# THE INFLUENCE OF CONSTANT AND ALTERNATING TEMPERATURES ON THE DEVELOPMENT OF CERTAIN STAGES OF INSECTS.

*(With 7 figures in the Text.)*

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CONTENTS.



## A. INTRODUCTION.

A considerable amount of work has been published dealing with the effect of constant temperatures on the development of insects, but the influence

of fluctuating temperatures on this phase of insect activity has been relatively little explored ; the available data are insufficient to admit of other than very limited generalizations.

Notable evidence of the accelerating effects of alternating temperatures has accumulated from the studies of Shelford (1927) on the development of the Codling Moth, Cook (1927) on the growth of the first instar larvæ of cutworm *Porosagrotis orthogonia*, and Peairis (1927) on the development of the larvæ of Blow fly, *Calliphora vomatoria*. Of particular interest are the observations of Parker (1930) on the nymphs and eggs of *Melanoplus mexicanus*. The nymphs and more particularly the eggs showed a striking acceleration in the rate of development at a high temperature following a period of low temperature known to be below the threshold of development for this insect. The longer the period of exposure to low temperature, the greater was the acceleration produced. Moreover a low temperature of 0°C. produced greater acceleration than a temperature of 8°C. Ludwig (1928), from his studies on the Japanese beetle, concluded that the rate of development is accelerated only when one of the temperatures involved is below the threshold of development. In his recent investigations (1933) on the pupæ of *Drosophila melanogaster*, he has however expressed a different view. He considers that the acceleration recorded by various workers (excluding of course the diapause stages of insects), as a result of the alternating temperatures, one of which lies below the threshold, is due to a certain amount of development taking place even at the low temperature. He alternated temperatures of 10°C., 9°C., 8°C., and 7°C. with 20°C. and found that though 8°C. was below the *theoretical* threshold of development of the insect, the pupæ showed a 5% acceleration in development. But when the pupæ were exposed to alternating temperatures of 7°C. and 20°C. the rate of development was the same as that at a constant temperature of 20°C.

The number of instances of low temperatures retarding the rate of development are very few. Alternation of the eggs of *Ephestia kühniella* (Hase, 1927) and the grain aphid, *Taxoptera graminum* (Headlee, 1914), to varying ranges of temperature are the cases in point. These authors found that, in the instances quoted, constant temperatures resulted in more rapid development than fluctuating temperatures.

Considering apparently such different results, Uvarov (1931) suggested that in those cases where a retardation is produced, the insects are susceptible to low temperature, and the slackening in development is accompanied by obvious injuries and abnormal mortality, but if the temperature is not low enough to be injurious, the alternating temperatures produce an accelerating effect. Chapman (1931) emphasized that while it was not wise to make generalizations which would apply to all animals, it seemed very likely that temperature behaviour of an organism would correspond to the ecological conditions to which it was adapted in a state of nature.

The present study was undertaken with a view to investigate the influence

of both constant and fluctuating temperatures on the rate of development of the eggs and pupæ of certain species of insects.

## B. Material and Methods.

The experimental work was carried out with eggs of *Locusta migratoria migratorioides*, and pupæ of *Calliphora erythrocephala* and of *Muscina stabulans*. Cultures of these insects were set up in two hot-houses at the Entomological Field Station, Cambridge. The temperature of one of the rooms was maintained at 32°C. with a mean deviation of ±1°C. This was reserved for breeding locusts. The temperature of the other room was maintained at about 20°C., this being a suitable temperature for breeding the Diptera. During most of the time the relative humidity as registered by hygrograph was 60–70% in the locust room and 65–75% in the fly room.

1. *Breeding of* Locusta migratoria:—The original stock consisted of eggs laid near Khartoum by the locust in the phasis *gregaria*. The eggs were supplied in June, 1933 by the courtesy of Mr. H. Wood of the Gezira Agricultural Service. They were incubated in moist sand and the hoppers were bred on grass in the hot house. The sand in the cages in which the adults were liberated was examined at 8 A.M. and 5 P.M. for eggs. This enabled a supply of eggs laid within a known period of time.

Mention may be made of some of the difficulties experienced. The eggs are extremely susceptible to fungus attack, and the sand particles sticking to the eggs contain enough spores to infect the material. Moreover, the high humidity requirement of the eggs amounting almost to a saturated atmosphere is a factor very favourable to fungal growth. Dipping the eggs in alcohol or even in weak solutions of copper sulphate (0·5%) or corrosive sublimate (0·1%) is fatal to the eggs, and therefore could not be used for killing fungi. Thorough rinsing of the eggs with distilled water was thus resorted to. Freshly laid eggs being very delicate burst in attempting to remove sand particles. This difficulty very much limited the amount of material that could be prepared for experimental work in a reasonable time. Further, as the eggs were generally laid during the night there was the possibility of an error regarding the time of oviposition. This was eliminated by dividing each pod into 4 or 5 parts and comparing the results of treated batches with a control batch kept from the same pod. A known number of eggs was placed on moist cotton lint in sterilized tubes.

Hatching was noted at 9, 15, and 21 hours daily. Freshly hatched hoppers are pale yellow in colour and grow darker in one to two hours. It was thus possible to obtain accuracy in regard to the time of hatching by noting their coloration at the time of observation.

2. *Breeding of* C. erythrocephala *and* M. stabulans:—The Blow flies were collected locally and bred on meat at about 20°C. A consignment of *Muscina* pupæ was obtained from Cornwall University, America. The *Muscina*

larvæ were bred generation after generation on a uniform food consisting of a mixture of six parts alfalfa meal, one part dried milk, and one part soybean meal boiled with enough water to form a thick paste.

In order to prepare the flies for egg laying they were fed on a solution of sugar and yeast. The eggs were laid on the food material and larvæ lived in it till they were full fed.

Full grown larvæ were examined every two hours for getting freshly formed pupæ. In both the species freshly formed pupæ are white. They turn yellow and then red in 2-3 hours. This fact enabled the beginning of pupation to be noted with accuracy. Larvæ pupating during the night were rejected. The pupæ were sterilised by keeping them in 90% alcohol for 5 minutes. They were then washed with distilled water and transferred into glass tubes containing sterilised sand. The mouths of tubes were covered with gauze and the tubes placed in constant relative humidities and temperatures.

The sexes of emerging Blow flies were recorded in one case at 23°C. Out of 106 pupæ, 52 males emerged after a mean pupal period of 10·99 days and 54 females after 11·04 days. Similarly, out of 58 *Muscina* pupæ 28 males emerged after a mean pupal period of 9·40 days and 30 females after 9·42 days. Though the males emerged slightly earlier than the females, statistically the differences were not significant. In the experiments described hereafter the emergence of flies has, therefore, been recorded without distinguishing the sexes.

3. *Control of temperature and humidity* :—The method employed and the apparatus used for controlling temperature and humidity are described in detail in a previous paper (Ahmad, 1936).

Instead of the percentage relative humidity, the same values of saturation deficiency were employed at different constant temperatures because the latter give a better measure of the evaporating power of the air. This is supported by the evidence that optimal requirements of insects in terms of percentage relative humidity rise with the rise of temperature (Parker, 1930, Menusan, 1934). Kirkpatrick's conclusions (1923) on the resistance of insects to high temperature by high relative humidity also favour the employment of saturation deficiency.

Saturation deficiencies of 3, 9, 14, and 21 mm. employed in this investigation were obtained by using saturated salt solutions or solutions of sulphuric acid at different temperatures (Ahmad, *op. cit.*).

It may be mentioned that the measuring of relative humidity is as important as the measuring of temperature. Once a solution of a given chemical has been placed in a desiccator it should not be taken for granted that the required relative humidity will be maintained. In low humidity jars water is being constantly absorbed by salt solution while in the high humidity jars water is being given out, so that the addition of salt in the former case and that of water in the latter case is essential. This being so it is important to

test the humidity from time to time. Small hair hygrometers standardised with a whirling wet and dry bulb psychrometer were used for this purpose.

4. *Presentation of experimental data*:—In each of the three species studied, data on the rate of development at a series of constant temperatures and saturation deficiencies is presented first. This is followed by alternating temperature experiments, in which a period at a low temperature of 5°C. or 0°C. alternated with one at high temperature. In one set the insects were subjected to a single period of low temperature of 1, 4, or 8 days duration after 0%, 25%, and 50% development. The particular stage of development was obtained by allowing the eggs or pupæ to develop at high temperature for 0%, 25% or 50% of the total period required at that constant temperature, before subjecting them to low temperature. In the second set the material was placed for about 8 hours at low temperature and for 16 hours at high temperature daily.

The entire data have been treated statistically. The standard error and the analysis of the variance are based on the method of Fisher (1934).

