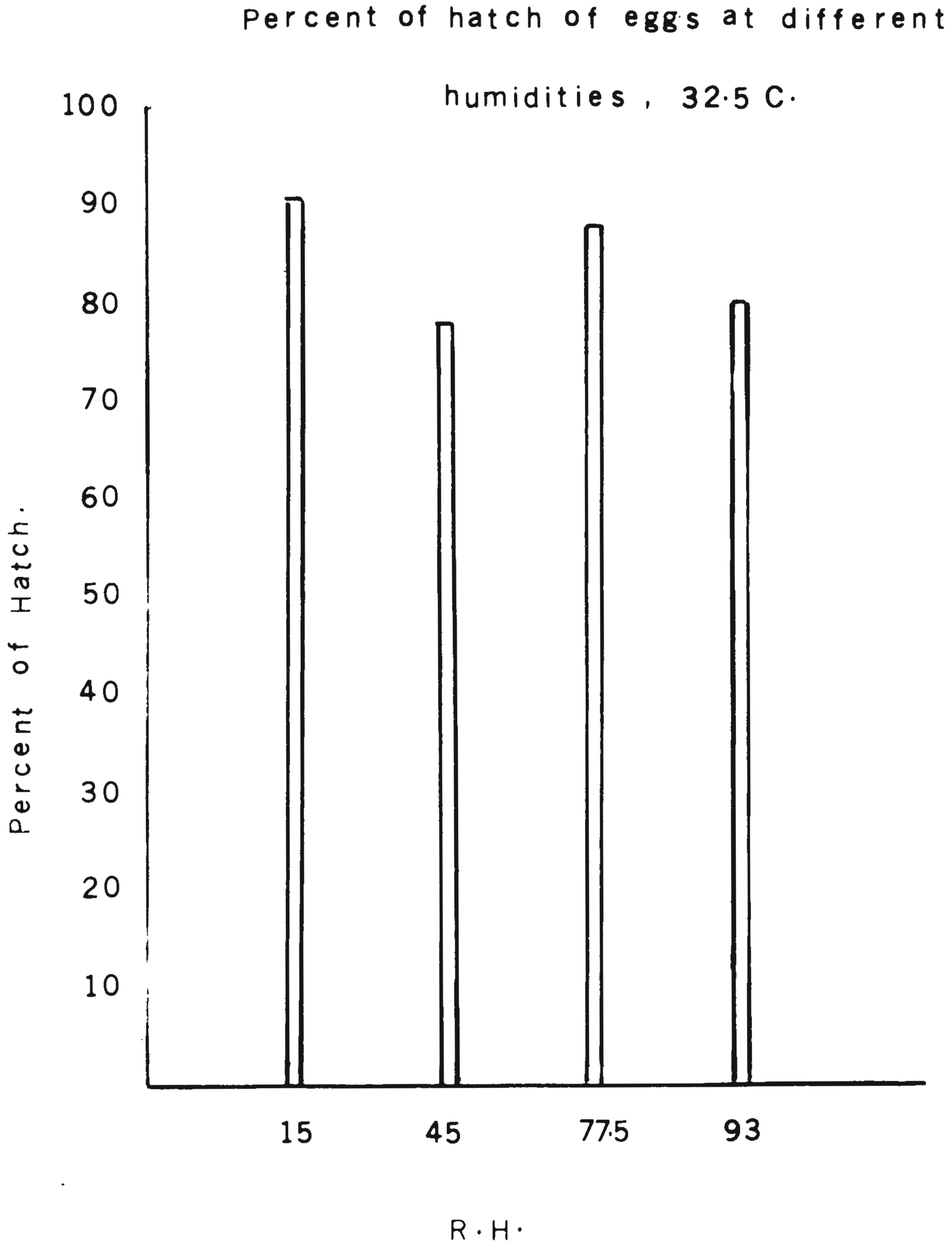


Figure 5. Hatchability of the eggs under different relative humidity and 32.5° C

Table 3. Duration of larval and pupal stage under three different temperatures and 32 percent relative humidity.

