TEMPERATURE INFLUENCE ON THE MORTALITY OF *CALANDRA GRANARIA L.* (CURCUL., COLEOPT.) FROM DDT.

by

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Received May 29, 1954.
(with 13 figs.)

I. INTRODUCTION

Several authors have shown that the mortality of insects from DDT poisoning quite often has a negative temperature coëfficient, that is to say that the mortality from a certain dose is higher at lower temperatures. (POTTER & GILLHAM, 1946; HÄFLINGER, 1948; PRADHAN, 1949; HOFFMAN & LINDQUIST, 1949; WOODRUFF, 1950; etc.). This applies especially to low concentrations of DDT, high concentrations generally showing a positive temperature effect.

FAN et al. (1948) working with *Aëdes-larvae* continually exposed to DDT in their medium, suggested that this negative correlation was due to greater adsorption of DDT on the chitinous cuticle at low temperatures. This explanation might apply to water insects which are continually exposed to DDT, but can hardly be applied to land insects exposed to residues and then removed from the toxic surface, or when DDT is applied topically.

PRADHAN (l.c.) and others consider that the rate of uptake of DDT is greater at higher temperatures, but that detoxification proceeds at a relatively higher rate at high temperatures. These two processes combined would result in an overall negative temperature coëfficient.

VINSON & KEARNS (1952) ,working with the "American Roach"*) also find a negative coëfficient. They draw attention to the fact that different species react in different ways and that generalisations are dangerous. They further show that the explanation must be more complicated than has been suggested earlier as animals still alive at high temperatures may contain larger quantities of

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[1]) No scientific name is given, but we suppose this to have been *Periplaneta americana L.*

unmetabolized DDT, than animals at low temperatures which show symptoms of poisoning.

They conclude that some process is involved which actually makes the insects more liable to the toxicant at lower temperatures.

We investigated the influence of temperature on the toxicity of DDT in the grain weevil, *Calandra granaria L.* The weevils were exposed to a DDT residue for a short time and then kept in a clean container to observe the results. It is characteristic of these experiments that the reaction to the DDT poisoning was very slow and that increasing mortality could be observed up to 15 days after the exposure. We could thus study the rate of mortality by counting dead weevils every day.

When observing a group of weevils for three weeks some deaths occur in untreated batches also, and at high temperatures this may amount to as much as 15%. We have not corrected our values for the mortality in the control as this sometimes leads to the unexpected result that the corrected mortality line descends in the latter part of the curve.

In other words, after some time of high mortality due to the toxicant, the mortality in the treated batches of weevils may become less than in the control. This leads to interesting considerations which we hope to discuss in a separate paper in the near future.

II. TECHNIQUE

The weevils were reared in glass containers of approximately 1 liter content, about three quarters full with wheat. The containers were closed with a lid with a round hole; over this hole a bit of wire gauze was fixed. The cultures were kept in a room of constant temperature ($\pm$ 25° C) and humidity ($\pm$ 70% R.H). To reduce the migration of mites *(Tyroglyphus farinae L.)* which always developed to some extent, the containers were put in flat dishes containing oil.

Experiments were done exclusively with weevils of 7–14 days old. To obtain these the grain was screened through gauze which retained the grain but let the weevils through. This grain was kept for a week and then screened again. All weevils obtained in this way (0—7 days old) were put in new pots with wheat and kept another week. They were then ready for use in the experiments.

Weevils were exposed to deposits of DDT made by pipetting 1 or 2 ml. of a solution of DDT in acetone into a petridish. The dishes were used within a few hours after preparation. The concentration was calculated to bring 4 different amounts of DDT per unit of surface area. They are further designated as follows:

$C_1 = 0.0104$ mg/cm²
$C_2 = 0.0209$ mg/cm²
$C_4 = 0.0417$ mg/cm²
$C_8 = 0.0834$ mg/cm²

In all cases the time of exposure was 1 hour. Each dish contained about 50 weevils. After the exposure the weevils were transferred to clean petridishes which contained a strip of moist filter paper and some wheat grains. Mortality was assessed daily. In cases of doubt whether a weevil was alive or dead it was held close to an electric lamp. If it did not react to the heat it was considered dead.

