Studies on the Lethal Action of Abnormally High Temperature on Insects.

XVIII. General Consideration and Conclusions on the Results of the Serial Studies published hitherto.

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Aim and Scope of the Present Studies

When we consult the literature on this problem, it will be found that not a few studies have been carried out by many workers. Of these, the more important studies are ones for estimating how resistant the insects are to heat, for demonstrating a great difference in resistance according to different developmental conditions, or to the difference in relative humidity in the environmental conditions and ones for searching for causes of lethal effect of heat upon insects. Also, it will be seen that many theories have been advanced to explain heat injury upon living organisms from the physiological points of view.

Although many theories on heat injury have been proposed through the physiological consideration, these are not yet sufficient for explaining heat death or heat resistance of insects, presumably because of the circumstances that many internal and external factors are concerned with the injurious effect of heat on insects: besides, the resistance of insect to heat may also be associated with the variation in the ability of insects to withstand heat.

The aim and scope of the writer's study is to analyse the lethal action of high temperature by means of the quantitative analysis and to explain the nature of resistance of insects to heat from the mass physiological and experimental autecological points of view, chiefly using the Azuki-bean weevils, Callosobruchus chinensis as the test insect. The present writer began this investigation in 1943 and the results were reported serially from 1950 to 1961. Since a considerable amount of data has been accumulated, the writer thinks it advisable for further studies to point out some gists from all data obtained hitherto and to discuss on them.

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Quantitative Method for estimating the Lethal Effect of High Temperature upon Insects

A statistical method of using a unit, so-called probit, has been proposed by Bliss (1935—1940) for the analysis of the experimental data in the field of bio-assay of
insecticides. It is possible to apply this method to the analysis of lethal action of high temperature on insects.

Principles for application of the probit-analysis to the experimental data. Lot of a certain number of Azuki-bean weevil adults were subjected to sudden exposure to a series of different temperatures ranging from 34°C to 52°C for various definite durations of exposure. The curve representing the relation between the exposure time and mortality of insects killed by high temperature in these experiments, that is to say, the so-called the time-mortality curve is a sigmoid curve; and that is analogous to the dosage-mortality curve which is observed in the bio-assay of insecticides. However, such a curve is not always a cumulative curve of the symmetrical normal curve for all of the range of temperatures used in the experiments. The sigmoid curve seems to be an asymmetrical one which varies to a certain extent according to the high temperature used for experiment. Hence, we shall first consider the initial frequency distribution of resistability to heat where the sigmoid curve is asymmetrical.

When insects are exposed to high temperature, the high temperature may increase the evaporation of water from insect body in accordance to the exposure time. Consequently, the lethal action of high temperature would be the result of interaction of the primary action of heat itself and the secondary action of desiccation of insect body; and these are two lethal factors which can not be distinguished from each other by the shape of the initial distribution curve. Thus, the initial distribution curve seems to be a compound one. So, the writer is inclined to interpret that the time mortality curve would be a compound cumulative frequency distribution curve which varies to a certain extent according to change in manner of lethal action of heat and desiccation acting together.

If the value on the abscissa $X$ in the time-mortality curve is transformed into a suitable value which is a function of the change in manner of lethal action, it would be possible to take off from the time-mortality curve the part which is contributed by the change in manner of lethal action and thus to show the real cumulative distribution curve which represents the effect of high temperature only. For this purpose, we need to examine more fully the change in lethal action of high temperature in accordance with the exposure time.

Thus, starting from the physiological consideration, the writer has assumed that the lethal effect of heat changes in proportion to the $k$-th power of the amount of lethal stimuli, i.e., time required to kill, and also that $k$ has different values for different temperatures. In other words, the writer believes that the frequency distribution of resistance to heat may be normal against the time in $x^k$-scale; and that $k$ in $x^k$ may change in accordance to rise in temperature.

Hence, in conforming to the above hypothesis, the mortalities of insects for various exposures are converted to probit and plotted against the $x^k$-scale of exposure time. To the coefficient $k$ in $x^k$-scale such numerical values as $1/4$, $1/2$, $1$, $2$, $4$ are

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*We have inferred indirectly that the time-mortality curve is a cumulative curve of frequency distribution and that the resistability of insects to heat is distributed normally against the $K$-th power of the time $X$. Now, in order to determine the distribution of resistability to heat directly, the time required to kill each individual in a given population has been measured, respectively; and the frequency distribution curve of heat-resistance have been constructed from the data.