Temperature	No. larvae incubated	Ave. duration larval stage (days)	Ave. duration pupal stage (days)	Percent pupation
		Pollen		
80	216	20.1 ± .6	7.8 ± .13	91.2
27.11 ± 1.2	187	40.5 ± .3	10.5 ± .73	46.4
21.9 ± 1.6	193	+	-	-
		Enriched Flour		
80	137	(x)	-	-
27.11 ± 1.2	100	(x)	-	-
21.9 ± 1.6	119	(x)	-	-

(+) = larvae grew up to prepupal stage but failed to pupate.
(x) = larvae molted once or twice and then died.
(-) = negative result, no duration of pupal stage.

Table 4. Duration of larval and pupal stage under different constant humidities and 30° C

Relative humidity percent	No. larvae incubated	Ave. duration larval stage (days)	Ave. duration pupal stage (days)	Percent pupation
		Pollen		
93	100	Fungus growth	-	-
77.5	100	20.5 ± .7	7.5 ± .7	9.1
45	100	21.5 ± .2	7.8 ± .3	93
15	100	22.3 ± .13	7.5 ± .12	90
		Enriched Flour		
93	100	(x)	-	-
77.5	100	(x)	-	-
45	100	(x)	-	-
15	100	(x)	-	-

(+) = larvae grew up to prepupal stage but failed to pupate.
(x) = larvae molted once or twice and then died.
(-) = negative result, no duration of pupal stage.