III. RESULTS

We distinguish between temperature of exposure and the

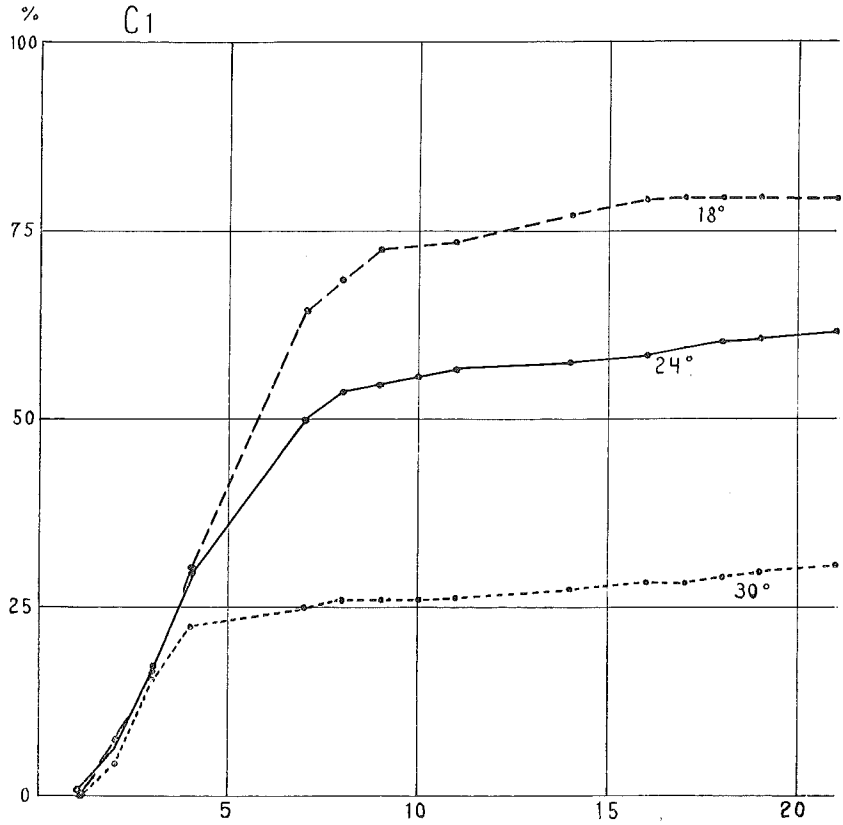

Fig. 1. Abscis: time in days; ordinate: percentage mortality: DDT concentration: C_1. Temp. of exposure = temp. of reaction.

temperature at which the weevils were kept after treatment, and which we have called temperature of reaction. The different combinations of temperature of exposure and temperature of reaction that were examined can be found in Table I.

TABLE I.

Concentration	Exposure temperature 18° C	24° C	30° C	
0	18	24	30	temperature of reaction.
C_1	18	24	30	
C_2	18	24	30	
C_4	18—24—30	24	18—24—30	
C_8	18—24—30	24	18—24—30	

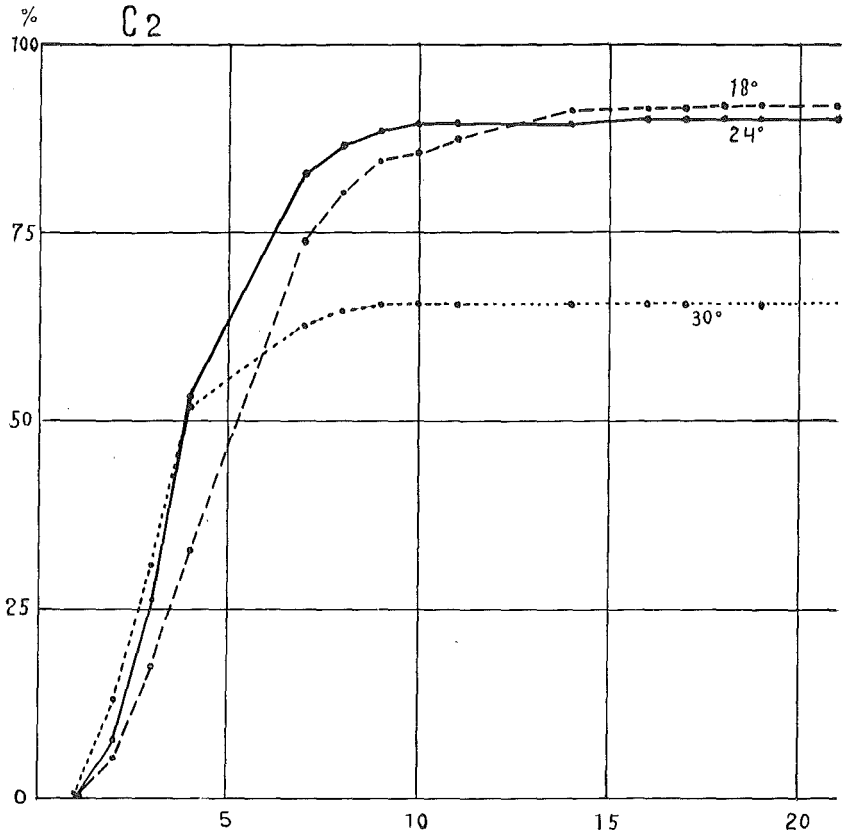

Fig. 2. as fig. 1; DDT concentration: C_2

The results are plotted in fig. 1—8. The abscis is the time axis, in days.

On the ordinate we find the mortality in percentages of the total number of weevils in the experiments. Each line is based on ca 250 individuals, exposed and observed in groups of ca 50 each.

The time of exposure was always one hour; the length of time during which the results were observed at different temperatures was at least 14 days.

In this way we obtained cumulative time-mortality curves, showing for each day of observation the total number of weevils which had died up to that moment (expressed in percentages of the initial number of weevils).

If we consider a certain number of weevils, the reaction of each weevil to a certain dose of the toxicant will depend on a great number of factors.