If $x$ = individual observation,
$f$ = frequency,
$n$ = number of observations,
$\bar{x}$ = mean,
$\sigma$ = standard error,
$\bar{\sigma}$ = standard error of the mean,

then $\bar{x} = \frac{\epsilon fx}{n}$,

$$\sigma = \frac{\epsilon fx^2 - \frac{(\epsilon fx)^2}{n}}{n-1},$$

and $\bar{\sigma} = \frac{\sigma}{n}$.

The difference between the treatment mean ($\bar{x}_1$) and the control mean ($\bar{x}$) is only significant when

(i) '$Z$' test shows significance

i.e. $\frac{1}{2} \log e \frac{\sigma^2 \text{ between treatments}}{\sigma^2 \text{ within treatments}}$ is higher than the values given for $n_1$ and $n_2$ in the '$Z$' table;

and (ii) '$t$' is significant

i.e. $\frac{\bar{x}_1 - \bar{x}}{\sigma \text{ of difference of means}}$ is greater than the values of '$t$' given in the '$t$' table.

## C. The Incubation Period and Mortality of *L. migratoria* Eggs at Constant and Alternating Temperatures.

1. *Effect of constant temperatures*:—Since locust eggs can develop only in an almost saturated atmosphere, the incubation period has been studied at

0 mm. saturation deficiency at different constant temperatures ranging from 20°C. to 37°C. The results are set out in Table I. The mean incubation period varies from 58 days at 20°C. to 9·42 days at 37°C. The index of development and hyperbolic curves are shown in Fig. 1. The theoretical threshold of development lies at 17°C. The viability of eggs is at its maximum between 33°C. and 37°C. It is markedly reduced below 23°C.

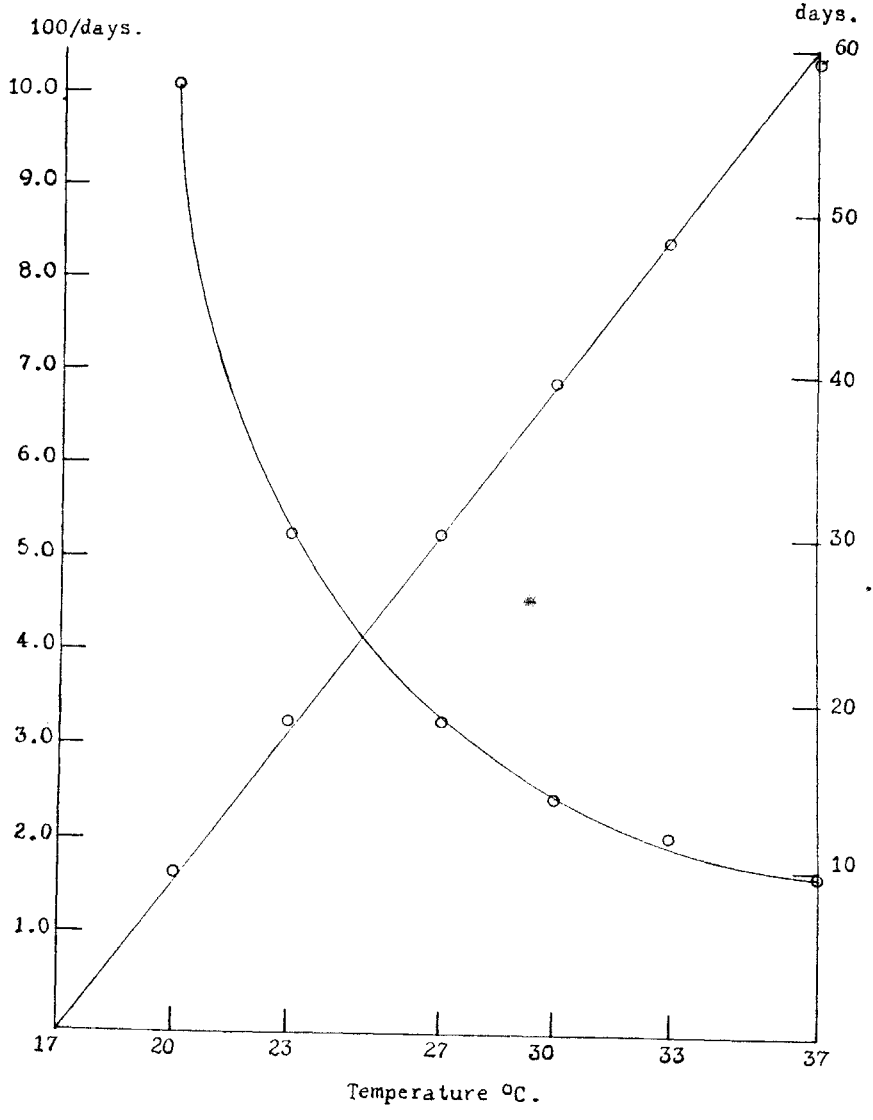


FIG. 1. Time-temperature and temperature-rate curves of the incubation period of *L. migratoria* eggs.

TABLE I.

*The incubation period and % viability of* L. migratoria *eggs at constant temperatures and 0 mm. saturation deficiency.*

| Temperature (°C). | INCUBATION PERIOD. | | NUMBER OF EGGS. | | % viability. |
|---|---|---|---|---|---|
| | (Days). | Standard error. | Total. | Viable. | |
| 37 | 9·42 | 0·0004 | 445 | 324 | 72·8 |
| 33 | 11·94 | 0·0009 | 432 | 285 | 66·0 |
| 30 | 14·73 | 0·0070 | 230 | 128 | 51·3 |
| 27 | 18·90 | 0·0012 | 825 | 459 | 55·6 |
| 23 | 30·34 | 0·0007 | 240 | 89 | 37·1 |
| 20 | 58·00 | .. | 50 | 9 | 18·0 |

2. *Effect of a single period of low temperature* :—The eggs in these experiments were transferred to 5°C. for periods varying from 1 to 16 days at different stages of their development, and kept at a constant high temperature of 27°C., 30°C., 33°C. or 37°C. during the remaining period. The mean period of transition from a high to a low temperature and *vice versa* lasted about $\frac{1}{3}$ hour. Since 5°C. is far below the threshold of development and there is no possibility of any development taking place at this temperature, the differences between the mean periods at high temperature alone and the mean periods required at the corresponding constant temperatures representing the influence of alternating temperatures are shown in Table II. This is graphically shown in Figs. 2 and 3.

It will be noticed that the eggs of *L. migratoria* do not show such a striking acceleration in development as Parker observed in the eggs of *M. mexicanus*. Nevertheless the small differences from control are in many cases statistically significant. At the high temperatures tried, there is a definite acceleration when the eggs are subjected to a low temperature at an early stage of development and a retardation when the eggs are exposed to low temperature after 50% development. The maximum acceleration occurs when the period of exposure to low temperature is one day. This amounts to 17 hours (0·72 day) at 27°C. The rate of acceleration diminishes as this period of exposure to cold increases, till an exposure of 8 days or more to 5°C. results in a marked retardation. A maximum retardation of 35·5 hours (1·48 days) occurs when eggs are subjected to 5°C. for 16 days after 50% development at 30°C. It will be further noticed that the injurious effect becomes more marked as the difference between the low and high temperature increases. Thus at 37°C., there is no significant acceleration in any of the treatments ; on the other hand there is a relatively well-marked retardation.

TABLE II.

*The rate of development and viability of* L. migratoria *eggs incubated at different high temperatures and exposed to a low temperature of 5°C., at different stages of development.*