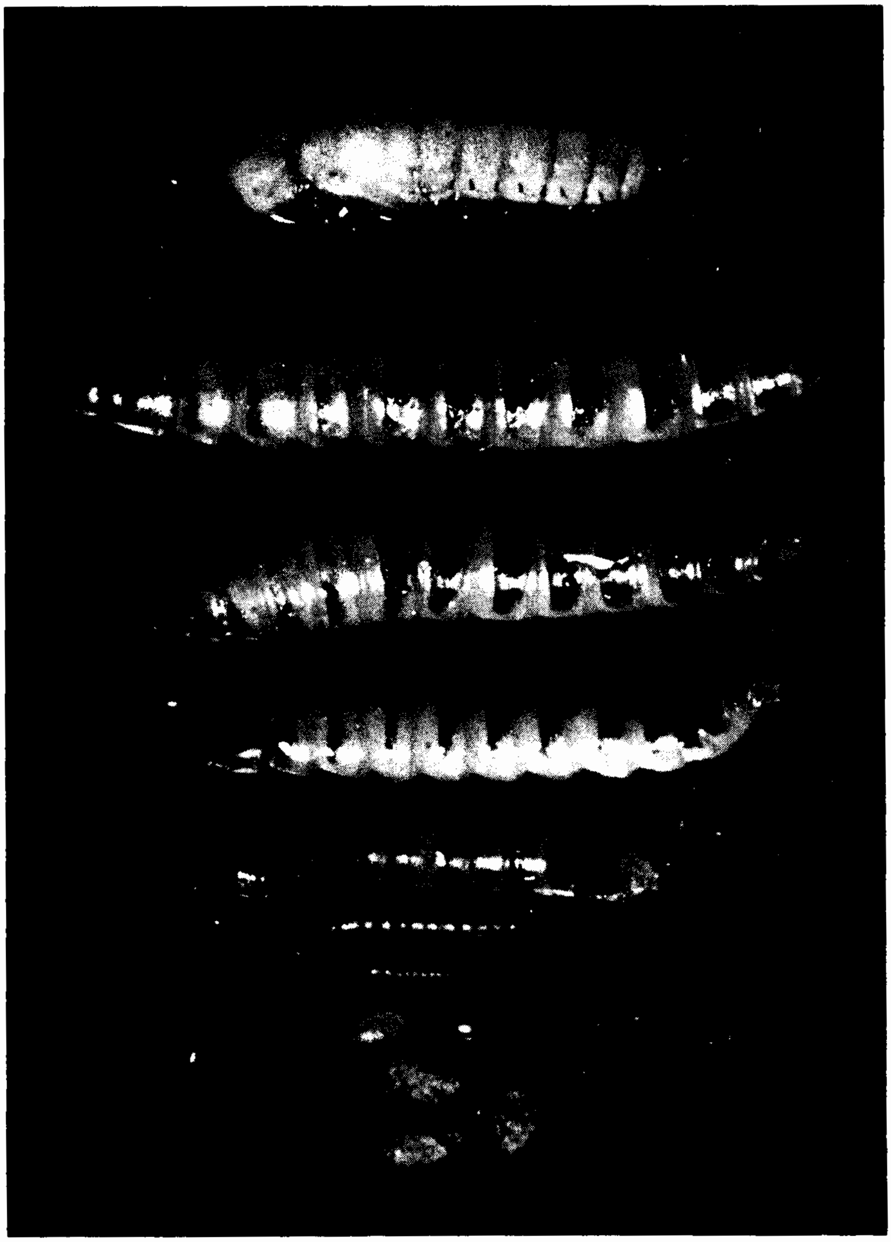


Figure 6. Developmental stages of Tribolium brevicornis. From top to bottom: pupal stage, sixth instar, fifth instar, fourth instar, third instar, second instar, first instar, ordinary eggs, egg coated with particles of medium (pollen).

Figure 7. Adult stage of Tribolium brevicornis

and 77.5 percent. This indicates that the humidity used does not have an important effect on the larval and the pupal development.

Table 5 shows the number of eggs recovered from two treatments which were replicated 10 times. This experiment was established to test the hypothesis that marked and the unmarked eggs have an equal chance of being destroyed by the beetles. The total of marked and unmarked eggs introduced and the total number of the eggs recovered are presented in Table 5. A "t" test (Table 6) did not show a significant difference between the two means, which indicates that the susceptibility of marked and unmarked eggs to destruction by the beetles is almost equal. This result is similar to the findings of Rich (1956) and Sonleitner (1960) with <u>Tribolium castaneum</u>.

Table 5. Marked and unmarked eggs recovered after 48 hours

Treatment	1	2	3	4	5	6	7	8	9	10	Total
I	87	136	101	107	116	103	107	114	112	98	1081
II	99	107	108	106	126	103	82	105	113	111	1060

"T" test of equal susceptibility of marked and unmarked to destruction by the beetle.

Table 6. Analysis of variance marked and unmarked egg recovered

Treatment	Total eggs introduced	Total eggs recovered	t value	t .95	P
Marked Eggs	1000	1081	.256	2.101	.05
Unmarked Eggs	1000	1060			

Table 7 shows the record of the recovery of marked and unmarked eggs in relation to densities of the beetles. The means of the values, the standard deviations (S D), and the coefficients of variation (C.V) were calculated. The means of the numbers of marked eggs recovered decreases with increasing densities of the beetles. This is shown graphically in Figure 8 which indicates that total cannibalism increases with increasing densities of the beetles. It is also evident from Table 7 that the means of the records of the unmarked eggs recovered increase with increasing densities of the beetles.

Table 7. Recovery of marked and unmarked eggs

Density of beetles	Marked eggs recovered			Unmarked eggs recovered		
	Means	S.D.	C.V. %	Means	S.D.	C.V. %
2	98.33	1.86	189	3.50	3.89	97
4	91.00	12.43	14	4.16	.76	18
8	85.17	11.27	13	10.67	3.56	33
16	80.00	12.70	15	21.00	9.76	46
32	70.67	9.61	14	37.83	18.78	50
64	70.00	15.28	22	70.00	20.33	29

The original figures (records of the recovery of marked and unmarked eggs) were transformed into the rate of cannibalism and the rate of oviposition, the values of which are shown in Table 8. The means of the values, the standard deviations (S D), and the coefficient of the variations (C.V) were calculated. The analysis of variance