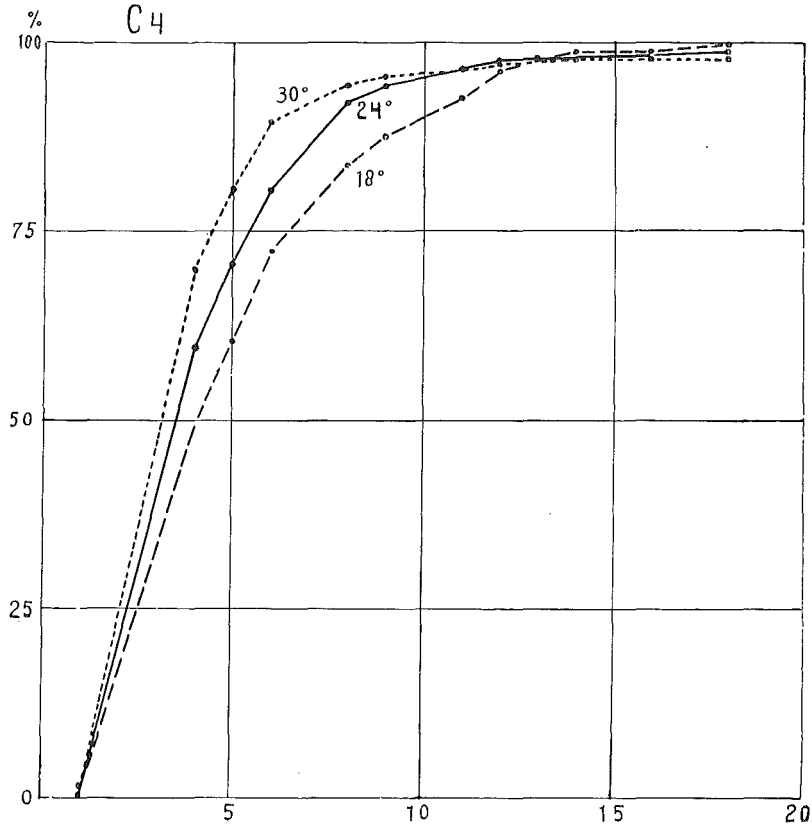

Fig. 3. as fig. 1; DDT concentration: C_4

If the action of the toxicant is constant in intensity the time-mortality curve would show us how the susceptibility of the weevils is distributed in the population. We would then expect to find a smooth sigmoid curve which might be transformed into a straight line in the usual way by plotting the results on logarithmic-probability paper. (BLISS, 1935, FINNEY 1952, etc.)

The action of the toxicant may however not be constant. If we apply a certain dose to each animal the external concentration is reduced as more is taken up by the animal, and detoxification in the animal may further effect the results. Diversion from the expected curve will therefore tell us something about changes in the condition of the toxicant. For instance, if the mortality line stops rising and changes into a more or less horizontal we can conclude that the action of the toxicant has come to an end.

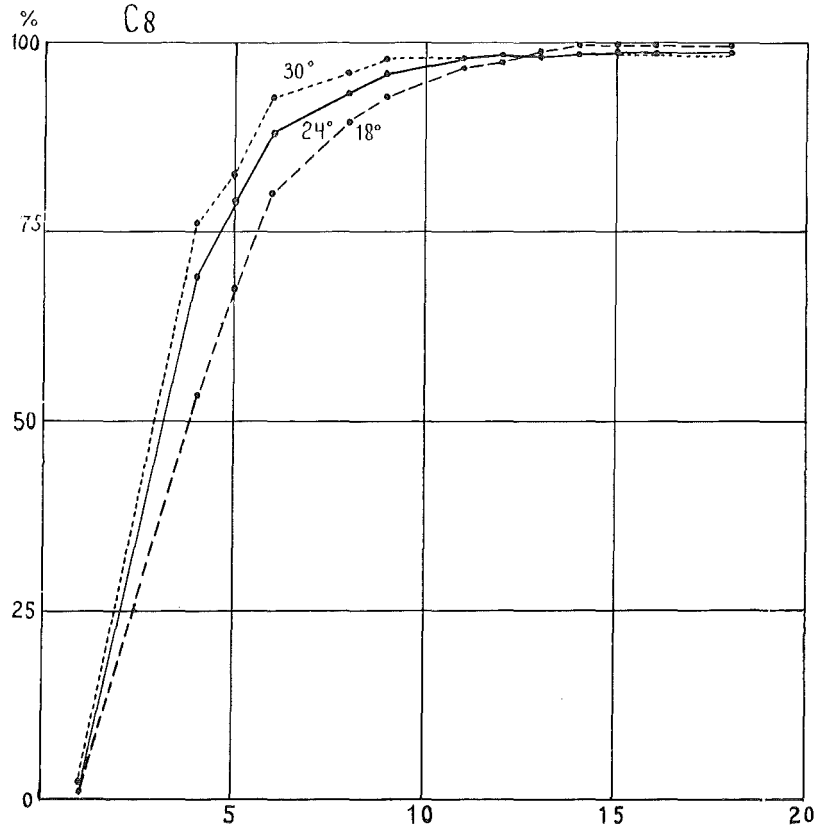

Fig. 4. as fig. 1; DDT concentration: C_8

In this way we can interpret the mortality curves as giving a description of changes in action of the toxicant.

We will now describe the results of the experiments and after that see whether the assumptions made here can be maintained in the light of the results.

a. Experiments in which exposure temperature and reaction temperature were the same.

In fig. 1, 2, 3 and 4 the graphs are arranged for comparison of the influence of the temperature on mortality at certain concentrations of DDT.

