Reports

Acquired Tolerance of Leaves to Heat

Abstract. When bean, cowpea, cucumber, fig and tobacco leaves were heated 15 to 30 seconds at 50°C, 12 to 48 hours later they tolerated a temperature of 55°C up to three times as long for the same degree of heat injury as did leaves which were not previously heated.

The phenomenon of acquired tolerance to cold has been clearly demonstrated in higher plants (*1*), acquired tolerance to heat has been clearly demonstrated in microorganisms and higher animals (*2*), but acquired tolerance of higher plants to heat has apparently not been reported.

Entire plants, twin leaves, half leaves, or leaf pieces of bean (*Phaseolus vulgaris* L. var. Pinto), cowpea [*Vigna sinensis* (Torner) Savi var. Blackeye], cucumber (*Cucumis sativus* L. var. National Pickling), fig (*Ficus carica* L. var. Calimyrna), and tobacco (*Nicotiana tabacum* L. var. Turkish) were immersed in water for various times at 45° to 55°C, and at various intervals after the first treatment, these plants and appropriate controls were subjected to 55°C for various periods. The heat tolerance of the species differed, being least with bean and greatest with fig. With bean the dosage required for 50-percent injury (50-percent reduction in green weight) was about 400 sec at 45°C, 80 sec at 50°C, and 16 sec at 55°C though these values varied with time of day, age of plants, and undetermined causes. Of these three temperatures, the optimum for the development of acquired heat tolerance (AHT) was 50°C, and the optimum time period for a single treatment was about 25 sec. Heat tolerance was tested by immersing the treated and nontreated leaves in water at 55°C for 5 to 30 sec. Injury was recorded after 2 days on a decimal scale of 0 (no injury) to 10 (death); it was shown that this rating of injury was well correlated with injury as based on green weight. The dosage required for 50-percent injury (ED_{50}) was observed directly or by interpolation on a standard curve relating heat dosage to injury. The ED_{50} for the treated leaves divided by the ED_{50} for the control leaves is the index of acquired heat tolerance (IAHT). Values less than 1 would indicate cumulative injury, values of 1 would indicate no effect, and values greater than 1 would indicate acquired heat tolerance.

In a typical twin leaf comparison, one primary unifoliate leaf of a pinto bean plant 10 days from seeding was heated 25 sec at 50°C at 5 P.M. on 11 May. At 7 A.M. on 13 May this leaf and the twin leaf control were heated 12 sec at 55°C. Two days later the control leaf was almost dead (injury rating, 9), and the treated leaf showed only slight injury (injury rating, 1). The dosage response curve for injury to control leaves follows approximately the relation

$$I = 21.7 \log x - 22$$

where I is the injury and x is the number of seconds of heat dosage at 55°C. Thus, in the above comparison the ED_{50} for the control leaf was 8.5 sec, and the ED_{50} for the treated leaf was 18.5 sec. The index of acquired heat tolerance was therefore $18.5/8.5 = 2.2$. A similar response to multiple treatments is illustrated in Fig. 1. The data and conclusions of this study are based on 370 such paired comparisons.

Acquired heat tolerance from a single treatment of 25 sec at 50°C was barely apparent when the injury treatment followed the treatment by 10 min (that is, the index was slightly but significantly greater than 1), was greatest at about 24 hours (the index ranged from 1 to 3.5), and lasted until at least 72 hours after treatment. Thus the AHT effect lasted more than 10^4 times as long as the treatment.

Acquired heat tolerance was demonstrated with bean, cowpea, cucumber, fig, and tobacco. Only with bean was acquired heat tolerance demonstrated with entire plants. The other species were tested only as leaves. With primary leaves of bean and cowpea and with cucumber cotyledons, acquired heat tolerance was demonstrated with twin leaves. With secondary leaves of cucumber, fig, and tobacco, acquired heat tolerance was demonstrated with half leaves and even smaller parts of leaves. The greatest degree of acquired heat tolerance ($IAHT = 3.1$) from single treatments so far has been with fig.