| Stage of development. | Days at low temp. | Difference from control (days). | Standard error. | '$t$' at 2%. | Number of eggs. Total. | Number of eggs. Viable. | % viability. |
|---|---|---|---|---|---|---|---|
| | | | (i) *High temperature of* 27°C. | | | | |
| 0% | 1 | +0·72 | 0·175 | *S | 120 | 61 | 50·8 |
| | 4 | +0·14 | 0·09 | I | 100 | 55 | 55·0 |
| | 8 | −0·07 | 0·073 | I | 100 | 35 | 35·0 |
| 25% | 1 | +0·51 | 0·091 | S | 100 | 50 | 50·0 |
| | 4 | +0·43 | 0·086 | S | 90 | 49 | 54·5 |
| | 8 | −0·02 | 0·100 | I | 110 | 49 | 44·5 |
| | 16 | −0·61 | 0·154 | S | 115 | 42 | 36·6 |
| 50% | 1 | −0·55 | 0·109 | S | 140 | 84 | 60·0 |
| | 4 | −0·61 | 0·191 | S | 120 | 66 | 55·0 |
| | 8 | −0·75 | 0·153 | S | 150 | 77 | 51·4 |
| | 16 | −1·46 | 0·237 | S | 130 | 40 | 30·8 |
| | | | (ii) *High temperature of* 30°C. | | | | |
| 0% | 1 | +0·62 | 0·122 | S | 80 | 49 | 61·1 |
| | 4 | −0·01 | 0·02 | I | 140 | 64 | 45·7 |
| | 8 | −0·63 | 0·132 | S | 70 | 8 | 11·4 |
| 25% | 1 | +0·42 | 0·107 | S | 90 | 51 | 56·7 |
| | 4 | +0·19 | 0·065 | S | 130 | 70 | 53·8 |
| | 8 | +0·12 | 0·129 | I | 100 | 48 | 48·0 |
| | 16 | −0·66 | 0·106 | S | 120 | 28 | 23·3 |
| 50% | 1 | −0·39 | 0·132 | I | 90 | 46 | 51·1 |
| | 4 | −0·65 | 0·115 | S | 110 | 55 | 50·0 |
| | 8 | −0·70 | 0·144 | S | 130 | 59 | 45·4 |
| | 16 | −1·48 | 0·386 | S | 70 | 7 | 10·0 |
| | | | (iii) *High temperature of* 33°C. | | | | |
| 0% | 1 | +0·42 | 0·126 | S | 100 | 57 | 57·0 |
| | 4 | −0·46 | 0·079 | S | 80 | 50 | 62·5 |
| | 8 | −0·69 | 0·202 | S | 60 | 9 | 15·0 |
| 25% | 1 | +0·43 | 0·110 | S | 70 | 48 | 68·6 |
| | 4 | +0·22 | 0·059 | S | 90 | 51 | 56·8 |
| | 8 | +0·06 | 0·102 | I | 120 | 53 | 44·2 |
| 50% | 1 | −0·33 | 0·09 | S | 110 | 63 | 57·3 |
| | 4 | −0·40 | 0·112 | S | 120 | 70 | 58·3 |
| | 8 | −0·86 | 0·152 | S | 140 | 66 | 47·1 |
| | | | (iv) *High temperature of* 37°C. | | | | |
| 0% | 1 | +0·05 | 0·09 | I | 90 | 61 | 67·8 |
| | 4 | −0·57 | 0·095 | S | 100 | 50 | 50·0 |
| 25% | 1 | +0·17 | 0·063 | S | 140 | 89 | 63·6 |
| | 4 | −0·26 | 0·068 | S | 140 | 77 | 55·0 |
| | 8 | −0·35 | 0·077 | S | 130 | 63 | 48·5 |
| 50% | 1 | −0·21 | 0·0073 | I | 100 | 56 | 56·0 |
| | 4 | −0·76 | 0·169 | S | 90 | 53 | 58·9 |
| | 8 | −0·97 | 0·275 | S | 95 | 45 | 47·4 |

* S=Significant. I=Insignificant.

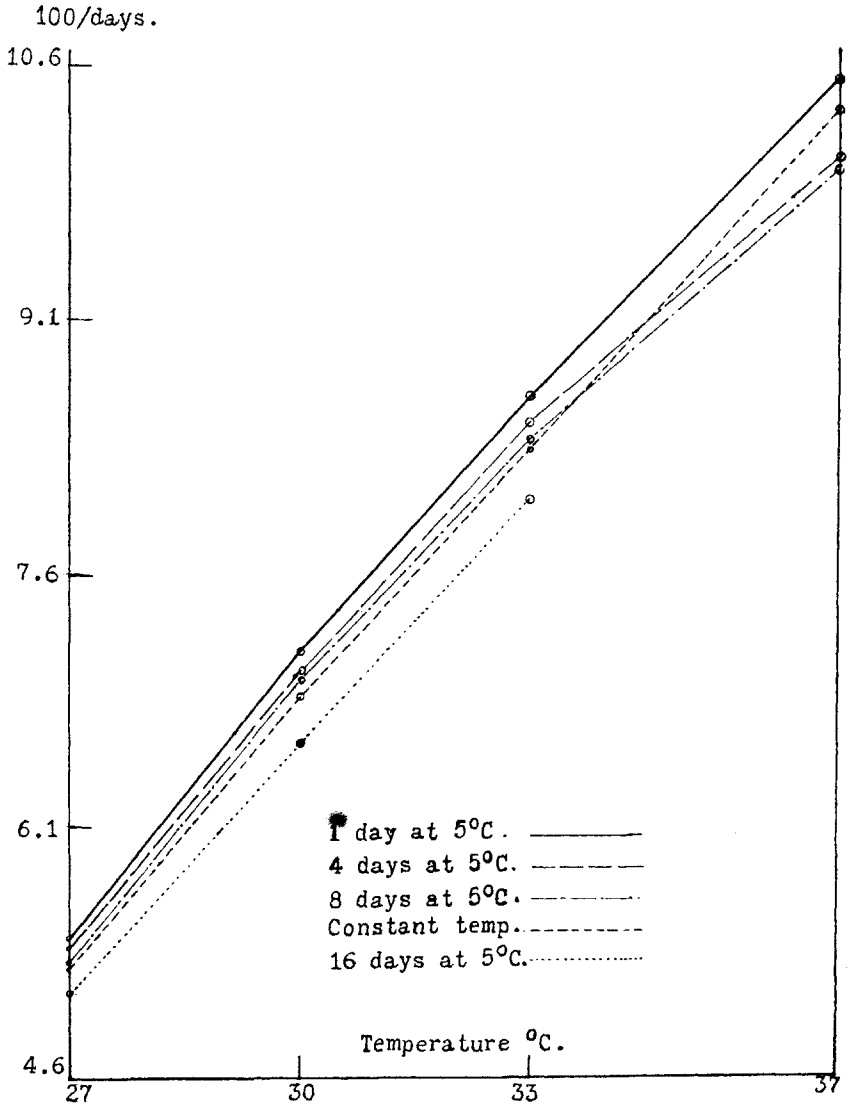


FIG. 2. Index of development of *L. migratoria* eggs subjected to 5°C. for 1, 4, 8 and 16 days after 25% development.

The percentage viability of the eggs under different treatments is inversely proportional to the duration of low temperature. The viability decreases when the difference between the low and high temperature increases. Thus, while the eggs that were kept for 16 days at 5°C., and subsequently incubated at 27°C. and 30°C., hatched out, those that were subsequently incubated at 37°C. could not complete their development. Eggs kept for 32 days at 5°C., however,

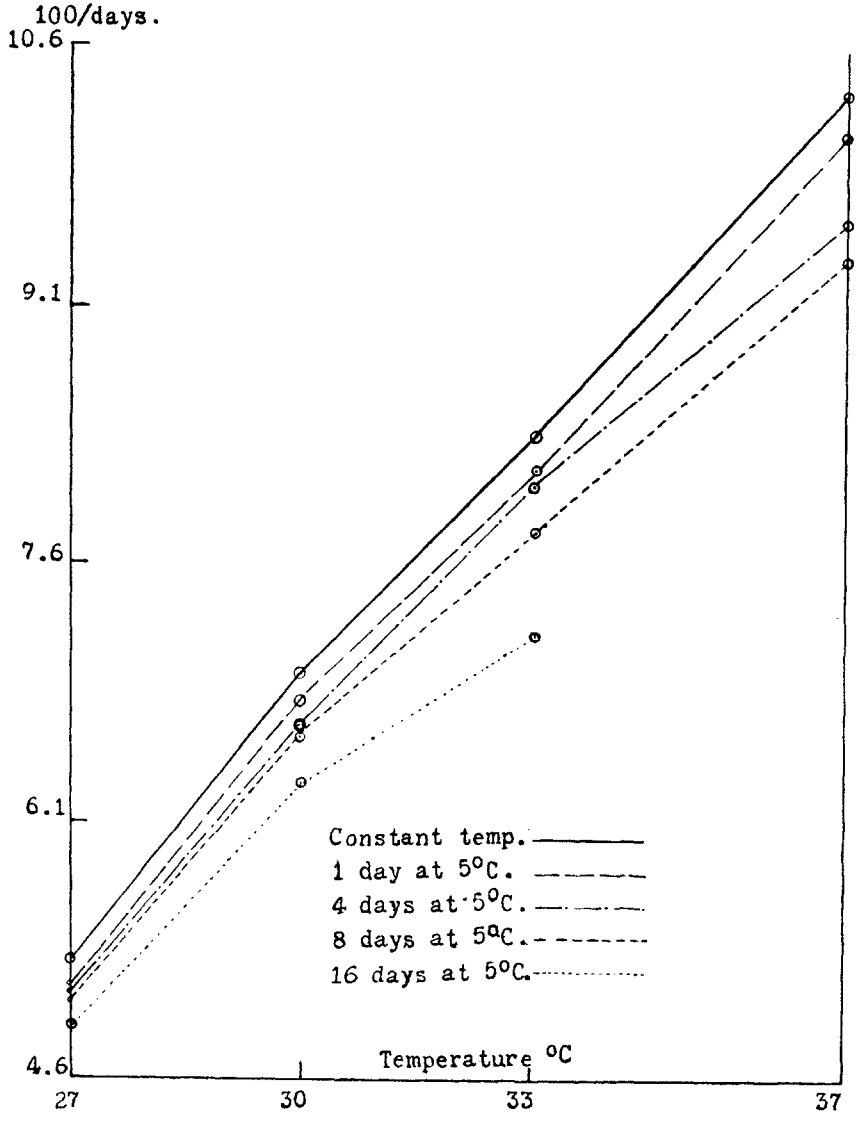


FIG. 3. Index of development of *L. migratoria* eggs subjected to 5°C. for 1, 4, 8 and 16 days after 50% development.

seemed to be too seriously injured and could not complete development at any of the higher temperatures.