According to these experimental results, the distribution curve of heat-resistance, as measured on the usual scale, has been found to be markedly skew. But, it has been possible to obtain a distribution curve which is approximately normal by adopting the simple transformation of the scale of the time as mentioned above. For this purpose, the numerical value of $k$ in $x^k$ to be used for transformation must be varied as $1/4$, $1/2$, $1$, $2$ with the rise of temperature.

Thus, the writer is convinced of the interpretation that the resistance to heat, in general, is distributed normally against the $k$-th power of the time $X$ for which the insects exposed to heat and that $k$ in the $x^k$-scale varies with the rise in temperature used for experiment.
assigned \textit{a priori} with increase in temperature. Using such a transformation of the scale on X axis (X'-transformation method), the time-mortality curve has been found to be expressible by a straight regression equation with great accuracy in all of the experiments. Thus the time-mortality curve could be calculated according to the calculation of dosage-mortality curve proposed by Bliss (1935\textsuperscript{a})\textsuperscript{9} except that x'-scale was used on the time axis instead of log x.

**Quantitative Theory of the Lethal Action of High Temperature upon Insects\textsuperscript{16-18\textsuperscript{26}}**

(a) **Theory on the relation between exposure time and temperature.** Many previous investigators have devoted their attentions to this problem.\textsuperscript{11} Their attempts have been made to represent the relation between them by a simple equation, but we must bear in mind that this relation is brought about by the sum of a considerable number of biological reactions and that there is a great variation in the heat-resistance. Therefore, it seems that the problem should be approached from the biostatistical point of view.

According to the writer's investigations from the bio-statistical point of view, the frequency distribution of heat-resistance is normal in regard to $X_1'$, where $X_1$ represents the exposure time, and it is also normal in regard to $X_2'$ where $X_2$ represents temperature to kill. The values of k and $k'$ vary for various ranges of temperature. These facts can be expressed by the following two regression equations: $Y = a_1 + b_1 \cdot X_1'$ and $Y = a_2 + b_2 \cdot X_2'$, respectively, where $Y$ is mortality in probit. As a representation of the joint effect of time and temperature, therefore, a plane represented by an equation $Y = m + b_1 \cdot X_1' + b_2 \cdot X_2'$ may be suggested. If the numerical value of $Y$ be 5, the equation becomes finally $\log X_1 = \log A - B \log X_2$ or $X_1 = A/X_2^B$. The constant A and B in the equation vary for the different ranges in temperature because of the fact that k and $k'$ in $X_1'$ and $X_2'$ vary according to the temperature. The equation here arrived at is not a new one, but the writer has been able to formulate it rationally from the bio-statistical considerations.

In order to examine the fitness of this equation to the experimental data, we plot the logarithm of the median lethal time on the ordinate and logarithm of temperature on the abscissa for all of the ranges of temperature used for the experiments: and the relations between them are found to be represented by five intersecting straight lines and the values of the coefficient $B$ change at the critical temperatures 36°C, 40°C, 44°C and 50°C, respectively. The result, therefore, seems to agree well with the equation mentioned above and the coefficient $B$ in the equation takes five different values for five sections of temperature into which the whole range of temperatures used in the experiment is divided. This result suggests that the lethal action of high temperature upon insects changes at the critical temperatures mentioned above.

When the relation between temperature and coefficient $B$ which is interpreted as the temperature-coefficient of heat-injury is examined, it is found that the temperature-coefficient $B$ is the largest in the temperature range from 48°C to 50°C and it becomes smaller when the temperature rises or falls. \textit{Judging the mode of variation in B, a equation, $X_1 = A_1(X_2 - m)^{-1} + A_2(X_2 - m)^{-2} + A_3(X_2 - m)^{-3} + A_4(X_2 - m)^{-4}$ has been constructed theoretically for all of the range of temperatures, where m is a critical temperature of heat death of insects. The experimental results agree well with the equation described above.}

(b) **Theory on the relation between variation coefficient and temperature.** The
variation coefficient $1/k^2b^2m^2$, where $b$ is the regression coefficient of probit-time regression equation, $m$ is the median lethal time and $k$ is the exponent in $X^k$, has been used for estimating the deviation of variation in resistability to heat of insects.