With bean and cowpea, acquired heat tolerance has been demonstrated with as many as 15 successive heat treatments of the same specimens over periods as long as 4 days, but for the dosages so far studied, successive treatments after the first one have raised the index of acquired heat tolerance only slightly.

Only two trials were made with plant pathogens, and single AHT treatments have not yet been tested with them. Leaves inoculated with bean rust (the uredinial stage of *Uromyces phaseoli* in living beans) were treated 10 sec at 45°C at 109, 119, 134, 144, 156, 167, and 181 hours after inoculation, and

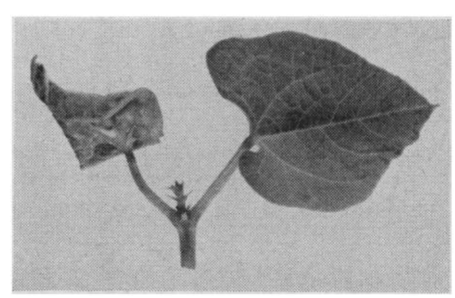

Fig. 1. Acquired heat tolerance in bean. The right leaf was treated for 20 sec at 50°C at 6 P.M. on 28 April, 7 A.M. on 29 April, 7 A.M. on 30 April, 6 A.M. on 1 May, and 8 P.M. on 1 May. On 2 May both leaves were heated for 15 sec at 55°C at 8 A.M. Photographed 10 A.M. on 4 May. The right leaf shows acquired heat tolerance and the left leaf (killed) shows severe heat injury.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

these leaves and control leaves were treated for 4, 6, 8, 10, or 12 sec at 50°C at 205 hours after inoculation. The ED_{50} for the treated rust was 8 sec at 50°C, and for the control rust it was 5.5 sec at 50°C, as indicated by continuation of mycelial growth and spore production.

It is believed that acquired heat tolerance may be an important factor in the ecological heat tolerance of plants and their pathogens (*3*).

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References and Notes

1. O. F. Curtis and D. G. Clark, *Plant Physiology* (McGraw-Hill, New York, 1950).
2. C. L. Prosser, Ed., *Physiological Adaptation* (American Physiological Society, Washington, D.C., 1958).
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29 May 1961

Thermal Reinforcement and Thermoregulatory Behavior in the Goldfish, Carassius auratus

Abstract. Goldfish in a warm environment can cause a small drop in the temperature of their environment by pressing a lever. The fish regulate the temperature of their environment, keeping the temperature between 33.5° and 36.5°C most of the time.

The rate of activity and metabolism of poikilotherms is largely determined by the temperature of their environment. Yet thermal adaptation in these animals tends to reduce the effects of temperature and poikilotherms can also change their body temperature by moving from one environment to another.

The process of temperature selection has been investigated in a number of poikilotherms, including the goldfish. Fry (*1*) has found that goldfish, when placed in water containing a temperature gradient, spend most of their time in water within a certain temperature range. This finding suggests that temperature might be used to reinforce learning in these fish. If a goldfish is placed at a temperature that is considerably different from its preferred temperature, will it perform some arbitrary response in order to bring the temperature of its environment closer to its preferred temperature? Furthermore, if temperature change can be used as a reinforcement, will the fish regulate its body temperature by regulating the temperature of its environment? Weiss and Laties (*2*) have shown that the albino rat, when placed in a cold environment, will press a lever for heat reinforcement. No similar experiment has been performed with a poikilotherm. In the experiment presented here, it is demonstrated that goldfish will work to produce certain temperature changes in their environment, and that, when given the opportunity to control their body temperature, they will do so to a certain extent.