3. *Effect of daily alternation of low and high temperatures* :—The eggs in this series were daily incubated from 5 P.M. to 9 A.M. at a high temperature of 27°C., 30°C. or 37°C. and for the rest of the day they were kept at 5°C. Table III shows the period required at each temperature and the difference

from the control. In every case the development is accelerated. The amount of acceleration varies from 3·6 hours (0·15 day) at 37°C. to 19 hours (0·79 day) at 27°C. ; thus an increase in the difference between low and high temperature reduces the acceleration.

TABLE III.

*Influence of daily alternation of about 8 hours at 5°C. and 16 hours at a high temperature on the rate of development and viability of* L. migratoria *eggs.*

| High temperature °C. | Low temperature °C. | Period (days) at high temperature. | Period (days) at low temperature. | Difference from control (days). | Standard error. | 't' at 2%. | Total No. of eggs. | No. of viable eggs. | % viability. |
|---|---|---|---|---|---|---|---|---|---|
| 37 | 5 | 9·55 | 4·03 | +0·15 | 0·084 | *S | 75 | 49 | 65·3 |
| 33 | 5 | 11·40 | 5·24 | +0·50 | 0·109 | S | 100 | 57 | 57·0 |
| 27 | 5 | 18·25 | 8·05 | +0·79 | 0·111 | S | 116 | 52 | 44·8 |

*S = Significant.

It will be noticed that while a continuous long exposure to low temperature was injurious to the eggs and caused a retardation, daily alternation of low and high temperature, in which the total period of cold amounted to about 5 days when high temperature was 30°C. and 8 days when high temperature was 27°C., produced significant acceleration in development. This suggests that while the eggs of *L. migratoria* are capable of withstanding nocturnal low temperatures alternating with warm days, they are not adapted to resist continuous long spells of cold.[1]

## D. THE DEVELOPMENT AND MORTALITY OF *C. erythrocephala* PUPÆ UNDER DIFFERENT CONDITIONS.

1. *Effect of constant temperatures and saturation deficiencies:*—The development and percentage viability of the pupæ have been determined at 6 constant temperatures and 4 saturation deficiencies (Table IV). The mean duration at 9 mm. saturation deficiency and different temperatures is graphically shown in Fig. 4. The length of pupal period depends mainly on the temperature and only to a very small extent on the saturation deficiency of the air. It is interesting to note that at each temperature the pupal duration as well as the percentage mortality are minimum at a saturation deficiency of 9 mm. An increase as well as a fall in the saturation deficiency slightly lengthen

[1] When this paper was going through the press a paper on the ecology of *L. Migratoria* by A. G. Hamilton (*Trans. Roy. Soc. London*, vol. 85, Pt. I, pp. 1–60) has been received. This work will be discussed separately.

the pupal period, but produce marked increase in the mortality. Thus both in regard to the rate of development and the viability, the saturation deficiency of 9 mm. represents the optimum condition. Under favourable conditions of moisture and temperature more than 90% of the pupæ can complete their development. Temperatures higher than the optimum seem more injurious than those below it.

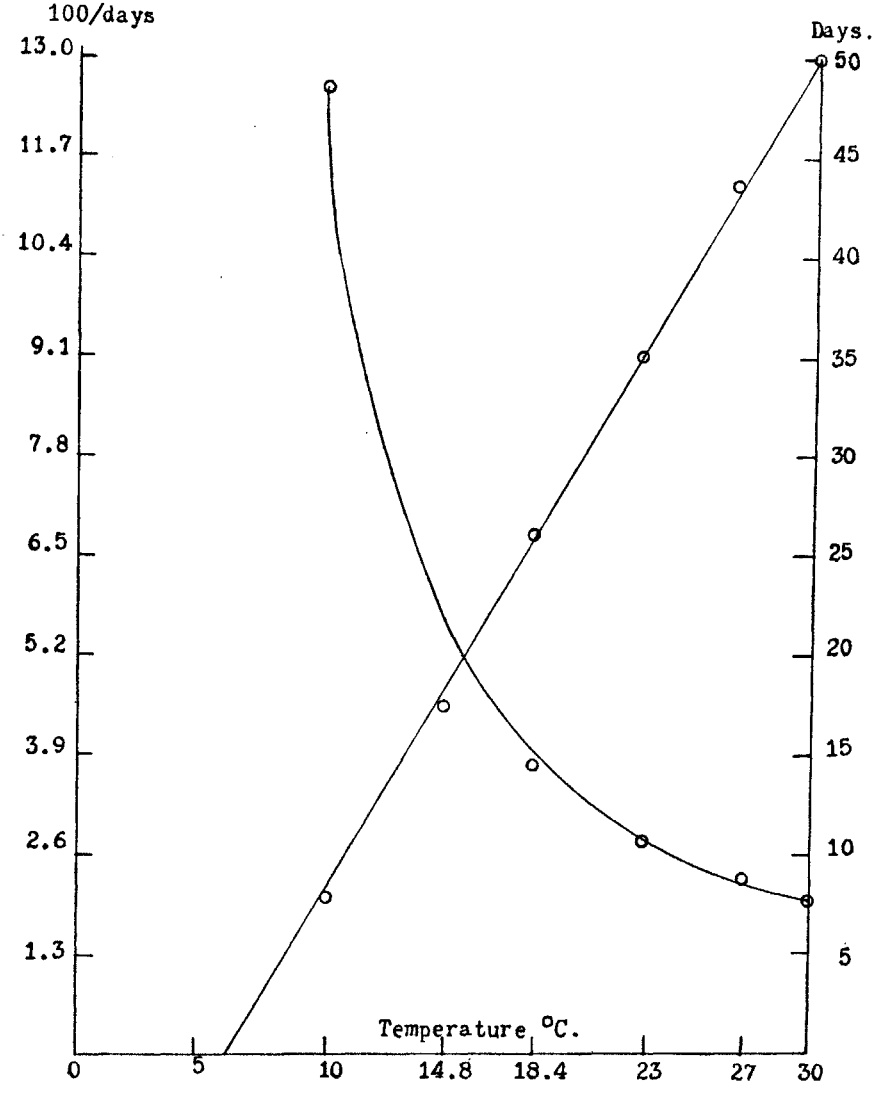


FIG. 4. Time-temperature and temperature-rate curves of pupal period of *C. erythrocephala* at constant temperatures and 9 mm. saturation deficiency.

TABLE IV.

*The rate of development and viability of* C. erythrocephala *pupæ at constant temperatures and saturation deficiencies.*

| Saturation deficiencies. | | Temperature °C. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10·5 | 14·8 | 18·4 | 23·0 | 27·0 | 30·0 |
| 3 mm. | Mean period .. | 49·04 | 23·53 | 14·61 | 11·01 | 8·85 | 7·87 |
| | Standard error.. | 0·629 | 0·108 | 0·029 | 0·044 | 0·023 | 0·126 |
| | No. of pupæ .. | 25 | 80 | 150 | 145 | 238 | 128 |
| | No. of flies .. | 4 | 43 | 138 | 106 | 62 | 10 |
| | % viability .. | 16·0 | 53·8 | 92·0 | 73·1 | 26·1 | 7·8 |
| 6 mm. | Mean period .. | 48·58 | 22·10 | 14·58 | 10·92 | 8·81 | 7·80 |
| | Standard error.. | 0·044 | 0·06 | 0·042 | 0·033 | 0·037 | 0·014 |
| | Number of pupæ | 65 | 189 | 177 | 142 | 96 | 112 |
| | Number of flies | 42 | 117 | 168 | 129 | 48 | 33 |
| | % viability .. | 65·0 | 62·2 | 94·9 | 91·1 | 50·0 | 29·5 |
| 14 mm. | Mean period .. | .. | .. | 14·92 | 11·0 | 8·86 | 7·85 |
| | Standard error.. | .. | .. | 0·07 | 0·041 | 0·041 | 0·031 |
| | Number of pupæ | .. | .. | 97 | 100 | 108 | 141 |
| | Number of flies | .. | .. | 76 | 69 | 43 | 36 |
| | % viability .. | .. | .. | 78·4 | 69·0 | 39·8 | 25·5 |
| 21 mm. | Mean period .. | .. | .. | .. | 11·24 | 9·03 | 7·83 |
| | Standard error.. | .. | .. | .. | 0·055 | 0·04 | 0·074 |
| | Number of pupæ | .. | .. | .. | 98 | 110 | 108 |
| | Number of flies | .. | .. | .. | 58 | 37 | 21 |
| | % viability .. | .. | .. | .. | 59·2 | 33·6 | 19·4 |

TABLE V.