The writer has pointed out a definite relationship of the variation coefficient with the temperature used for kill; The variation coefficient is, in general, large at a critical temperature where the lethal action of heat changes. In the temperature range between one critical temperature and another, the relation between temperature and variation coefficient is expressed by a equation, $U = M/2 (a^{-kX^k} + bX^k)$, where $U$ is variation coefficient, $X$ is temperature, $M$, $t, a$ and $b$ are constants. The equation shows a catenarian line.

The season for existence of these relationship is presumed to be the circumstance that the evaporation of water from insect body, which is the secondary factor that produces heat death, increases as the temperature rises, so that the lethal action upon insects changes with the increase of the secondary lethal factor and the deviation of variation in resistance to heat varies corresponding to such a change in the lethal action when temperature becomes higher.

Relative Humidity as a Factor influencing the Heat Resistance of Insects

Studies concerning this problem have been published. Nevertheless, the problem is so important that further experiments have been performed by the present writer to solve some question by means of Probit-Analysis. For this purpose, the experiments for estimating heat resistance of insects have been carried out under several different humidities which were expected to control differently the evaporation of water from insect body. And some time-mortality data have been obtained.

When some time-mortality data for different relative humidities under a constant temperature of 48°C were compared with one another, the difference between the time-mortality curves under different humidities were significant within limits of experimental and sampling errors. From this fact, we are able to infer that the lethal effect of high temperature varies in accordance with the different in saturation deficiency. Moreover, we have been able to ascertain that the time-mortality curve mentioned above is a compound cumulative frequency distribution curve representing the changes in lethal action of temperature and saturation deficiency. Such a time-mortality curve, of course, can be analysed by the $X^k$-transformation method as we mentioned already. Three points of the conclusion are given as follow:

(a) The variation coefficients at the critical saturation deficiencies are always high. In the range of saturation deficiency between a critical saturation deficiency and another, the relation between variation coefficient and saturation deficiency can be expressed by a catenarian line as described in (2)-b.

(b) Decrease in the median lethal time which accompany the rise in saturation deficiency is approximately expressed by a straight line. Strictly speaking, however, the median lethal time increases under the extreme condition of high or low saturation

**In these experiments, the exponent $k$ in $X^k$-scale, which we used to represent the time-scale in the time-mortality curve, takes different values with increasing temperature. If the variation coefficient $\sigma/m$, which statisticians ordinarily use is adopted for estimating the variation in resistibility of insects, we cannot appraise the difference between the variation observed in one temperature range and that in another temperature range in which $k$ takes different values. Therefore, a new index $1/k^2b^2m^2$, by which we are able to estimate the variation for all of the range of examined temperatures, has been adopted.
deficiency. The reason for decrease in the median lethal time under dry conditions may be that the greater evaporation in dry condition results in killing the insect by the desiccation and heat, and that for increase in the median lethal time under extreme dry condition may be that the insects are able to cool themselves for a time by evaporation.

(c) In order to express the changes in mortality due to the saturation deficiency and exposure time, the following procedure is adopted. The time required for a definite mortality of insect varies inversely proportional to the saturation deficiency of air; that is to say, the relation is represented by the equation \( X = a - bZ \), where \( X \) is time required to kill a definite percentage and \( Z \) is saturation deficiency. Moreover, the constants \( a \) and \( b \) in this equation are proportional to the mortality in probit. Namely, the equations for the constants are \( a = -76.047 + 29.393Y \) and \( b = -0.461 + 0.179Y \), where \( Y \) is the mortality in probit. The relation between saturation deficiency, mortality in probit and time required for kill is, therefore, expressed by the equation, \( X = -76.047 + 0.461Z + 29.393Y - 0.179ZY \). Finally, the equation, \( Y = \frac{76.047 - 0.461Z + X}{29.393 - 0.179Z} \), is obtained.

**Probit-Analysis of the Interaction of Temperature and Saturation Deficiency upon Insects**

For insects, the atmospheric moisture is an important factor and the effect upon insects differs from the heat. The effect of the former is modified by the change in the latter. The relative influences of heat and moisture on insects have been studied utilizing the thermohydrograph by the previous workers. On the other hand, the writer has analysed the influences through the Probit-Analysis of multipule regression equation. A definite number of adults were placed in different environments which were maintained constant in regard to both temperature and moisture, and the numbers of dead insects were counted repeatedly at a definite interval of time. A series of temperatures consisting of five grades ranging from 30°C to 42°C were used and the relative humidities were from 19% R. H. to 100% R. H., respectively.