From fig. 1, which shows the results at the lowest concentrations of DDT used (C_1), a lower mortality at higher temperature is obvious. We also see that the time during which the mortality continues to increase is shorter at high temperatures.

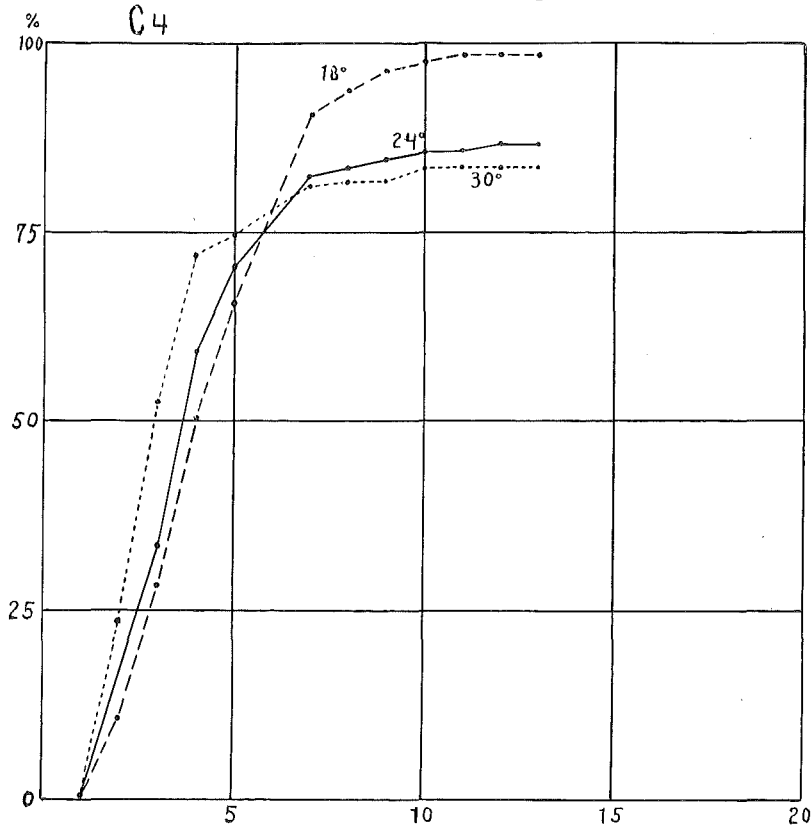

Fig. 5. as fig. 1; DDT concentration: C_4; temp. of exposure 18° C; temp. of reaction 18°, 24° C and 30°.

Fig. 2 shows the results at the next higher concentration (C_2). We see that the rate of mortality increases much more quickly and that this increase is most marked at higher temperature. However the highest temperature still produces the lowest final mortality, as the increase of mortality again stops sooner than at lower temperatures.

In fig. 3 (C_4) the same shift continues, the rate of increase of mortality being highest at 30° C. What happens after the eleventh day is not very clear. The final mortality is again lowest at 30° C and highest at 18° C. The differences are very small, however, and certainly not reliable. The very quick rise in mortality has obliterated the lower final mortality found at lower concentrations.

In fig. 4 (C_8) the same can again be seen, mortality increasing even more quickly than at lower DDT concentrations.

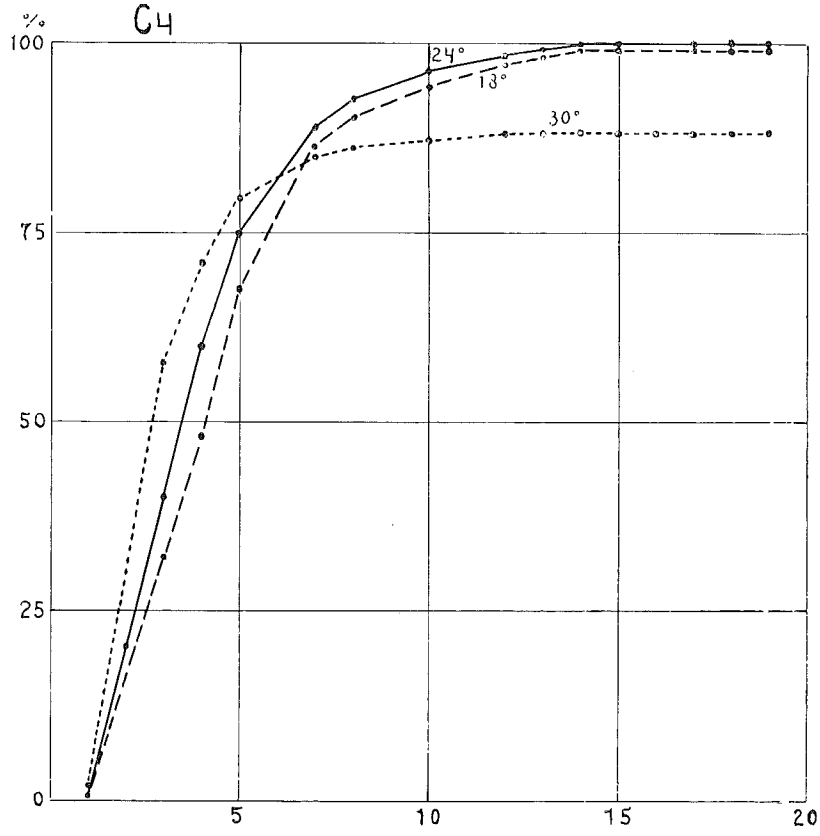

Fig. 6. as fig. 5; DDT concentration: C_4; temp. of exposure 18°, 24° and 30°.C

If we compare the four concentrations at one single temperature, (which means comparing the line of equal temperatures from the 4 figs.), we see that at 18° the lines run very near to each other; at 24° they diverge more widely, while at 30° the "fanning out" of the graphs is most obvious. This is the same as what WOODRUFF (1950) described when studying the effect of nicotine on the Milkweed Bug *(Oncopeltus fasciatus)*. All this fits well into the general picture of DDT poisoning.