*The development and viability of* C. erythrocephala *pupæ kept at a low and a high temperature.*

| Temperature °C. | | Exposure to low temp. (days). | Pupal period at high temp. | Difference from control (days). | Number of pupæ. | | % viability. |
|---|---|---|---|---|---|---|---|
| Low. | High. | | | | Total. | Viable. | |
| 5 | 14·8 | 1 | 21·89 | +0·21 | 83 | 48 | 57·8 |
| | | 4 | 21·06 | +1·04 | 315 | 178 | 56·5 |
| | | 8 | 19·98 | +2·12 | 314 | 198 | 63·1 |
| 5 | 18·4 | 1 | 14·40 | +0·18 | 232 | 197 | 84·9 |
| | | 4 | 13·73 | +0·85 | 578 | 379 | 65·6 |
| | | 8 | 13·13 | +1·45 | 473 | 300 | 63·4 |
| 5 | 23·0 | 1 | 10·72 | +0·20 | 250 | 229 | 91·6 |
| | | 4 | 10·33 | +0·59 | 280 | 246 | 87·9 |
| | | 8 | 10·07 | +0·85 | 634 | 366 | 57·7 |
| 5 | 27·0 | 1 | 8·86 | −0·05 | 101 | 26 | 25·7 |
| | | 4 | 8·76 | +0·05 | 235 | 70 | 29·8 |
| | | 8 | 8·30 | +0·51 | 399 | 104 | 26·1 |
| 5 | 30·0 | 1 | 7·82 | −0·02 | 313 | 42 | 13·4 |
| | | 4 | 7·80 | 0 | 406 | 51 | 12·5 |
| | | 8 | 7·66 | +0·14 | 288 | 31 | 13·0 |

2. *Effect of a single period of exposure to low temperature of 5°C. on pupal development* :—Pupæ were exposed to a low temperature of 5°C. for 1, 4 or 8 days after they had completed 0%, 25% or 50% development. During the remaining period they were exposed to a temperature of 14·8°C., 18·4°C., 23°C., 27°C. or 30°C., with a saturation deficiency of 9 mm. The mean values with different treatments (Fig. 5), their standard errors and the percentage viability of pupæ are given in Table V. The analysis of the variance and tests of significance are set out in Table VI.

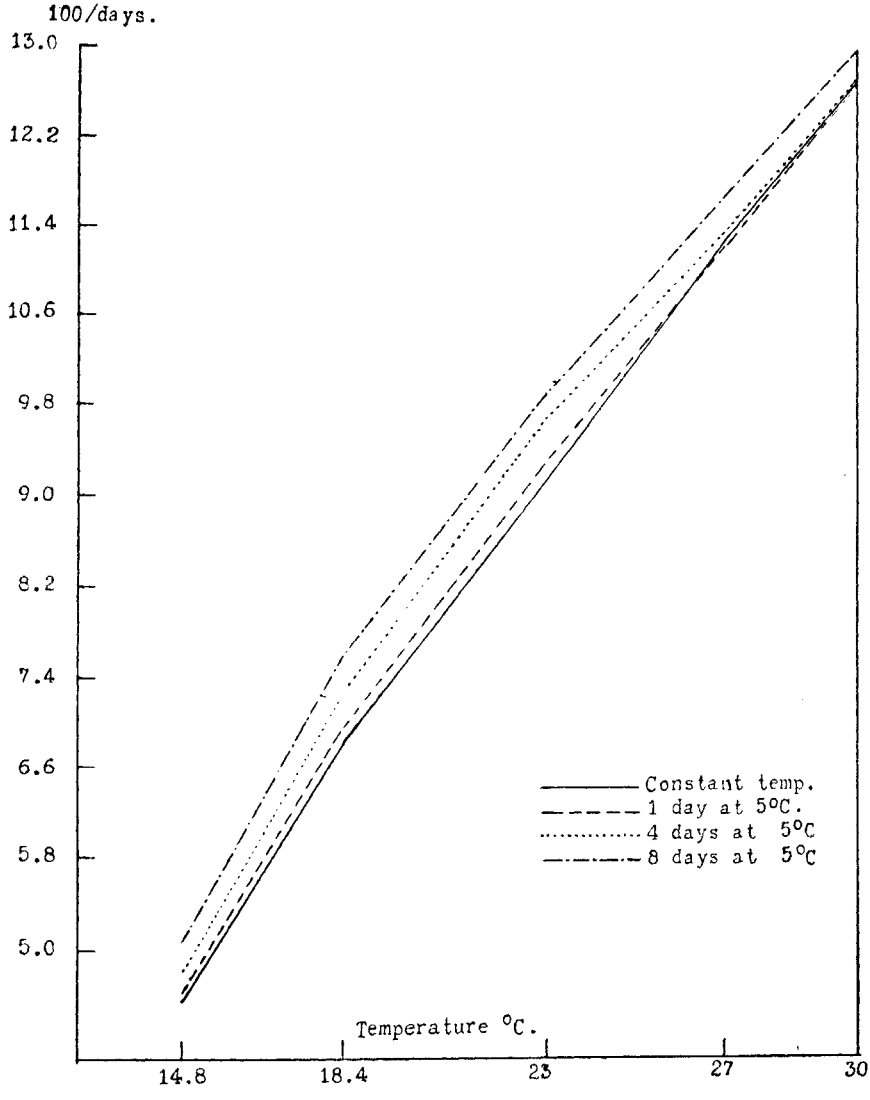


FIG. 5. Index of development of *C. erythrocephala* pupæ subjected to 5°C. for 1, 4 and 8 days.

TABLE VI. *The analysis of variance and the test of significance based on the data presented in Table V.*

| High temp. (°C). | Analysis. | | Degrees of freedom. | Sum of squares. | Variance. | '$Z$' | Significance of '$Z$' at 1%. | Significance of '$t$' at 2%. |
|---|---|---|---|---|---|---|---|---|
| 14·8 | Between treatments .. | *1 | 3 | 385·9356 | 128·6452 | 2·836 | †S | Significant. |
| | | 2 | 1 | 0·5556 | 0·5556 | 0·113 | I | .... |
| | | 3 | 2 | 3·1076 | 1·5538 | 0·627 | I | .... |
| | | 4 | 2 | 1·7797 | 0·8898 | 0·348 | I | .... |
| | Total .. | | 8 | 391·3785 | | | | |
| | Within treatments (error) | | 532 | 235·6477 | 0·4429 | | | |
| | Grand total .. | | 540 | 627·0262 | | | | |
| 18·4 | Between treatments .. | *1 | 3 | 255·4296 | 85·1432 | 2·882 | S | Significant. |
| | | 2 | 2 | 12·7318 | 6·3659 | 1·586 | S | Insignificant. |
| | | 3 | 2 | 20·2014 | 10·1007 | 1·817 | S | Significant only between 0% and 25% development. |
| | | 4 | 2 | 11·7704 | 5·8850 | 1·546 | S | Insignificant. |
| | Total .. | | 9 | 300·1332 | | | | |
| | Within treatments (error) | | 1034 | 276·0732 | 0·2670 | | | |
| | Grand total .. | | 1043 | 576·2064 | | | | |
| 23·0 | Between treatments .. | *1 | 3 | 100·3098 | 33·4366 | 2·466 | S | Significant. |
| | | 2 | 2 | 1·3398 | 0·6699 | 0·511 | I | .... |
| | | 3 | 2 | 0·8325 | 0·4162 | 0·273 | I | .... |
| | | 4 | 2 | 2·2949 | 1·1474 | 0·780 | B.S. | .... |
| | Total .. | | 9 | 104·7770 | | | | |
| | Within treatments (error) | | 960 | 231·3942 | 0·2410 | | | |
| | Grand total .. | | 969 | 336·1712 | | | | |
| 27·0 | Between treatments .. | *1 | 2 | 13·5059 | 6·7529 | Significant. | | Insignificant, except between the control and 8 days of low temp. |
| | | 3 | 2 | 0·1133 | 0·0566 | Insignificant. | | .... |
| | | 4 | 2 | 0·2333 | 0·1166 | Insignificant. | | .... |
| | Total .. | | 6 | 13·8525 | | | | |
| | Within treatments (error) | | 215 | 29·9118 | 0·1391 | | | |
| | Grand total .. | | 221 | 43·7643 | | | | |
| 30·0 | Between treatments .. | *1 | 3 | 1·0943 | 0·3648 | Significant. | | Insignificant. |
| | | 2 | 2 | 0·2517 | 0·1258 | Insignificant. | | .... |
| | | 3 | 2 | 0·1376 | 0·0688 | Insignificant. | | .... |
| | | 4 | 2 | 0·1057 | 0·0528 | Insignificant. | | .... |
| | Total .. | | 9 | 1·5893 | | | | |
| | Within treatments (error) | | 147 | 10·2819 | 0·0699 | | | |
| | Grand total .. | | 156 | 11·8712 | | | | |