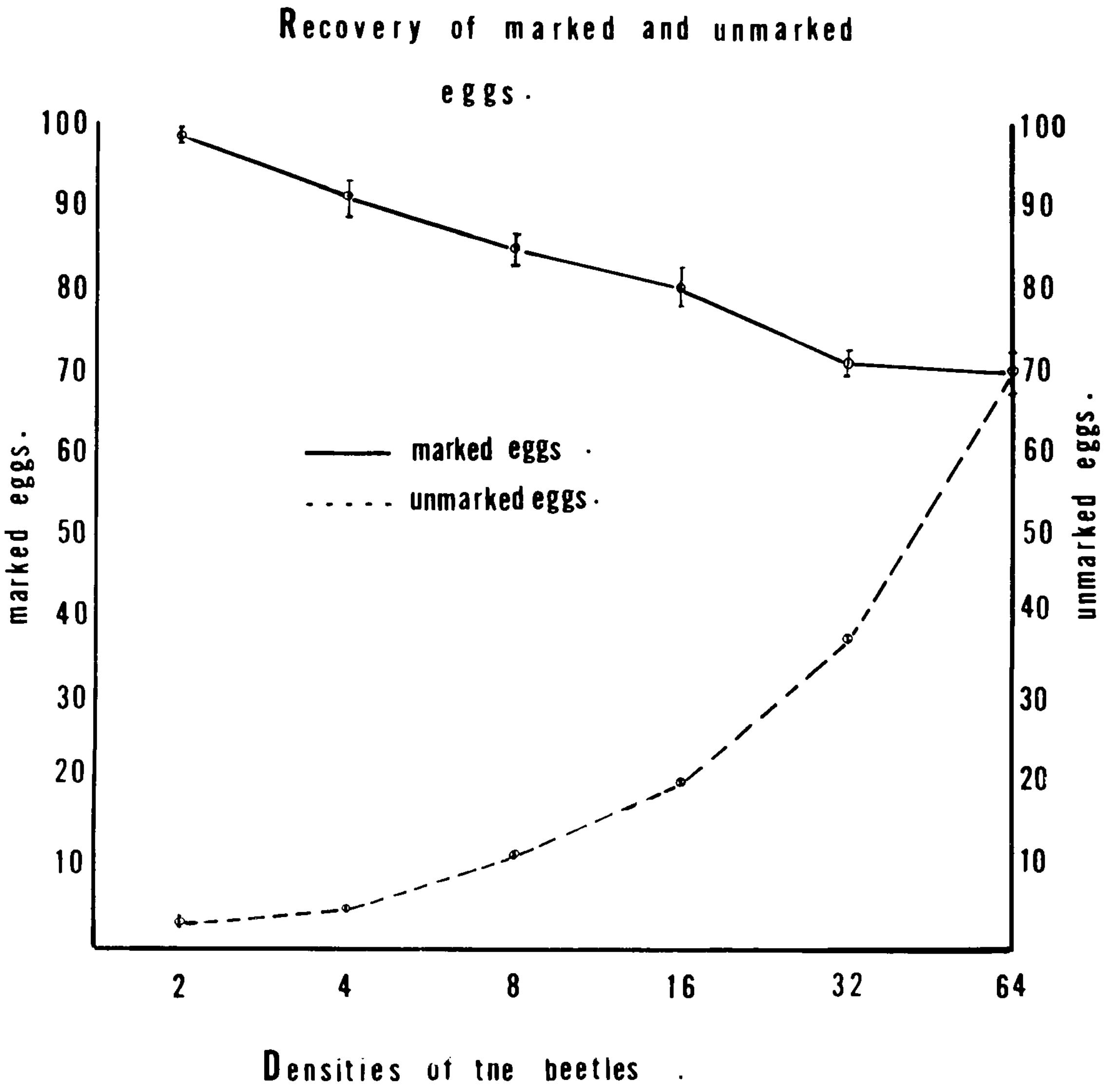


Figure 8. Recovery of marked and unmarked eggs in relation to population density

Table 8. Rate of cannibalism and oviposition

Density of beetles	Cannibalism rate			Oviposition rate		
	Means	S.D.	C.V. %	Means	S.D.	C.V. %
2	.0042	.0048	112.59	1.7735	1.7189	96.92
4	.0129	.0194	14.92	.9733	.4918	52.53
8	.0105	.0087	82.90	1.4470	.4910	33.93
16	.0073	.0052	71.42	1.4408	.5963	41.39
32	.0055	.0021	38.52	1.4325	.7658	53.46
64	.0029	.0017	58.24	1.3008	.3131	24.07

for the rate of cannibalism is shown in Table 9 and for the rate of oviposition in Table 10. Graphic illustrations for the rate of cannibalism and the rate of oviposition are shown in Figure 9 and 10 respectively. Analyses of variance for the untransformed data (Table 11 and 12), demonstrated that absolute cannibalism and oviposition increase with increasing population density. In Figure 9 the highest rate of cannibalism falls in the density of four beetles. It is difficult to explain the sharp break of the curve between the densities of two and four beetles in Figure 10, however, the lowest rate of oviposition was also associated with a density of four beetles (Figure 10). The analysis of variance for the rate of cannibalism (Table 8) and the rate of oviposition (Table 9) did not show significant differences, but because the coefficients of variation were very high, the hypothesis that the densities of the beetles affect the population of the eggs cannot be rejected.

Table 9. Analysis of variance-rate of cannibalism

Source	d.f.	Sum of squares	Mean square	F	F.99	P
Total	35	.0030				
Treatments	5	.0004	.00009	1.0446	3.70	.01
Error	30	.0026	.00008			

Table 10. Analysis of variance-rate of oviposition

Source	d.f.	Sum of squares	Mean square	F	F.99	P
Total	35	24.4052				
Treatment	5	2.0171	.4034	.5406	3.70	.01
Error	30	22.38881	.7463			

Table 11. Analysis of variance-absolute cannibalism, original figures not transformed

Source	d.f.	Sum of squares	Mean square	F	F.99	P
Total	35	7654.9800				
Treatment	5	3795.4600	759.0920	5.9004	3.70	.01
Error	30	3859.5200	128.6507			

Table 12. Analysis of variance-absolute oviposition, original figures not transformed

Source	d.f.	Sum of squares	Mean square	F	F.99	P
Total	35	24264.9700				
Treatment	5	19836.4700	3967.2940	26.8757	3.70	.01
Error	30	4428.5010	147.6167			