We can see that at high temperatures the process of poisoning is much quicker (as shown by the high-concentration curves), but that detoxification is much more effective, when low concentrations are used.

Obviously these experiments cannot tell us anything about other factors involved, such as those mentioned by VINSON & KEARNS (l.c.). It is interesting to compare our graphs with those given by

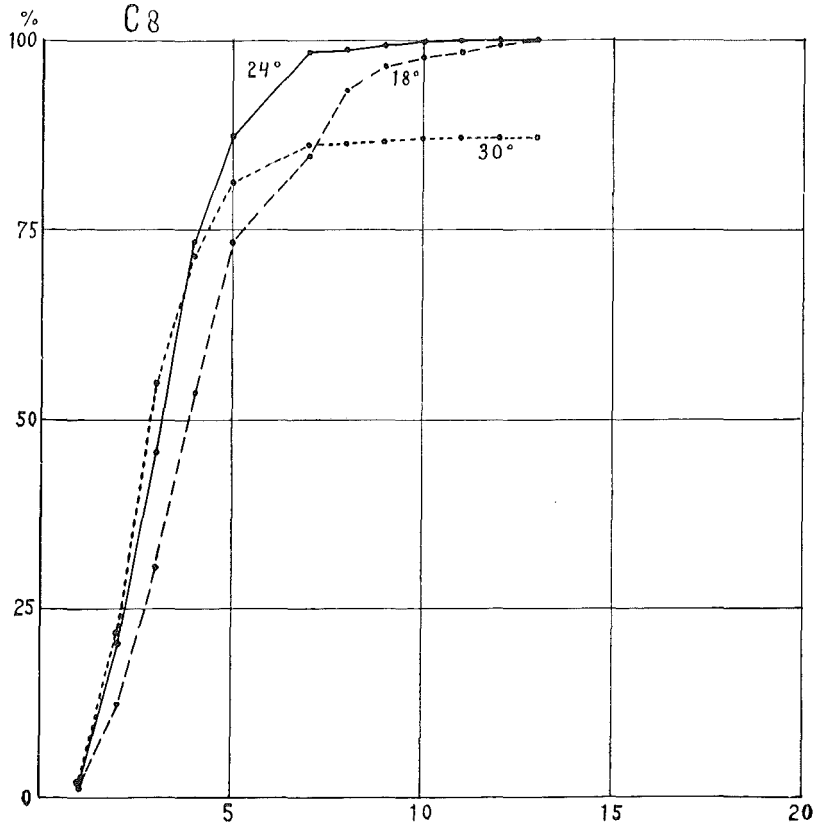

Fig. 7. as fig. 5; DDT concentration C_8; temp. of exposure: 18° C.

ARMSTRONG, BRADBURY & BRITTEN (1952) which show a very slow rate of penetration of DDT into the insect from a stable level of DDT on the outside of the weevils. This stable level was obtained by continous exposure to a DDT-treated surface. It would be interesting to make similar studies in weevils which had been exposed for a short time and to compare penetration and mortality data in the same experiment.

b. *Experiments in which there was a difference between exposure temperature and reaction temperature.*

While in the previous experiments the same temperature effected both the uptake of DDT and the reaction of the weevils to the DDT, in these experiments the two temperatures were different. Batches of weevils were either exposed at the same temperature but kept at different