In this case, lethal factors are temperature \( T \), saturation deficiency \( Z \) and duration of exposure time \( X \), so that the mortality \( Y \) of insects may be expressed by the equation, \( Y = G(T,Z,X) \). In order to analyse the relation represented by the equation, we have considered the relation between two of these four variables when the others were fixed. Besides, the relation between three of these four variables has been considered when another variable was fixed.

To put the problem in other words, the objects of the writer are to analyse the relations of (a) time-mortality, (b) temperature-mortality, (c) saturation deficiency-mortality, (d) temperature-duration of exposure time, (e) saturation deficiency-

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*** In any experiment in which insects were subjected to a fatal combination of heat and desiccation, there was a question as to which of these two factors actually caused the death of insects. It is, therefore, important to answer the question by experimentation. These experiments were: (a) test of the relation between index of water loss from insect body and the median lethal time, (b) test of the percentage of water loss from insect body and of water content in insects at the time when fifty percent mortalities are produced under given conditions of temperature and humidity.

Main points of the conclusion arrived at are as follow: Some insects are considered to die as the result of desiccation rather than that of heat when temperature is lower than 37°C. Nevertheless, the water loss from insect body or desiccation is not the principal cause of the heat death of insects at temperatures above 37°C.

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duration of exposure time, (f) temperature-saturation deficiency and the relation of
g) duration of exposure time-temperature-saturation deficiency to cite an example
of relation between three variables. Of course, these relations were analysed one
after another.

Important points in the study of the interaction of temperature and saturation
deficiency upon insects, however, are the analysis of the relation of time-mortality
and that of the relation of duration of exposure time-temperature-saturation deficien-
 cy. In this report, therefore, we shall describe the results of these two analyses only
in a condensed form.

(a) Time-mortality relation. The effect of a single factor, for example, temperature or saturation
deficiency modifying the mortality of insects has been expressed by the equation, \( Y_1 = a_1 + b_1 X \) or
\( Y_2 = a_2 + b_2 X \), where \( Y \) is mortality in probit, \( X \) is exposure time. If these two factors act together
on insects independently, \(^6\) the relation can be theoretically expressed by the equation \( Y_{1,2} = a + \beta(1+\omega)X \), where \( \omega \) is relative potency. As an example, two time-mortality equations that could be obtained
from two sets of experiments conducted at a temperature of 37°C and a relative humidity of 24% R.H.
have been calculated. These experimental equations are \( Y_1 = 1.17 + 0.96X \) and \( Y_2 = 1.87 + 0.91X \),
respectively. Hence, the theoretical equation \( Y_{1,2} = 1.250 + 1.44X \) has been constructed from the two
experimental equations described above for the case where two factors act together independently.

Now, the experimental equation actually obtained from the experiments with combination of two
lethal factors — temperature 37°C and relative humidity 24% R.H. — has been found to be
\( Y_{1,2} = 1.287 + 1.76X \). This differs from the above theoretical equation. Therefore, it is evident that these
factors do not act independently. If the time-mortality curve is constructed theoretically for the interaction
of two factors, it may be expressed by an interaction equation, \( Y_{1,2} = a + \beta(1+\omega \pm \mu \sqrt{\omega})X \), where \( \mu \)
is the coefficient of interaction. When the value of coefficient of interaction is 0.5 and the interaction
equation is constructed from the two experimental equation \( Y_1 = 1.17 + 0.96X \) and \( Y_2 = 1.87 + 0.91X \),
the resultant interaction equation is \( Y_{1,2} = 1.250 + 1.78X \). This theoretical equation agrees very nearly
with the experimental equation \( Y_{1,2} = 1.287 + 1.76X \) described above. From the mathematical consideration
mentioned above, the writer has been able to point out that the lethal action of temperature and saturation
deficiency on insects is not independent, but that it is dependent joint action.