* 1 denotes the analysis between various periods of low temperatures, while 2, 3 and 4 denote the analysis between different stages of development with 1, 4 and 8 days at low temperature respectively. † B.S. = Barely significant, S = Significant, I = Insignificant.

Some of the important features characterizing the behaviour of *Calliphora* pupæ towards the treatments are :—

(i) In all these experiments the pupæ subjected to a low temperature of 5°C. show statistically significant acceleration in development at high temperatures of 14·8°C., 18·4°C., and 23°C. The longer the period of exposure to low temperature the greater is the difference from the control individuals. Thus, the acceleration produced when the pupæ are subjected for one day to 5°C. and for the remaining period to 14·8°C., 18·4°C., and 23°C. amounts to 0·21, 0·18 and 0·20 day respectively, but when subjected for 8 days to 5°C. it amounts to 2·12, 1·45 and 0·85 days respectively.

(ii) An alternation of very high temperatures, viz. 27°C. and 30°C. with the low temperature produces no acceleration. It will be recalled from constant temperature series (Table IV) that 27°C. and 30°C. are positively injurious to the insect, and appreciably reduce the viability of the pupæ.

(iii) The stage of development at which the pupæ are exposed to low temperature has no significant effect on the rate of acceleration produced. This is clearly borne out by the analysis of the variance given in Table VI. While both the '$Z$' and '$t$' tests show marked significance within the various periods of low temperatures, they are either both or '$t$' at any rate, insignificant within the different stages of development for any one period of low temperature. This being the case, figures for mean periods alone, irrespective of the three stages of development, are shown in Table V.

(iv) The positive differences between the alternating temperature experiments and the corresponding controls have different significance according to the high temperature employed. In order to make these results comparable, the differences from control have been converted into the index of acceleration by first dividing the total difference by the number of days of low temperature and then by the period required for complete development at different constant temperatures (Table VII). An examination of this data reveals that :—

(*a*) At 14·8°C., the difference from control per day of low temperature increases from 0·21 (5 hours) to 0·265 (6·4 hours) day with increase in the duration of exposure to cold.

(*b*) At 18·4°C., the acceleration per day of low temperature remains approximately the same (about 4·3 hours) irrespective of the length of exposure to cold.

(*c*) At 23°C. the acceleration expressed per day of low temperature decreases from 0·15 (3·6 hours) to 0·11 (2·6 hours) day as the period of exposure to cold increases.

(*d*) At 27°C. and 30°C. there is no significant acceleration with any duration of exposure to low temperature.

(*e*) The mean index of acceleration for all the different periods of low temperature (last column, Table VII) increases from 0·011 to 0·014 as the temperature rises from 14·8°C. to 23°C. but suddenly falls at 27°C.

TABLE VII.

*The pupal period and index of acceleration of* C. erythrocephala *pupæ under different conditions.*

| High temperature °C. | Pupal period at constant temperature (days). | Exposed to a low temperature of 5°C. for different periods. | | | | | | | | | Mean acceleration per day of low temp. | Index of acceleration. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | One day. | | | Four days. | | | Eight days. | | | | |
| | | Pupal period at high temp. (days). | Difference from control. | Difference per day of low temp. | Pupal period at high temp. (days). | Difference from control. | Difference per day of low temp. | Pupal period at high temp. (days). | Difference from control. | Difference per day of low temp. | | |
| 14·8 | 22·10 | 21·89 | +0·21 | +0·21 | 21·06 | +1·04 | +0·26 | 19·98 | +2·12 | +0·265 | +0·245 | 0·011 |
| 18·4 | 14·58 | 14·40 | +0·18 | +0·18 | 13·73 | +0·85 | +0·21 | 13·13 | +1·45 | +0·18 | +0·190 | 0·013 |
| 23·0 | 10·92 | 10·72 | +0·20 | +0·20 | 10·33 | +0·59 | +0·15 | 10·07 | +0·85 | +0·11 | +0·150 | 0·014 |
| 27·0 | 8·81 | 8·86 | −0·05 | −0·05 | 8·76 | +0·05 | +0·01 | 8·30 | +0·51 | +0·06 | +0·036 | 0·004 |
| 30·0 | 7·80 | 7·82 | −0·02 | −0·02 | 7·80 | 0 | 0 | 7·66 | +0·14 | +0·02 | 0 | 0 |

(v) The viability of the pupæ is, on the whole, not appreciably affected by the period of exposure to cold.

These results indicate that with alternating temperatures the acceleration and viability are maximum when the high temperature approaches the optimum.

3. *Effect of a single period of exposure to low temperature of* 0°C. *on pupal development* :—Experiments on the effect of different periods of low temperature of 0°C. on the pupal duration at high temperatures of 14·8°C., 18·4°C. and 23°C. showed a small amount of acceleration at 14·8°C., but this disappeared altogether at 18·4°C. and 23°C. A temperature of 0°C. thus seems to be injurious, or in other words, lies below the limit at which beneficial influence of alternating temperatures is manifested. The percentage viability, however, is affected in the same manner as when treated with 5°C.

4. *Effect of daily alternation of low and high temperatures on pupal development* :—The results of experiments on the daily alternation of low temperature of 5°C. and 0°C. with a high temperature of 18·4°C., 23°C. and 27°C. are given in Table VIII and Fig. 6. When the low temperature is 5°C. and the high temperature is 18·4°C. or 23°C., there is a marked acceleration in the rate of development amounting to 1·43 days at 18·4°C. and 0·85 day at 23°C. The acceleration at 27°C. is hardly appreciable. The index of acceleration in the case of experiment with daily alternation of low and high temperatures amounting to ·017 day at 18·4°C. and ·02 day at 23°C. is relatively higher than with a single period of low temperature. Here again, a wider difference between the low and high temperature is less favourable to pupal development. With a low temperature of 0°C. the rate of development is not affected ; the small positive differences are statistically insignificant.

TABLE VIII.

*The influence of daily alternation of a low and a high temperature on the rate of development and viability of* C. erythrocephala *pupæ.*

| Low temperature °C. | High temperature °C. | Period at low temperature (days). | Period at high temperature (days). | Standard error. | 't' at 2% between treated and control. | Total difference from control (days). | Difference per day of low temperature. | Index of acceleration. | Number of pupæ. | Number of flies. | % viability. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 18·4 | 5·85 | 13·15 | 0·085 | *S | +1·43 | 0·245 | 0·017 | 97 | 64 | 65·7 |
| 5 | 23 | 3·93 | 10·07 | 0·042 | S | +0·85 | 0·220 | 0·020 | 121 | 102 | 84·6 |
| 5 | 27 | 3·39 | 8·73 | 0·073 | I | +0·08 | 0·024 | 0·003 | 100 | 22 | 22·0 |
| 0 | 18·4 | 5·12 | 14·55 | 0·081 | I | +0·03 | .. | .. | 96 | 71 | 74·9 |
| 0 | 23 | 4·45 | 10·88 | 0·058 | I | +0·04 | .. | .. | 66 | 54 | 82·1 |

* S=Significant. I=Insignificant.