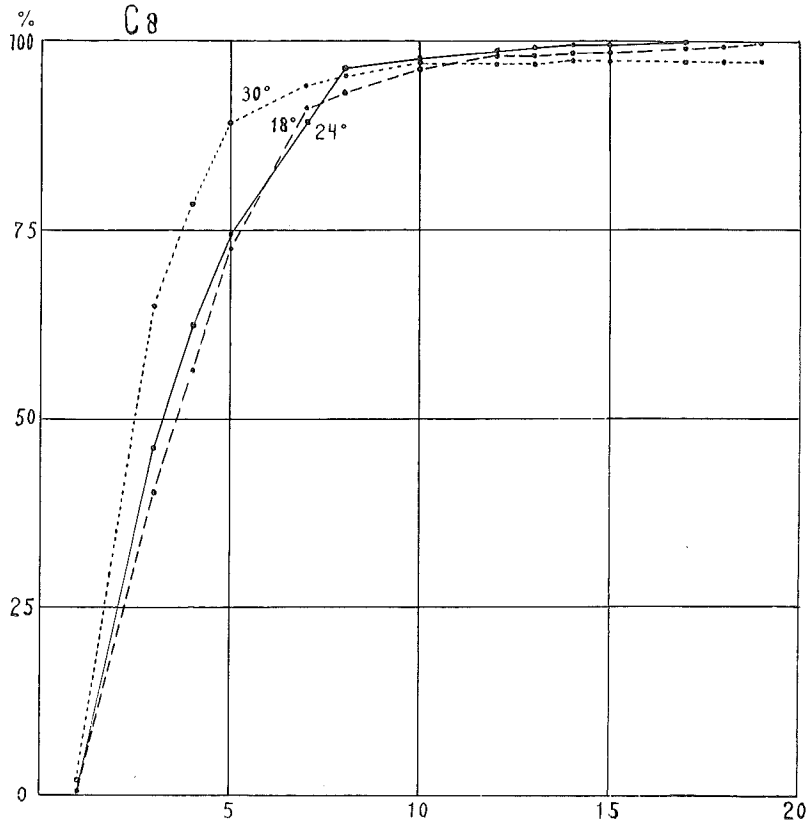

Fig. 8. as fig. 7; temp. of exposure: 30° C.

temperatures to observe the reaction, or the other way round. Exposure at 18° C. Concentration C_4 (fig. 5).

We see here the same difference between reaction temperatures as before (a) at the lower concentrations: at high temperatures the mortality develops more quickly, but the maximum is reached sooner than, and is not as high as, at lower temperatures.

At C_4 and exposure temp. 30° C (fig. 6) the amount of DDT taken up is evidently increased, no doubt mainly through the higher activity of the weevils at that temperature, but perhaps also because a greater amount can be taken up by the epicuticle at higher temperatures, resulting in all mortalities being higher. The rate of increase is not seen to be faster, the lines in fig. 5 and fig. 6 running parallel over the entire first straight part.

At the higher concentrations (C_8, fig. 7, 8) we see the same,

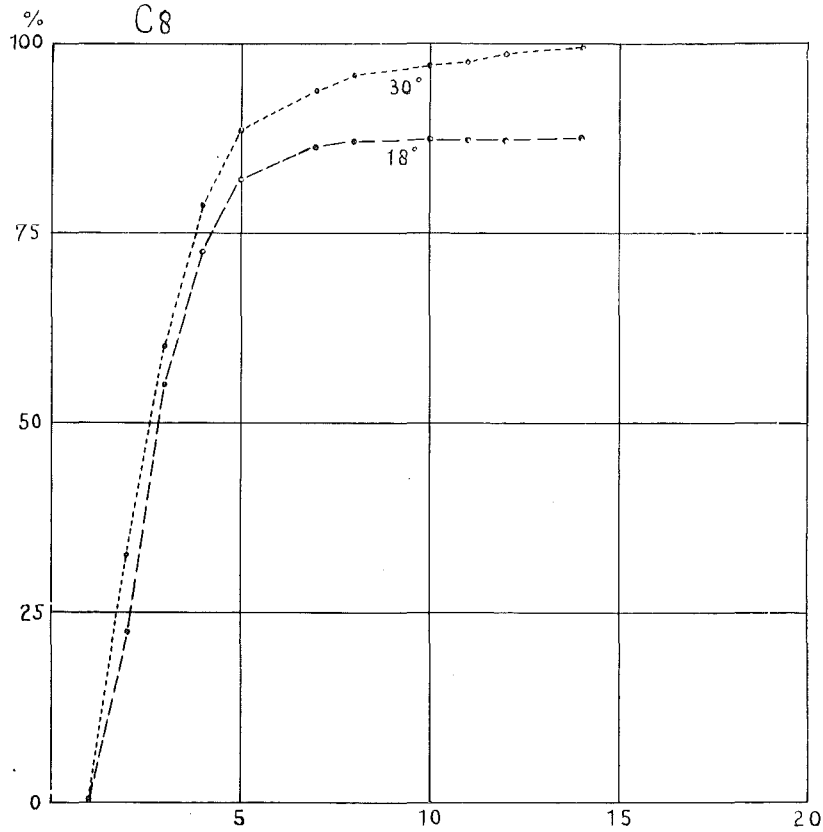

Fig. 9. DDT concentration C_8; temp. of exposure 18° and 30° C, temp. of reaction 30° C.

though the differences are less clear, the high mortality in all cases obscuring them. One thing, however, is quite obvious. At this higher concentration, we again find the shift already noticed when comparing fig. 1, 2 and 3, namely that at the higher concentration the 24° line runs higher than the 18° and high concentration plus high exposure temperature (fig. 8) combine to increase this effect so that the 30° line runs highest.

Here again, then, we find that as the concentration increases, the temperature must also increase if the animals are to survive.

By combining graphs from different figures we can also compare the effect of different exposure temperatures in the reaction at identical temperatures. Applying this to one case (fig. 9), we see that high exposure temperature results in high mortality. This is indeed what we could expect. We have refrained from giving any further figures as the results are not interesting. Only in two cases the results are different from expectation and we then see that the