(b) The relation between exposure time, temperature and saturation deficiency. The procedure
of analysis of this interaction is as follows: If the temperature \( T \) is fixed, the median lethal time \( X \)
which is obtained from the probit-time in \( X^2 \)-scale regression equation is inversely proportional to the
saturation deficiency \( Z \) for all levels of temperature: \( X = a - bZ \). The two regression coefficients \((a,b)\)
in this equation are linear function of the logarithm of temperature, \( \log T : a = 40.224 - 23.175 \log T \),
\( b = 0.849 - 0.514 \log T \). Therefore, the final regression equation of \( X \) for \( \log T \) and \( Z \) is represented by
the multiple regression equation, \( X = 40.224 - 23.175 \log T - 0.849Z + 0.514 \log T \cdot Z \). The coefficient
0.514 in the equation represents the interaction of temperature and saturation deficiency. A surface
that represents such a response may be described in geometric term as a hyperbolic paraboloid. The
multiple correlation coefficient \( R \) is 0.872; and it is highly significant statistically.

Using the multiple regression equation described above, the writer has been able to point out that
the lethal action of high temperature and saturation deficiency upon insects is the interaction of these
two factors and that the relative effect of temperature, saturation deficiency and interaction of the two
factors on insects can be shown quantitatively. Judging from the standard partial regression coefficients
in the multiple regression equation, it is found that the relative effect is 1:1.07:1.01.

Physiological Factors affecting the Resistance of
Insects to Heat\(^{20,21,23,24,26,27}\)

It has been generally supposed that the resistance of insects to heat is often dif-
ferent according to the differences in age, sex and physiological conditions of insects
even if they belong to the same race. Nevertheless, few studies had been carried out
hitherto. The writer has been able to point out some evidences on this problem
through the present investigations.

(a) Difference in the heat resistance of insects reared under different temperatures.
The Azuki-bean weevils reared from the egg stage to adult under different temperatures 22°C, 25°C, 30°C and 35°C were exposed to a high temperature of 46°C. Differences in the time-mortality relations observed in the experiments were significant statistically, so that differences among four sets of median lethal times and of regression coefficients calculated from the time-mortality equations were also significant between any two sets of the median lethal times or of the regression coefficients. The degree of heat resistance was compared one another by the ratio of values of the median lethal time to the median lethal time obtained at 22°C, and also by the ratio of values of regression coefficient to the regression coefficient obtained at 35°C. The former ratio was found to be 1.00:1.11:2.07:2.77 and the latter was 1.40:1.06:1.27:1.00 in the increasing order of rearing temperature.

When we examined the relation between the median lethal time and rearing temperature, it was found that the relation was represented nearly by a straight line. Next, when we examined the relation between variation coefficient of the resistability of insects to heat and the rearing temperature, it was found that the relation could not be represented by a straight line.

(b) Differences in the heat resistance of insects reared under different population densities.

In these experiments, two lots of insects whose population density differed from each other were used. In the one lot of high density, the number of eggs deposited per bean was from 10 to 15, while in the other of low density, it was from 3 to 5. From each set of the experimental data which were obtained on the relationship between exposure time and mortality of insects using the adults of these two lots, two probit-time regression lines were calculated. The test of parallelism of these regression lines was made by comparing the sum of X²-values for two sets. The mean probit difference was calculated. From these results, it was possible to conclude that the insects reared under low density can withstand high temperature better than those reared under high density and that a measure of the difference between two series of observation, mean probit difference was 0.416±0.096. The variation in resistibility to heat did not vary significantly by the rearing under two different population densities.

(c) Differences in the heat resistance of insects whose preimaginal stages were reared with food of different moisture contents and under different atmospheric moistures.

The rearing was performed under two different conditions, namely, wet condition and dry one. In the former case, the water content in the bean was approximately 19% and the atmospheric relative humidity during the rearing period was approximately 95%, whereas in the latter, the water content of the food was approximately 13% and the atmospheric humidity was approximately 55%.

Since the probit regression lines obtained from these two series of experiments were not parallel when statistically tested, the comparison of their heat resistance was somewhat difficult. No simple method of comparing the resistance was known, so that the comparison was made at a specific level. Namely, we adopted the median lethal time and the regression coefficient in the initial regression equations which were calculated from the results of experiment. Thus, the ratio of median lethal time obtained under dry condition to the median lethal time under wet condition and similarly, ratio of regression coefficients were compared, respectively. It has been found that the former is 1.65:1, and the latter is 1.73:1. The following conclusion may be drawn from these results: The insects reared under dry condition are 1.65 times as resistant as those reared under wet condition at the level of fifty percentage mortality, whereas the lethal efficiency of heat (regression coefficient h) on the insects bred under dry condition is 1.73 times as large as that upon those bred under wet condition.