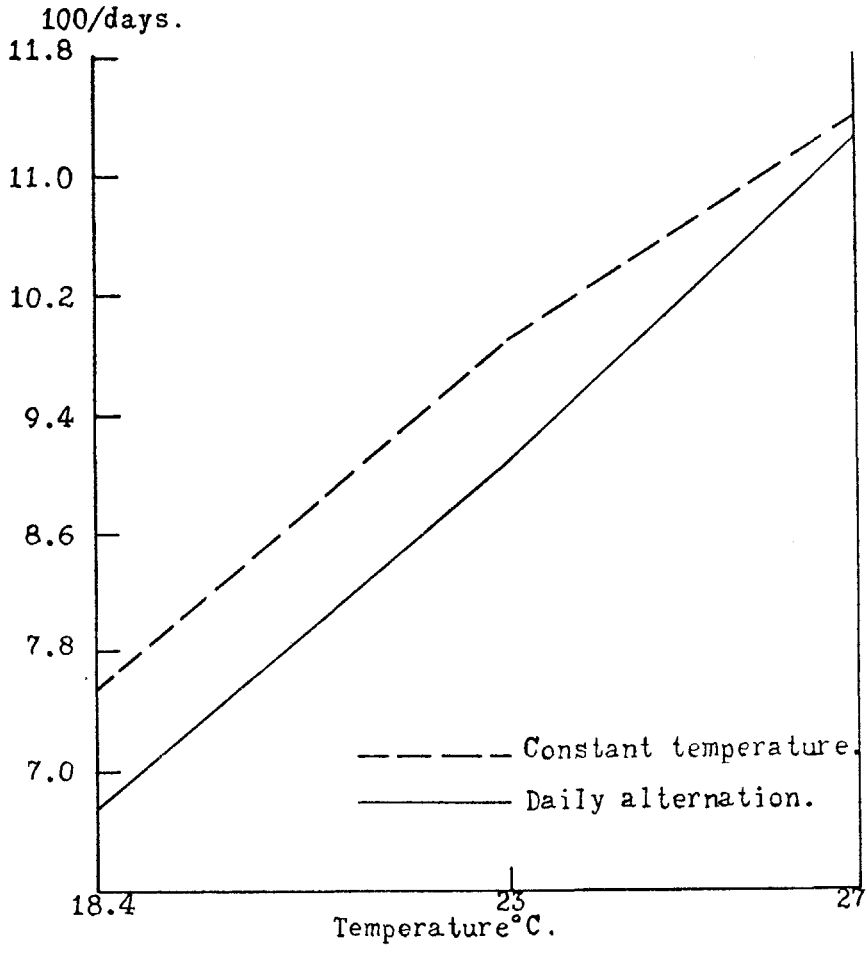


FIG. 6. Index of pupal development of *C. erythrocephala* subjected for about 8 hours to 5°C. and 16 hours to a high temperature daily.

## E. THE DEVELOPMENT AND MORTALITY OF *M. stabulans* PUPÆ UNDER DIFFERENT CONDITIONS.

1. *Effect of constant temperatures and saturation deficiencies* :—The rate of development (Fig. 7) and percentage viability of *M. stabulans* pupæ at constant temperatures and saturation deficiencies are shown in Table IX. Like *C. erythrocephala* the rate of development and mortality of *M. stabulans* pupæ are affected by temperature and saturation deficiency. The minimum duration and maximum viability are both obtained at 3 mm. saturation deficiency at all the temperatures tried.

These observations on *C. erythrocephala* and *M. stabulans* emphasize the desirability of using similar values of saturation deficiency instead of relative humidity in studying insect development.

TABLE IX.

*The rate of development and viability of* Muscina stabulans *pupæ at constant temperatures and saturation deficiencies.*

| Saturation deficiencies. | | Temperature °C. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 14·8 | 18·4 | 23·0 | 27·0 | 30·0 | 32·0 |
| 3 mm. | Mean period .. | 23·04 | 13·20 | 9·35 | 7·03 | 6·35 | 5·99 |
| | Standard error | 0·206 | 0·044 | 0·05 | 0·03 | 0·03 | 0·037 |
| | Number of pupæ | 150 | 85 | 54 | 78 | 55 | 107 |
| | Number of flies | 12 | 68 | 48 | 67 | 44 | 46 |
| | % viability .. | 8·0 | 80·0 | 88·9 | 85·9 | 80·0 | 43·0 |
| 9 mm. | Mean period .. | .. | 13·22 | 9·41 | 7·05 | 6·36 | 6·21 |
| | Standard error | | 0·04 | 0·04 | 0·034 | 0·033 | 0·037 |
| | Number of pupæ | 74 | 155 | 75 | 65 | 84 | 74 |
| | Number of flies | 0 | 125 | 58 | 52 | 61 | 23 |
| | % viability .. | 0 | 80·6 | 77·3 | 80·0 | 72·6 | 31·1 |
| 14 mm. | Mean period .. | .. | 13·83 | 10·13 | 7·12 | 6·71 | 6·23 |
| | Standard error | .. | .. | 0·036 | 0·031 | 0·027 | 0·035 |
| | Number of pupæ | .. | 45 | 80 | 71 | 61 | 150 |
| | Number of flies | .. | 27 | 47 | 44 | 29 | 36 |
| | % viability .. | .. | 60·0 | 58·7 | 61·8 | 47·5 | 24·0 |
| 21 mm. | Mean period .. | .. | .. | 10·75 | 7·33 | 6·75 | .. |
| | Standard error | .. | .. | 0·032 | 0·071 | .. | .. |
| | Number of pupæ | .. | .. | 83 | 102 | 80 | 60 |
| | Number of flies | .. | .. | 14 | 24 | 4 | 0 |
| | % viability .. | .. | .. | 16·9 | 23·5 | 5·0 | 0 |

TABLE X.

*The development and viability of* M. stabulans *pupæ kept at a low and a high temperature.*

| Temperature °C. | | Exposure to low temperature (days). | Pupal period at high temperature. | Difference from control (days). | No. of pupæ. | | % viability. |
|---|---|---|---|---|---|---|---|
| Low. | High. | | | | Total. | Viable. | |
| 5 | 18·4 | 1·0 | 13·19 | +0·03 | 203 | 148 | 72·9 |
| | | 4·8 | 13·05 | +0·17 | 193 | 128 | 66·3 |
| | | 8·0 | 12·91 | +0·31 | 219 | 142 | 64·8 |
| 5 | 23·0 | 1·0 | 9·53 | +0·08 | 326 | 293 | 89·9 |
| | | 4·0 | 9·33 | +0·28 | 294 | 203 | 69·0 |
| | | 8·0 | 9·05 | +0·56 | 434 | 234 | 53·9 |
| 5 | 30·0 | 1·0 | 6·36 | 0 | 395 | 290 | 73·4 |
| | | 4·0 | 6·37 | −0·01 | 316 | 105 | 33·2 |

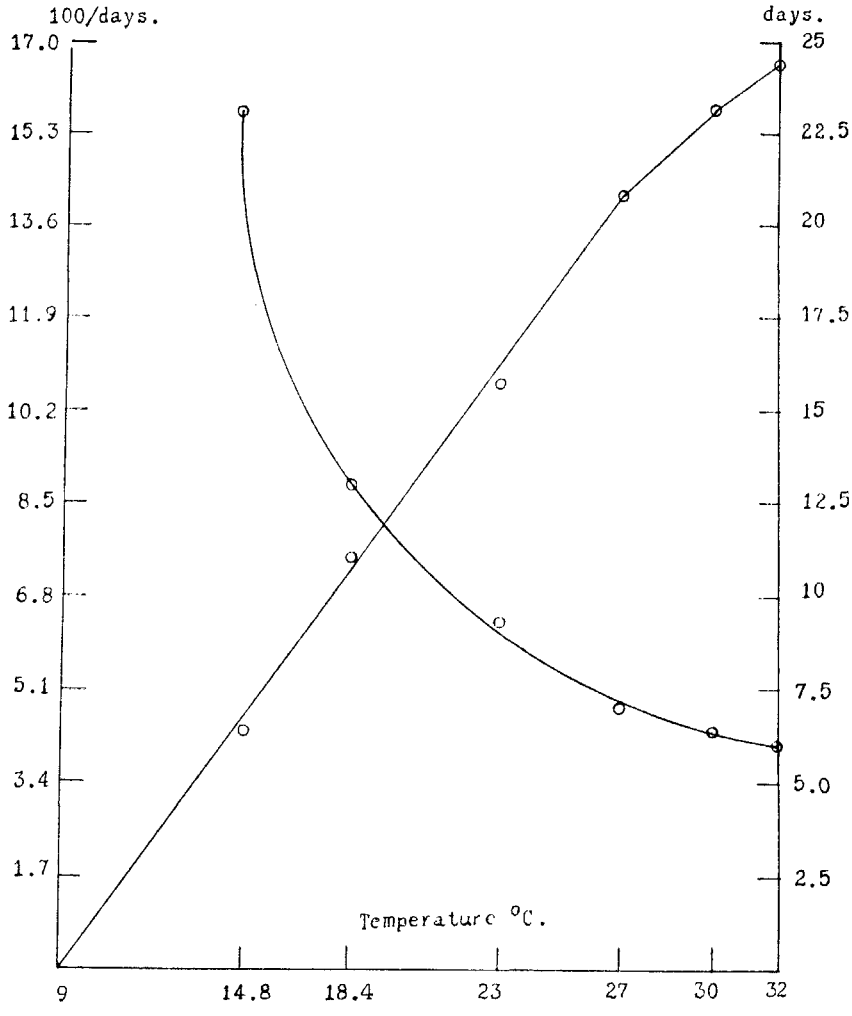


FIG. 7. Time-temperature and temperature-rate curves of pupal period of *M. stabulans* at constant temperatures and 3 mm. saturation deficiency.