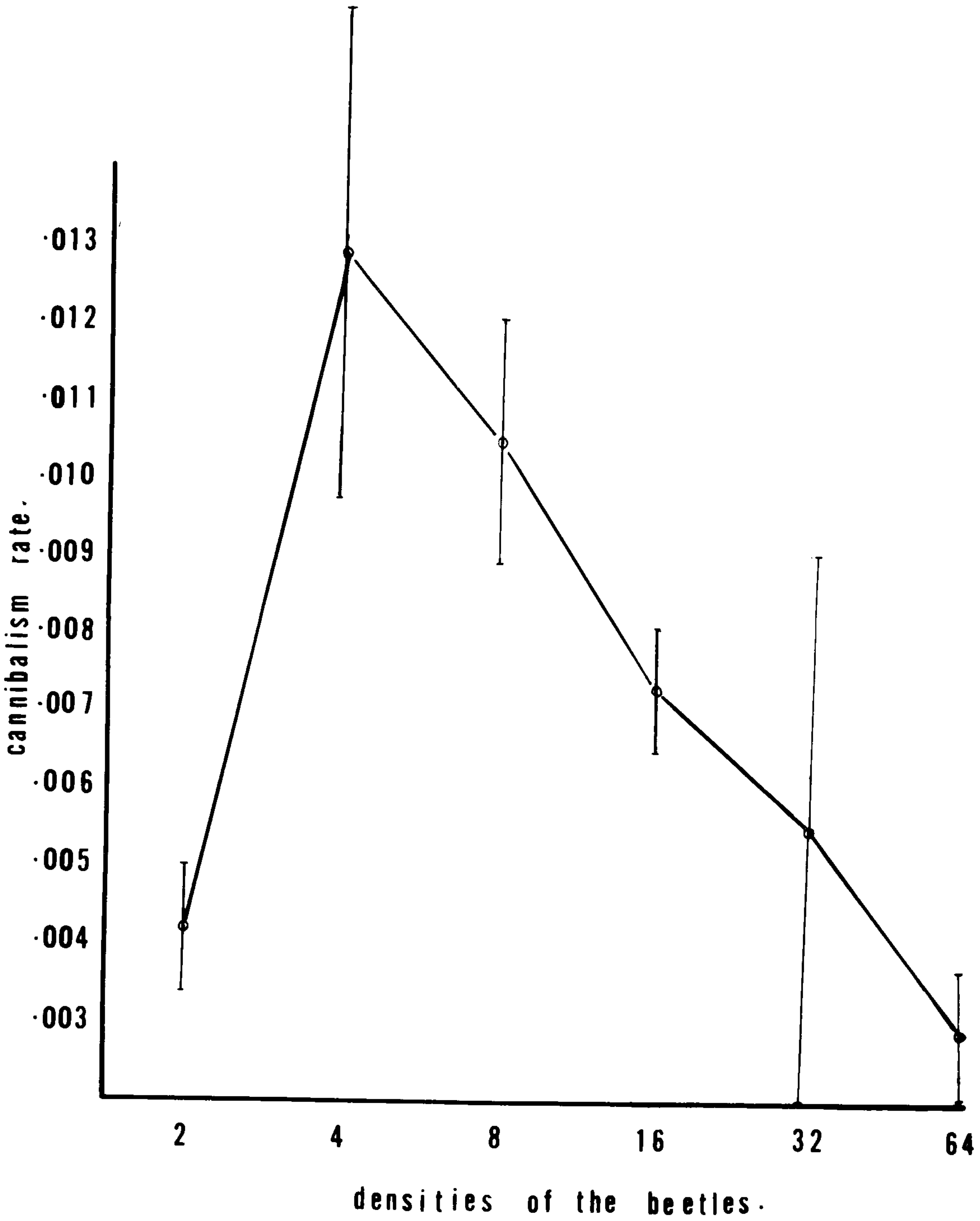


Figure 9. Cannibalism rate in relation to population density

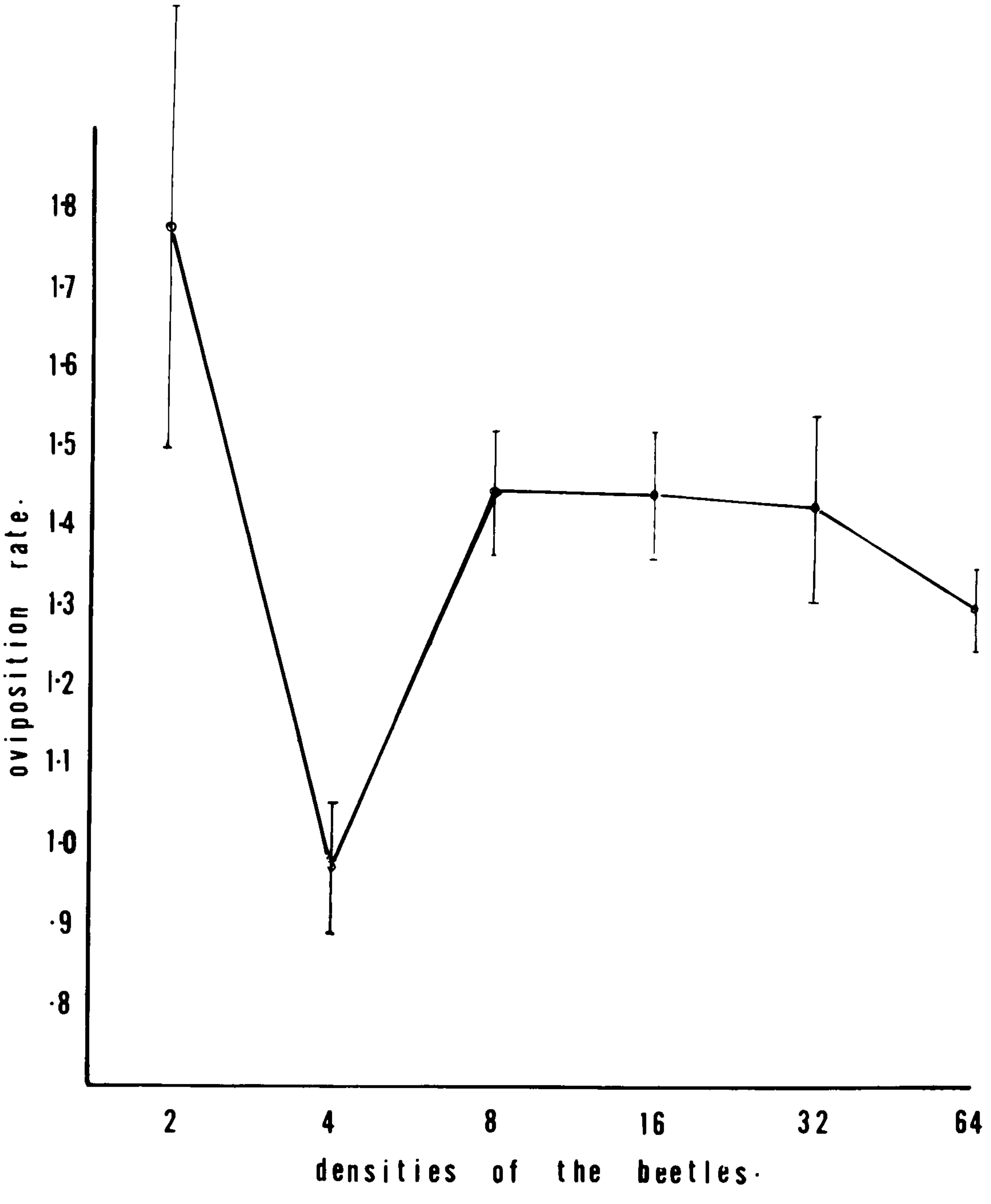


Figure 10. Oviposition rate in relation to population density

SUMMARY AND DISCUSSION

The effect of temperature on the hatchability of the eggs was determined within the range of 17.5 to 37.5° C with the step of 2.5° C. The optimum temperature for the egg hatching was found to be between 32.5 and 35° C.

Different levels of humidities (15 percent, 45 percent, 77.5 percent and 93 percent) were tested on the hatchability of the eggs. These levels of relative humidities did not seem to have a significant effect upon the hatchability of the eggs.

The duration of larval and pupal stages were determined at three levels of temperature (21.9 ± 1.6, 27.11 ± 1.2 and 30° C). The duration of the larval stage at $\overset{3}{8}0^\circ$ C was shortened twice as at 27° C. Ground pollen used was found suitable for rearing the larval unlike enriched flour which did not favor the development of the immature stages of the insect.

A method of preparing marked and unmarked eggs was used to determine the effect of cannibalism and oviposition on the population growth of these beetles. The total cannibalism as well as the total oviposition were found to increase with increasing densities of the beetles. The hypothesis that the rate of cannibalism increases with increasing densities and the rate of oviposition decreases with increasing densities was neither rejected nor accepted.

The hypothesis that the marked and unmarked eggs have the same chance of being destroyed was proved.

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