The experimental apparatus is shown in Fig. 1. A small goldfish (3 to 8 g) was placed in a 1-pint container of water. This container rested in a constant-temperature water bath. During training the bath was initially at a temperature of 24.5° ± 0.5°C. The home container of the fish was kept at 23° ± 1°C. The fish was given 10 minutes to adapt to the experimental container, and then the temperature of the water bath was gradually raised to 41°C over a period of about ½ hour. The lethal temperature for these goldfish is approximately 41°C (*3*). When the temperature in the experimental container reached between 30° and 35°C, training was begun. Measured amounts of cold water were introduced into the container at irregular intervals. Each cold reinforcement consisted of a 1-sec flow of cold water (2 to 3 ml) from the distilling tube mounted above the container (see Fig. 1) and produced a transient drop in temperature of approximately 0.3°C. A small light bulb mounted above the container was lighted during the 1-sec reinforcement period. Each fish received approximately 50 reinforcements in each of two training sessions.

In the third session, the lever was placed in its appropriate position, and the lever target was located behind the hole in a Plexiglas lever guard (Fig. 1). In order to actuate the lever, the fish had to insert its head through the hole and push at the target. The lever guard prevented chance operation of the lever by the swimming movements of the fish. When the temperature rose to above 30°C, training for lever pressing was begun. The method of "successive approximations" was employed (*4*). In this method, the reinforcement is first given whenever the animal is near the lever, then when the animal touches the lever, and finally only when the animal presses the lever. Most fish learned to press the lever within 2 hours after the onset of training. Seven small goldfish were trained.

The fish were then placed in a "titration" situation. The temperature of the water bath gradually rose and leveled off at 41°C. By pressing the lever for squirts of cold water, the fish could lower the temperature in its container. The temperature was maintained at 41°C for the entire session, once it had reached this level. Thus, a constant temperature stress was provided for the fish.

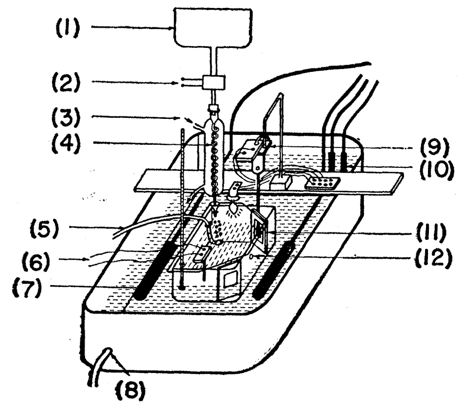

Fig. 1. Device for the study of regulatory behavior in the goldfish. 1, Water supply; 2, electric valve; 3, cold water; 4, distilling tube; 5, air line; 6, wires from thermistor; 7, heater; 8, "constant level" hole; 9, lever assembly; 10, thermostats; 11, lever guard; 12, "constant level" hole.

Two procedures were employed in experimental sessions. In the first procedure, the temperature of the experimental container was raised to 38°C before the fish was permitted access to the lever; the fish was then given access to the lever for 2 hours. The lever-pressing responses and temperature in the container were recorded continuously

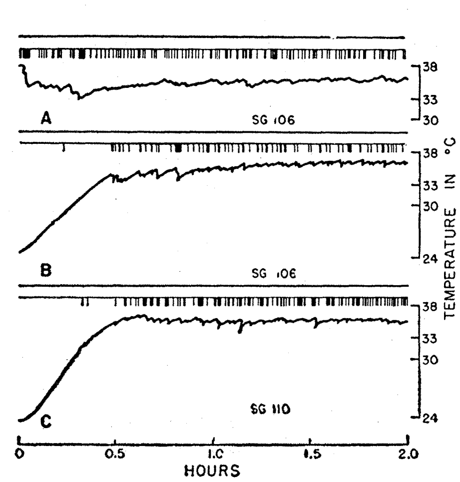

Fig. 2. Typical records of lever-pressing responses of goldfish and temperature in the container. *A*, Fish drives down environmental temperature. *B* and *C*, Fish prevent the temperature from rising above 35° to 36°C.