2. *Effect of a single period of exposure to 5°C. on pupal development:*— Results of *Muscina* pupæ subjected for 1–8 days to a low temperature of 5°C. and kept for the remaining period at 18·4°C., 23°C. or 30°C., are set out in Table X. At 18·4°C. and 23°C. the alternating temperatures produced acceleration amounting to a maximum of 0·31 and 0·56 day respectively. The amount of acceleration is, however, much less than that in the case of the Blow fly pupæ. Pupæ kept at 5°C. and then at 30°C. showed no acceleration in comparison to those kept at a constant high temperature.

The percentage mortality of the pupæ is affected by the period of exposure to low temperature; the longer is this period the higher is the mortality. The injury caused by low temperature is greater in the case of freshly formed pupæ than in those which have undergone some development.

3. *Effect of a single period of exposure to 0°C. on pupal development*:—The rate of development at constant and alternating temperatures in this series remained approximately the same. The adverse effect of exposure to 0°C. on the viability of the pupæ was also about the same as that of exposure to 5°C.

4. *Effect of daily alternation of low and high temperatures*:—Pupæ kept daily for about 8 hours at 5°C. and for 16 hours at a high temperature of 18·4°C., 23°C. or 30°C. developed faster than those kept at constant temperature, or those subjected only once to low temperature. When, instead of 5°C., a low temperature of 0°C. was used, daily alternation did not produce any measurable acceleration. This is illustrated by Table XI.

TABLE XI.

*Influence of daily alternation of about 8 and 16 hours at low and high temperatures on the development and viability of* M. stabulans *pupæ.*

| Temperature (°C.) | | Period (days) at | | Difference from control (days). | Standard error. | '$t$' at 2%. | Acceleration per day. | No. of pupæ. | | % viability |
|---|---|---|---|---|---|---|---|---|---|---|
| Low. | High. | Low temp. | High temp. | | | | | Total | Viable. | |
| 5 | 18·4 | 5·61 | 12·97 | +0·25 | 0·046 | *S | 0·04 | 87 | 69 | 79·3 |
| | 23·0 | 3·98 | 9·22 | +0·39 | 0·052 | S | 0·10 | 78 | 62 | 79·0 |
| | 30·0 | 2·56 | 6·12 | +0·24 | 0·035 | S | 0·09 | 104 | 90 | 86·5 |
| 0 | 18·4 | 6·42 | 13·29 | −0·07 | 0·030 | I | .. | 90 | 78 | 86·7 |
| | 23·0 | 3·81 | 9·55 | +0·06 | 0·031 | I | .. | 100 | 71 | 65·1 |
| | 30·0 | 2·24 | 6·30 | +0·06 | 0·024 | I | .. | 101 | 45 | 44·5 |

* S = Significant. I = Insignificant.

## F. Discussion.

The results of the effect of alternating temperatures on the rate of development of insects dealt with here would be better understood by a brief consideration of their ecological habitats. Of the three insects studied, *Locusta migratoria* is an inhabitant of a more or less tropical region. The winter is passed in the adult stage and egg-laying begins early in spring. The egg-stage is not subjected in nature to any long continuous spells of cold, although during the night it may be subjected to temperatures below the threshold of its development. From the present investigation we find that the eggs are not only able to withstand daily alternation of low and high temperatures but such eggs develop faster in comparison with those kept at constant high

temperatures. A single exposure to low temperature for 24 to 96 hours also produced acceleration when acting in the beginning of embryonic development, but caused an unfavourable and retarding effect on eggs which had undergone about 50% development. In nature the first oviposition by the over-wintered adults usually takes place in the second and third weeks of February. Assuming that no development takes place during the night, it will be noticed that eggs would not reach a stage of 50% development until the middle of March, and while continuous periods of low temperature are possible during February, they are very unlikely after the middle of March. This may be the reason why freshly laid eggs can withstand low temperature while the half developed eggs do not. Again, long and continuous periods of exposure to cold in my experiments proved to be injurious to the developing embryo at any stage of development and caused a retardation during subsequent incubation at high temperatures. In *M. mexicanus* and allied species studied by Parker (*op. cit.*) the passing of winter in the egg stage is a general phenomenon which explains the accelerating effects of long durations of low temperature in those species.

The Blow fly *C. erythrocephala* is adapted to a cold habitat and possesses a theoretical threshold of development at about 6°C., which is 11 degrees below that of *L. migratoria* eggs. The pupæ are exposed to long spells of cold in nature (Graham-Smith, 1916); the alternating temperatures, in which the low temperature is below the threshold of development are, therefore, more in line with its natural evironment. In the experiments described here alternating temperatures accelerated the pupal development without affecting their viability, provided of course the low temperature was not too low to be positively injurious. It can be argued from Ludwig's (*op. cit.*) observations on *Drosophila melanogaster*, and there is no reason to disbelieve it, that some development may be taking place at 5°C., since it lies so near the theoretical threshold and may be the cause of apparent acceleration. But the rate of development at 5°C., if any, must be a definite quantity; in other words the index of acceleration should remain approximately the same irrespective of the high temperature with which a period of 5°C. is alternated. Experimental evidence shows that in alternating temperature experiments, the extra amount of development which may have taken place at 5°C. increases, as the high temperature approaches the optimum, i.e. from 14·8°C. to 23°C. At 27°C. and 30°C., however, there is a sudden fall and no measurable development can be attributed to a period of even 4–8 days at 5°C. It is possible that in this case the pupæ are so injured by the wide difference in the temperatures employed that they cannot resume the course of development followed by normal pupæ at constant temperatures, and subsequent retardation more than covers the amount of development that took place at 5°C. Again, daily alternation of low and high temperatures indicates a relatively greater development than a low temperature of the same duration acting at a stretch. It may be further mentioned that the amount of development per day of the period

at 5°C. in the case of daily alternation with 23°C. is equivalent to that indicated by 10°C. in the constant temperature series. It is not implied that no development takes place at 5°C., but what is meant is that the acceleration in these experiments is more than the development which can take place at a temperature of 5°C. and must be at least partly due to the differences in treatment, viz. the fluctuation of temperatures employed.

The stable fly *M. stabulans* remains in and near houses during the adult stage. But the larval and pupal stages have been observed in a variety of environments consisting of decaying and decomposing vegetable and animal matter, growing plants, human fæces, etc. In the experiments described here, however, there is very little response of the pupal stage to alternating temperatures. In no case does notable acceleration in development occur and the viability is affected by long duration of cold.

The foregoing studies indicate that alternating temperatures would affect different insects differently depending very probably on the ecological environment to which the insect is acclimatized in nature. It would therefore appear that in the present state of our knowledge each species of insects demands an independent critical investigation of its ecology.

## G. Acknowledgments.

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## H. Summary.

The development of *Locusta migratoria* eggs has been studied at 100% relative humidity and constant temperatures ranging from 20°C. to 37°C. The threshold of development lies at 17°C., the optimum range of development between 33°C. and 37°C. With daily alternation of 5°C. and 27°C., 30°C. or 37°C., significant acceleration of development amounting to 19 hours, 12 hours and 3·6 hours respectively is produced. A single exposure of 1–4 days to 5°C. at the beginning of embryonic development also produces an acceleration, with a maximum of 17 hours when the temperature of incubation is 27°C. Exposure to 5°C., after 50% development has occurred, results in retardation of development and lowering of viability.

The pupal development of *Calliphora erythrocephala* and *Muscina stabulans* is influenced mainly by temperature and to a small extent by saturation deficiency. The development as well as viability are optimum at saturation deficiency of 9 mm. in the former and 3 mm. in the latter insect. Alternating temperatures accelerate the development of *C. erythrocephala* pupæ. Acceleration in pupæ exposed to 5°C. for 8 days and then kept at 14·8°C., 18·4°C. and 23°C. is 2·12 days, 1·46 days and 0·85 day respectively. Daily alternation produces greater acceleration than a single prolonged exposure. Comparatively little acceleration is obtained by the fluctuations of any of the temperatures employed in the case of *M. stabulans* pupæ.

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