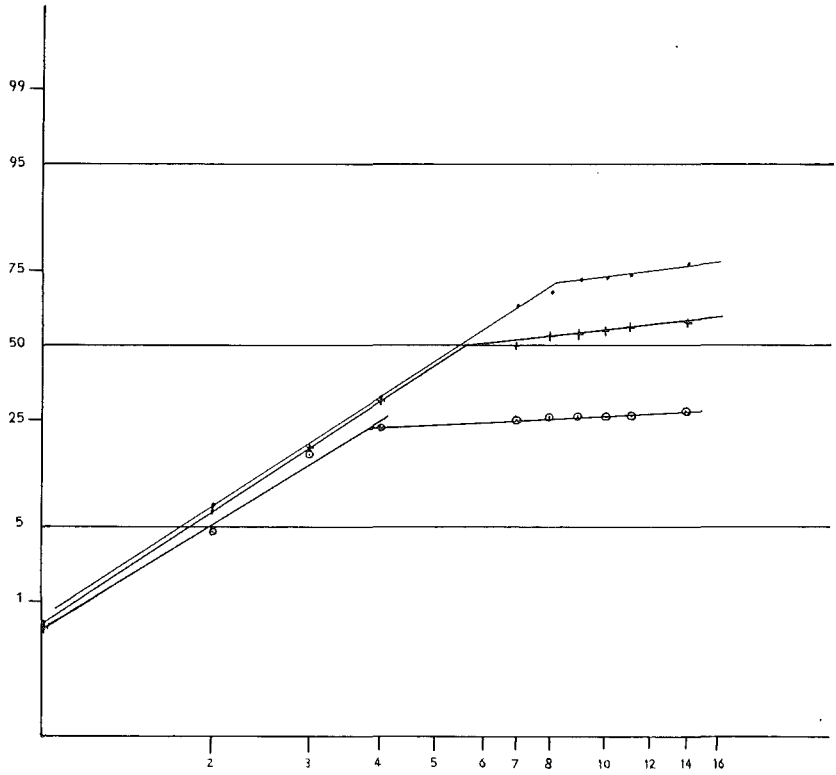

Fig. 10. Abscis: time in days, log. scale; ordinate: percentage mortality, probit-scale. Data as fig. 1. . . . = 18°, +++ = 24°, ooo = 30°.

weevils exposed at 18° C show a higher mortality than those exposed at 30° C. We have no explanation to offer for this result.

It now remains to test the validity of the assumptions made on p. 318 namely that these graphs are derived from normal distribution curves and that divergences are due to change in action of DDT. To test this assumption it is convenient to transform the data to a log-probit graph. This has been done for some experiments in fig. 10—13.

If we do this we find that in some cases a straight line can be fitted to the data without difficulty. In others, however, this is not the case and we find two groups of points, to each of which a straight line can be fitted.

Now it is important that one straight line can be fitted in the cases where we can expect continuous action of DDT, namely at high concentrations and low temperatures (fig. 11: t 18 and t 24; fig. 12: t 18; fig. 13: t 18 and t 24).

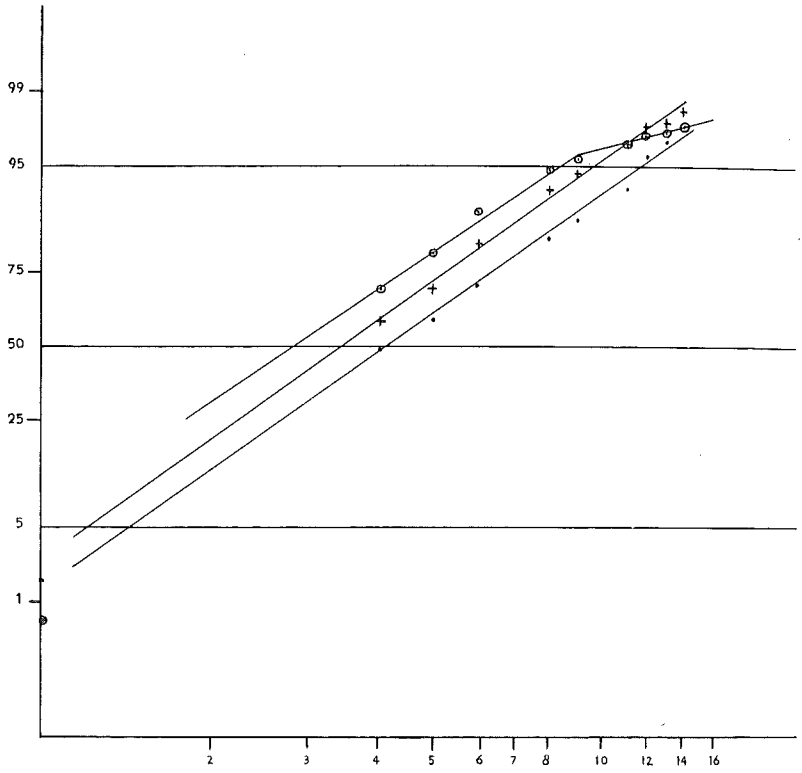

Fig. 11. as fig. 10; data as fig. 3. ... = 18°, +++ = 24°, ooo = 30°.

In the other cases, where the temperatures of reaction were higher or the concentration lower or both, we find two intersecting lines. The point of intersection gives us the time where the action of the DDT has come to an end, that is to say, where the toxicant, being gradually released into the insect from the store picked up during exposure, has been totally detoxified.

These transformed graphs give the impression that the endpoint of DDT-action is fixed exactly. This obviously is not the case, and only due to the fact that we try to fit *straight* lines to the plotted values. In some cases this seems a very good approximation but in others a curved line would seem to be more appropriate, as in fig. 13, 30° C etc. That would mean that the rate of absorption of DDT into the animal decreases gradually, as the store picked up at the beginning of the experiment is depleted.