(d) Differences in the heat resistance of insects reared on different kinds of food.

As the food of the larvae, soy bean and peas were used. As the control insects, the weevils obtained from the usual culture medium (Azuki-bean) were used. Since three time-mortality regression lines obtained from the experiments with different kinds of food seemed to be parallel, the heat resistance was estimated by the method of the parallel line assay. The summarizing the results, the following conclusion could be drawn: The variation in resistibility of insects to heat did not vary with the difference in their food, but the duration of exposure which the insect could withstand heat differed considerably. Namely, the adults reared on Azuki-bean withstood heat better than those reared on soy beans, while the latter was more resistant than the adults reared on peas. The differences in heat resistance between them were 0.1217 ± 0.053 and 0.2591 ± 0.065 in terms of the mean probit difference, respectively. The difference in resistance between the adults from Azuki-bean and those from pea was 0.4208±0.065.
(c) Differences in the heat resistance of insects in different ages.

The experiments were planned to indicate the difference in heat resistance due to different ages after emergence of adults. The heat resistance of insects were estimated by the mortality when they were exposed to a fixed temperature (46°C) for a definite duration of time (2h). From the result of experiment, it could be seen that the decrease in heat resistance of adults was closely related to the advance of age after adult emergence.

(f) Heat resistance of adult insects emerged on different days from the same stock of rearing.

Heat resistance of a group of adults that emerged in different periods from the same stock was examined several times during the emergence period of adults. No difference in the heat resistance was found among different groups of adults when compared with the adults appeared on the first day of emergence so long as the emergence occurred two or three days later, but it was definitely shown that the resistance decreased for adults emerged on the fourth day of emergence or later.

(g) Differences in the heat resistance of adults supplied with food and those not supplied.

Adults of the Azuki-bean weevil ordinarily do not take food and survive for several days. However, when they are supplied with sugar solution, they feed on it. Difference in the heat resistance between the adults that were fed on sugar solution and those which were not supplied with food was examined. The result of the experiment showed a marked difference in the heat resistance. Namely, the adults that were fed on sugar solution were more resistant than those which were not fed.

Physiological Characters common to Different Groups of Insects which are highly Resistant to Heat and the Physiological Significance of Heat Resistance of Insects

Significant difference in the resistance to heat were observed among adult insects which were reared from egg stage to emergence under different conditions of temperature and of nutrition. Next, we desired to know whether there is a relation between difference in heat resistance and physiological characters of insect, since the conditions of rearing affect the physiological characters of insects in a regular manner. The physiological characters that we examined in these experiments were (a) death rate during the growth period, (b) duration of adult life, (c) water content of insect body, (d) rate of water loss from insect body, (e) duration of adult life in a very dry air, (f) crude fat content in adult, (g) melting temperature of fat and (h) body weight of adults.

According to the result of experiment in which rearing was carried out under different temperatures, certain characters such as duration of adult life in a very dry air, rate of water loss from insect body, crude fat content in adult and melting temperature of fat seemed to be closely correlated with the heat resistance of insects. On the other hand, when conditions of rearing were varied as to nutrition, i.e., population density, water content in food and kind of food, such characters of insect as duration of adult life in a very dry air, longevity of adult, body weight of adult and crude fat content in adult could be looked upon as the characters which were common to highly resistant insects.

However, when heat resistance and characters of insects were examined throughout two sets of experiments of different rearing conditions mentioned above, we have found that the relationship between heat resistance and the characters was not always consistent. For example, longer duration of adult life in a dry air and larger amount of crude fat content in adults were found to be two characters which were common to all resistant individuals and at the same time closely related to the heat resistance, but the other characters could not be considered as characters which were common to resistant individuals.
Nevertheless, it would be difficult to decide whether the other characters can not be considered as the important characters related to the heat resistance. Since the heat resistance of insects seems to be such a complex physiological function and may possibly be influenced by many experimental factors that the character which affects the heat resistance may not always be the same according to the great difference in rearing conditions. For this reason, the relationship between heat resistance and certain characters of insects has been examined further using the same population reared under the same condition. These were the experiments using adults with different ages and those of different orders of emergence from the same stock. Besides, the difference between adults supplied with food and those not supplied and also difference between individuals that withstood a certain fixed high temperature and those killed in the same experimental sample were examined.

