content decreases in close proportion (*2*), and the life-span of the leaf is markedly reduced. Detached leaves are capable of incorporating labeled nitrogen and carbon into their protein (*3, 4*). Thus, their ability to synthesize protein is not altogether lost. However, they seem to have largely lost the ability to synthesize certain amino acids (*4*), and the ratio between protein synthesis and breakdown is greatly shifted in favor of the latter. Although this characteristic pattern of protein metabolism in detached leaves has led to extensive experimentation, attempts to modify it experimentally have not been successful.

In an attempt to control experimentally the survival and protein balance in detached leaves, we studied the effect of some plant regulators on these processes (*5*). Auxin (indole-3-acetic acid) sometimes reduced protein loss, but the effect was slight and erratic. Kinetin (*6*), in contrast, reduced protein loss in a consistent and striking manner.

The opposite primary leaves of young, vegetative *Xanthium pennsylvanicum* (cocklebur) plants were used in all experiments. Leaves that had reached full expansion or were quite close to reaching it were cut off and inserted with the petioles either into aqueous solutions of kinetin or into water. They were kept in bright, diffuse daylight and in a nearly water-saturated atmosphere (in glass-covered enamel trays) at a temperature of 22° to 25°C. The cuts were renewed every other day; the solutions, every fifth day.

Figure 1 shows the condition of the leaves at the end of an experiment. The leaves that were kept on water lost most of their chlorophyll, but those supplied with kinetin retained their green color.

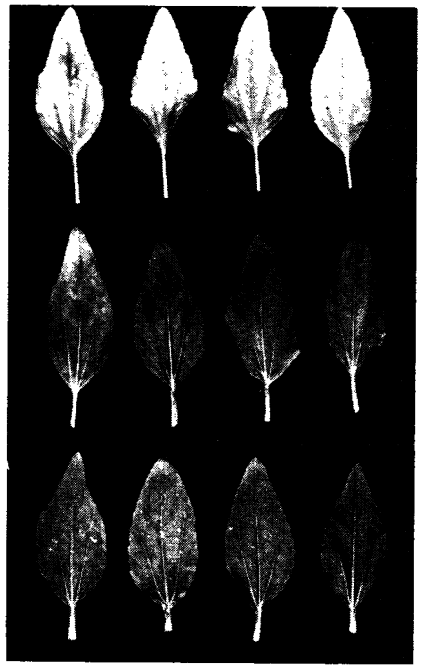

Fig. 1. Condition of detached *Xanthium* leaves after 10 days' culture on (from top to bottom) water, 1 mg of kinetin per liter, and 5 mg of kinetin per liter.

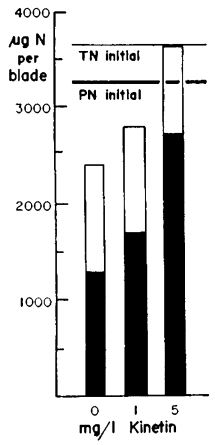


Fig. 2. Protein nitrogen (PN) and total nitrogen (TN) in detached *Xanthium* leaves (blades) after 12 days' culture on water and kinetin solutions. The total columns represent total nitrogen; the solid parts of columns represent protein nitrogen; and the horizontal lines show levels at the start of the experiment.

Figure 2 shows the protein nitrogen and total nitrogen content of the leaf blades after an experimental period of 12 days. The blades of the controls lost 60 percent of their initial protein content. In the blades of leaves that were kept on 5 mg of kinetin per liter, the loss amounted to only 15 percent and was of the same magnitude as it is in attached leaves of comparable age. Leaves kept on 1 mg of kinetin per liter lost 50 percent of their protein. As has been found before (*1*), the protein nitrogen lost by the blades appears as soluble nitrogen in the petioles and the major veins. The amount of soluble nitrogen (measured as difference between total nitrogen and protein nitrogen) in the blades was about the same in controls and treated leaves.

The effect of kinetin on condition and protein content of detached *Xanthium* leaves has so far been somewhat variable. In one experiment, the treated leaves were still fully green after a period of 20 days, while the controls were completely yellow and were dying at the tip and margins. In other experiments, the difference in the survival period was smaller. This variability, which seems to depend on the age of the leaves and on the growing conditions of the plant, should have further investigation. However, there is no doubt that kinetin is capable of reducing or preventing the accelerated protein loss that is typical of detached leaves; at the same time, it delays the loss of chlorophyll and extends the life-span of the leaf. The former effect is very likely the immediate cause of the two latter.

Kinetin was discovered as a regulator of cell division (*6, 7*). However, several authors (*8*) found that kinetin promotes the growth of leaf discs, and this effect was based solely on cell enlargement. In the blade of kinetin-treated, detached *Xanthium* leaves, new cell division cannot be observed either. Cell-division activity thus does not seem to be a premise for the effects of kinetin on the growth of leaf tissue and on its protein metabolism. Whether the last-named effect is an essential feature of the action mechanism of kinetin in growth responses will have to be decided in future work.

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## Temperature-Respiration Curve of Flour Beetles Exposed to Nonoptimal Temperatures

The ability of various species of poikilotherms to adapt to temperatures above or below their normal temperature range is well known (*1*). The adaptation may or may not be accompanied by a change in metabolic regulation (*2*). Bělehrádek (*3*) has shown that temperature coefficients often increase with protoplasmic adaptation to a higher temperature. Respiratory compensation in poikilotherms at subnormal temperatures is evidenced by a higher oxygen consumption, at any given temperature, compared with that of the organism at its normal environmental temperature. In supranormal temperatures, compensatory respiration is depressed (*1*).