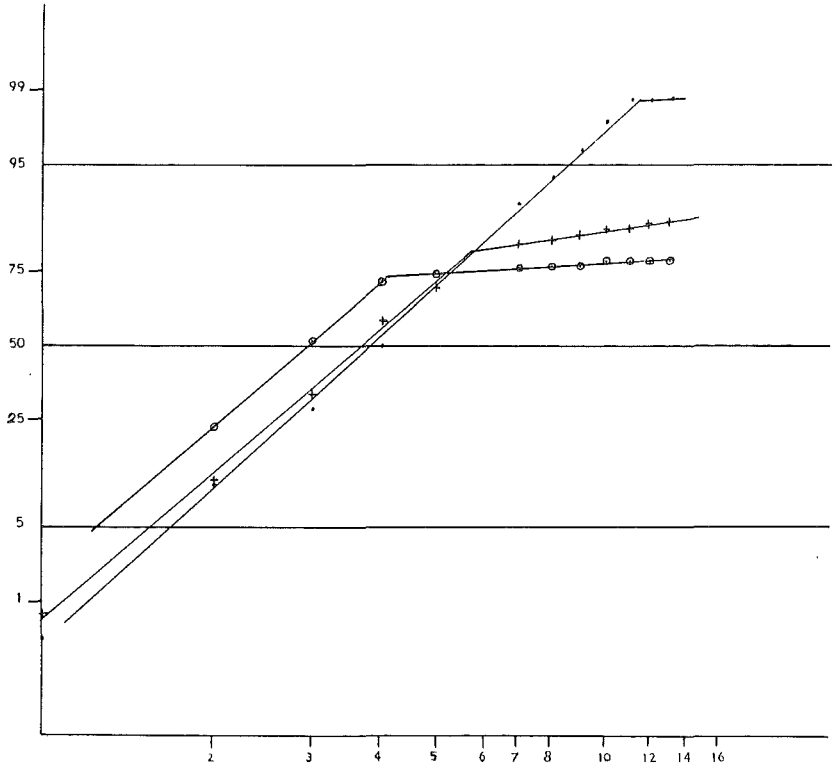

Fig. 12. as fig. 10; data as fig. 5. . . . = 18°, +++ = 24°, ooo = 30°

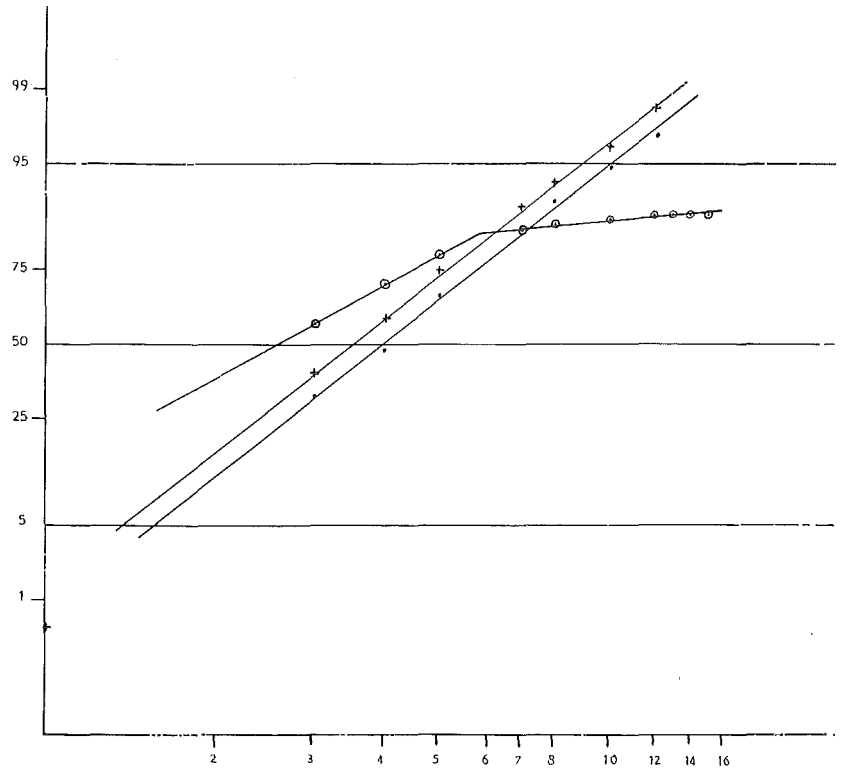

Fig. 13. as fig. 10; data as fig. 6. . . . = 18°, +++ = 24°, ooo = 30°

IV. CONCLUSION AND SUMMARY

The experiments with *Calandra granaria* described in this paper lead us to the following conclusions:

1. At high temperatures the symptoms of DDT-poisoning develop quicker than at low temperatures.
2. If the animals finally die, death occurs sooner at higher temperatures than at lower temperatures; if they recover the recovery occurs first at the highest temperatures.
3. At high temperatures more DDT is taken up by the insects than at lower temperatures. This is probably at least partly due to their greater activity at high temperatures.
4. As in most other studies on this subject we also found that with low doses of DDT more animals survive at high temperatures than at low.
5. The higher the dose, the higher must be the temperature if the animal is to survive.

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