From the results of the investigations designed above, a larger amount of crude fat content, heavier body weight and longer duration of adult life in a very dry air have been found to be three important characters which were closely related to heat resistance of insects and common to the resistant individuals.

Next, the present writer has interpreted three characters mentioned above as indices representing the strength of insect viability. Therefore, it has been presumed that the potency of insect to recover from heat injury may be interpreted as an important factor affecting the resistance of insects to heat.

**Increase and decrease in the heat resistance of natural insect populations and experimental analysis of their causes**

If insects are exposed to abnormally high temperature for a certain period of time, they are ordinarily killed by heat. However, we have already learnt that the resistance of insects to heat varies according to environmental conditions under which the insect grow. In view of these observations, a study on the seasonal variation in heat resistance of insects has been carried out.

As experimental materials, larvae of the mosquito, *Aedes albopictus*, which were thriving in a certain water tank in the open air throughout a year were employed and the larvae for the materials were so selected as to be in the same stage of development. These experiments of heat resistance consisted in dipping mosquito larvae suddenly for certain minutes into hot water which was kept constant at five different temperatures ranging from 42°C to 50°C. The median lethal time and coefficient of variation were calculated from the time-mortality data.

The relation between the median lethal time and temperature by which insects are killed could be expressed by the equation $X_1 = \frac{A}{X^{2\beta}}$ in all experimental results as mentioned in (2)-a. The coefficient $B$ in the equation varies according to different seasons. We kept the record on changes in temperature of the water during the progress of season in which insects lived; and we could notice the fact that the curve representing the reciprocal of temperature coefficient $B$ and the curve representing the average temperature of water in the water tank did not differ much in regard to the variation in a year. On other wards, the relation between them could be expressed by a straight line. On the other hand, the relation between the coefficient of variation in distribution of heat resistance and the average temperature of water did not represent a straight line. On other wards, the negative correlation between them was not high. However, we could recognized the fact that the coefficient of variation showed a tendency to become smaller in summer and larger in spring or autumn.
The two facts that heat resistance corresponds fairly well to the environmental temperature in which insects grow and that the coefficient of variation in heat resistance shows a tendency to become smaller when the environmental temperature become higher, agree quite well with the experimental results obtained with the laboratory population of Azuki-bean weevils mentioned in (5)-a. Why such a different heat resistance is observed is just the question that we desire to answer. In some cases, the development in heat resistance has been interpreted as the adjustment of insect individuals to high temperature. In other cases, it has been believed that insect populations become more resistant to heat owing to the disappearance of weak individuals among a population by the selection due to high temperature.

The death rate in percentage of the Azuki-bean weevils during the preimaginal life increases considerably with the rise in the rearing temperature. If the correlation between the coefficient of variation in resistability of insects to heat and the death rate during the preimaginal life is high, the increase in resistance of insects to heat may be explained mainly the selection hypothesis. As a matter of fact, the curves showing these relations are not linear. Hence, the increase in heat resistance can not be sufficiently explained by the selection hypothesis alone.

The crude fat content in the Azuki-bean weevils shows a tendency to increase with the rise in temperature under which the insects develop. When we illustrate the relation between the median lethal time as the measure of heat resistance and crude fat content, the relation is represented by a straight line. From these facts, it is presumable that the development of heat resistance is due to a kind of thermal adjustment which is brought about by the change in the crude fat in insect body.

To determine the primary cause of increase in the heat resistance, the writer has undertaken further investigations. The results are described below: The Azuki-bean weevils reared at 35°C from generation to generation are more resistant than those reared at 30°C. When insects are returned from the condition of 35°C to that of 30°C, and are reared at this temperature during one generation, their heat resistances are still high to a certain extent. But those reared at 30°C for two generations after returning to 30°C become less resistant.

The mortality during growth period of insects reared at 35°C shows a tendency to be slightly higher than that of insects reared at 30°C. The number of eggs laid by the insects reared at 35°C is smaller. Moreover, some of the eggs laid by these insects are abnormal and can not hatch. It is some times found that the variation in heat resistance is inversely proportional to the mortality during growth period. Therefore, we can not completely deny the hypothesis that the increase in heat resistance of insects is due to the disappearance of weak individuals among a population by the thermal selection.