The work described here represents a portion of a study made to determine the factors influencing the effects of temperature on adult flour beetles, *Tribolium confusum* Duval (*4*). The insects were taken from a culture kept at 30°C which has been maintained in our laboratory for 10 years. The food medium

Table 1. Respiration curve constants, with 95-percent confidence limits, for *T. confusum* with three different previous temperature histories.

| Previous temperature (°C) | Constant | Females | | Males | |
|---|---|---|---|---|---|
| | | Value | Confidence limits | Value | Confidence limits |
| 30 | log *a* | − 1.856 | ± 0.098 | − 1.968 | ± 0.125 |
| 30 | *b* | 1.490 | ± 0.072 | 1.540 | ± 0.092 |
| 18 | log *a* | − 2.707 | ± 0.204 | − 2.959 | ± 0.199 |
| 18 | *b* | 2.008 | ± 0.174 | 2.124 | ± 0.169 |
| 38 | log *a* | − 2.868 | ± 0.151 | | |
| 38 | *b* | 2.002 | ± 0.128 | | |

was whole wheat flour plus 3 percent of ground wheat germ. A large sample of insects aged at least 2 months from pupal emergence was placed in a constant-temperature cabinet at 18° ± 1.5°C, while a similar sample was placed in a cabinet 37.5° ± 1°C. All cultures were kept at 75-percent relative humidity. The insects were kept at the abnormal temperatures for a number of months, and larvae were continually removed so that the age of the insects was known.

Respiration determinations were made with Barcroft respirometers (*5*) in a constant-temperature bath. Ten insects of one sex were introduced into the manometer cup with small flour-paper strips. They were allowed to recover from the effects of handling for 24 hours prior to use. A series of measurements of oxygen consumption was made, using fresh insects for each experiment, between temperatures of + 5° and + 44°C. Determinations were made for males and females that had been kept at 30° and 18°C and for females that had been kept at 38°C. Five readings for oxygen consumption were made at each temperature.

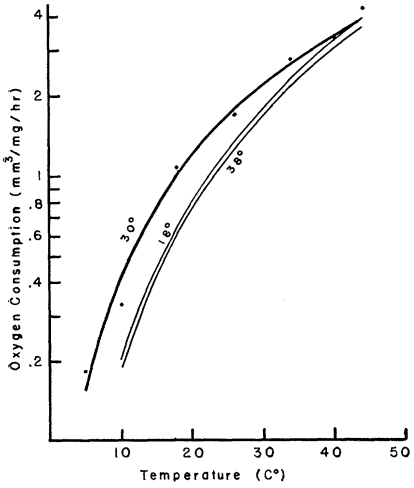


Fig. 1. Semilogarithmic temperature-respiration curves of adult, female, *T. confusum* with three different previous temperature histories. Each point represents the mean of five values for oxygen consumption of 30° insects at each experimental temperature.

The data obtained were found to fit a straight line when the logarithms of both variables were plotted. The regression line was fitted by the method of least squares and tested for goodness of fit by an analysis of variance and an *F* test. The lines of best fit for females have been expressed in semilogarithmic form in Fig. 1. The equation that best describes the data obtained between the temperature limits used is

$$y = ax^b$$

or

$$\log y = \log a + b \log x$$

where $y$ is oxygen consumption in cubic millimeters per milligram, per hour and $x$ is the temperature in degrees Centigrade.

The respiration curve for insects from both 18° and 38° temperatures is depressed below the curve for 30° insects. The values of the constants log *a* and *b* for the insects taken from the three experimental temperatures are given in Table 1. For the insects that were kept at the nonoptimal temperatures 18° and 38°C, *a* is significantly lower and *b* is significantly higher than the corresponding values obtained for insects kept at 30°C. However, corresponding constants for the 18° and 38° groups are not significantly different. The inverse relationship between the constants *a* and *b* when the previous temperature history is changed is the result of the curves' approaching a similar value for oxygen consumption at the maximum temperature. The log-log line "rotates" about the maximum value, thus resulting in an inverse relationship between the slope (*b*) and the intersection of the line with the vertical axis (log *a*).

Insects kept at 18°C increased in weight, although they had been originally put at this temperature as fully mature adults. Insects kept at 38°C lost weight. Regression of oxygen consumption on weight was not, however, sufficient to account for the differences in the *T-R* curves.

It seems probable that the apparent similarity between the 18° and 38° groups is superficial, the depression of the over-all metabolic rate of the latter being the result of heat injury, while depression of the 18° curve represents a depression of the metabolic rate without injury to the metabolic systems. This is suggested by the fact that the activity and general behavior of the 38° group appeared slow and poorly coordinated, while that of the 18° insects was normal, and indeed seemed more vigorous when the insects were disturbed.

Samples of insects from the three experimental temperatures were kept at − 3° ± 1°C, relative humidity 75 percent. Two days' exposure was sufficient to kill approximately 90 percent of those that had been acclimatized at 30°, while approximately 1 percent of the 18° insects were killed. All 38° insects were killed in 2 days. Males were found to have a statistically greater survival ability than females at this temperature.

Samples of insects from the three temperatures were kept at 40°C, relative humidity 75 percent. Eighty-four percent of 30° and 82 percent of 18° insects were killed in 6 days, while all 38° insects were killed in 6 days. There was no statistical difference between the survival abilities of females and males.

The curve data show a nonstatistical difference between the constants obtained for males and females of the same temperature group. There is, however, a trend in the direction of a greater oxygen consumption per unit weight in females. This difference, reported previously (*6*), cannot be considered significant.

It is concluded that, under the conditions described in the preceding paragraphs, respiratory adaptation to nonoptimal temperatures does not occur in *Tribolium confusum*, while the ability to survive at low temperatures may be enhanced by low-temperature adaptation (*7*).

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7. A description of further results obtained in connection with this study is in preparation.

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