Insects reared at 34°C for ten days after being hatch at 30°C become more resistant. But the degree of increase in heat resistance of these insects is lower than that of insects reared at 35°C from generation to generation. The increase in heat resistance mentioned above seems to be chiefly due to the thermal adjustment of insect individuals. When insects bred at 30°C are exposed to a high temperature of 48°C in some stage in development, and then they are reared at 30°C again, the survivals are more resistant. But the degree of increase in heat resistance in this case is not higher than in the former case.

Considering these experimental results, it may be concluded that the so-called thermal adjustment of insect individuals among a population to high temperature is the main cause of increase in heat resistance and that the so-called thermal regulation of a insect population owing to the disappearance of weaker individuals by the thermal selection is the secondary one.

Summary

The writer has dealt with the problem of lethal action of abnormally high temperature upon insects and resistance of insects to heat from the mass physiological and experimental autecological points of view. In this paper, however, the results obtained from these experiments have not been described in detail, but the writer's
interpretations and general conclusions regarding the problems mentioned above are based on the writer's data reported serially from 1950 to 1961.

**Literature Cited**

摘 要

おもにアズキソウムシ *Callosobruchus chinensis* を用いて実験し次の結論をえた。

（1）熱抵抗の頻度分布は高温の強さ（温度）にも、その曝露時間に対しても、ともに正規分布し、指数kは広い温度範囲において特定の変動をあらわす1群の数値であると考えた。この見解に基づいて適用の範囲を広げた“Probit-Analysis”により高溫の殺虫効果を判定することに、殺虫温度と曝露時間の間に成り立つ従来の温度公式を批判し、広い温度範囲に適用される1つの新しい温度式を通じた。また熱抵抗の分布の巾を示す特定の指標と殺虫温度との関にも新しく一定の公式（一種の変曲線式）をあたえた。

（2）高温の殺虫作用に及ぼす湿度の影響を検討して、(a)比較散布度は作用の軸移点で大きく、それら軸移点間において、それを分析の前に変曲線式がなりたかつたこと、(b)中央致死時間と結合の間には大体直線式が成り立つが厳密に言うと、結合が極度に高いときに低いためには結合の作用が異なることを認めた。そこで高温殺虫作用の結合による殺虫効果の変動の表現には、従来の2元式（中央致死時間と結合）よりも新しい3元式（中央致死時間、死亡率、結合）が適切であると考えて、そのための公式を提案した。次に高温と結合の共同作用の研究に、従来の（低温度関）を用いるより更に進んで新しく重相関プロビット解析の方法を適用することを試み、それに基づいて両作用の殺虫の役割を説明した。

（3）昆虫の発育中に環境要因を実験的に1つ1つ変化させて、熱抵抗の変化を統計学的に分析した。この実験における環境要因は飼育温度および湿度、幼虫の発育密度、幼虫食物の質（大豆、大豆、えんどう）であった。また各条件の異なる場合と、たとえば成虫期間中の若壮老齢、同一飼育区内における初期、中期、後期羽化虫間および同一期成虫で温度を取ったものとそうでないものの間の熱抵抗の変化を検討した。

上記各種条件の異なる成虫の熱抵抗の実験とともに昆虫の8種の物理的性質を調べ、これらの変化と熱抵抗の変化の関連を検討した結果、(a)乾燥環境の下で生存期間の長いこと、(b)脂肪含有率の高いこと、および(c)体重の重いことの3性質が熱抵抗の強い場合の共通性質であることが認められ、これら性質が熱抵抗の要因と見做される意義を論じた。

（4）彩来から動物の適応力として知られた現象を観察および実験によって検討し、昆虫類においては、(a)熱抵抗を示す指標とその昆虫の棲息環境の温度が正の相関を示すこと、(b)熱抵抗の分布の巾を示す指標と温度とは、完全でないが幾分の負の相関性を示すことを認めた。更に温度適応の機転にも論及び、その大要は次のとおりである。

温度適応の原因には2つの一現象が関与するが、その1つは個体群内の個体が高温に生理的反応をおこし、いわゆる個体の温度調整であり、いま1つは個体群が高温に対して弱い個体変異を消失させ、強いもののみになるといういわゆる個体群の温度融解である。