Suppression of Embryogenesis and Hatching in Meloidogyne javanica by Thermal Stress

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Abstract: Embryogenesis and hatching of eggs of *Meloidogyne javanica* were suppressed by brief heat treatment (46 C for 10 min). The period of suppression or arrested development differs according to the stage of development of the nematode when heat treatment is applied. The effect on hatching is much more pronounced than on embryogenesis. *Key Words:* root-knot nematode, biology, gastrula, temperature.

Recently, Steele (5), in his studies of the effects of hot water treatments on the survival of *Heterodera schachtii,* reported a delayed emergence of second-stage larvae (L_2) from

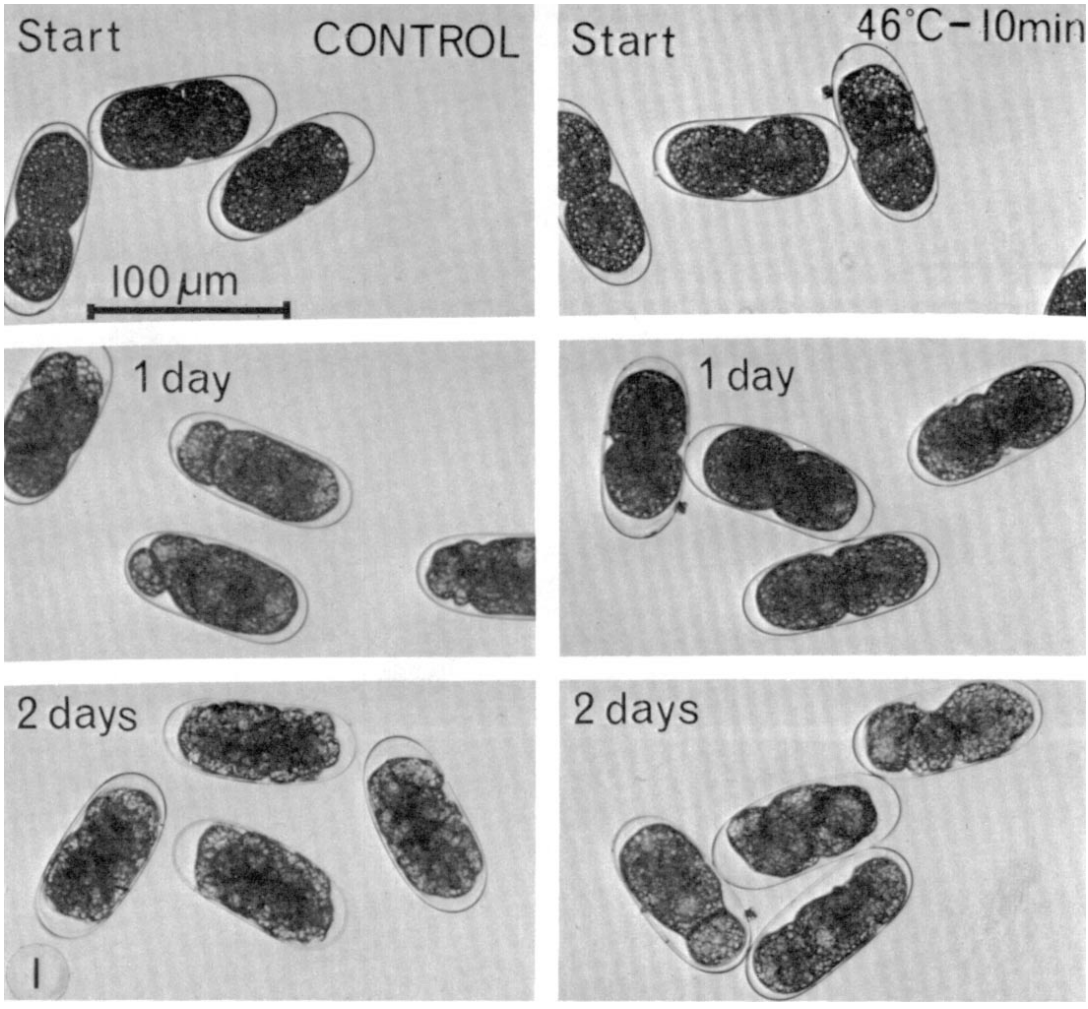

FIG. 1. The effect of heat stress on eggs in the two-celled stage of *Meloidogyne javanica* is to retard further development until the second day when cell division leading to normal development commences. The same group of eggs was photographed each time.

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heat-treated cysts. He concluded that this result is due either to a delay in the time taken for root diffusate to reach eggs in the center of the cyst compared with those at its periphery

or that "thermal stress at sub-lethal temperatures may temporarily suppress emergence of larvae which commences after a period of recovery".

In this paper I report the results of experiments on the effect of thermal stress on eggs of *Meloidogyne javanica* which support Steele's (5) second hypothesis.

MATERIALS AND METHODS

Egg masses of *M. javanica* were dissected from roots of tomato plants and the eggs freed

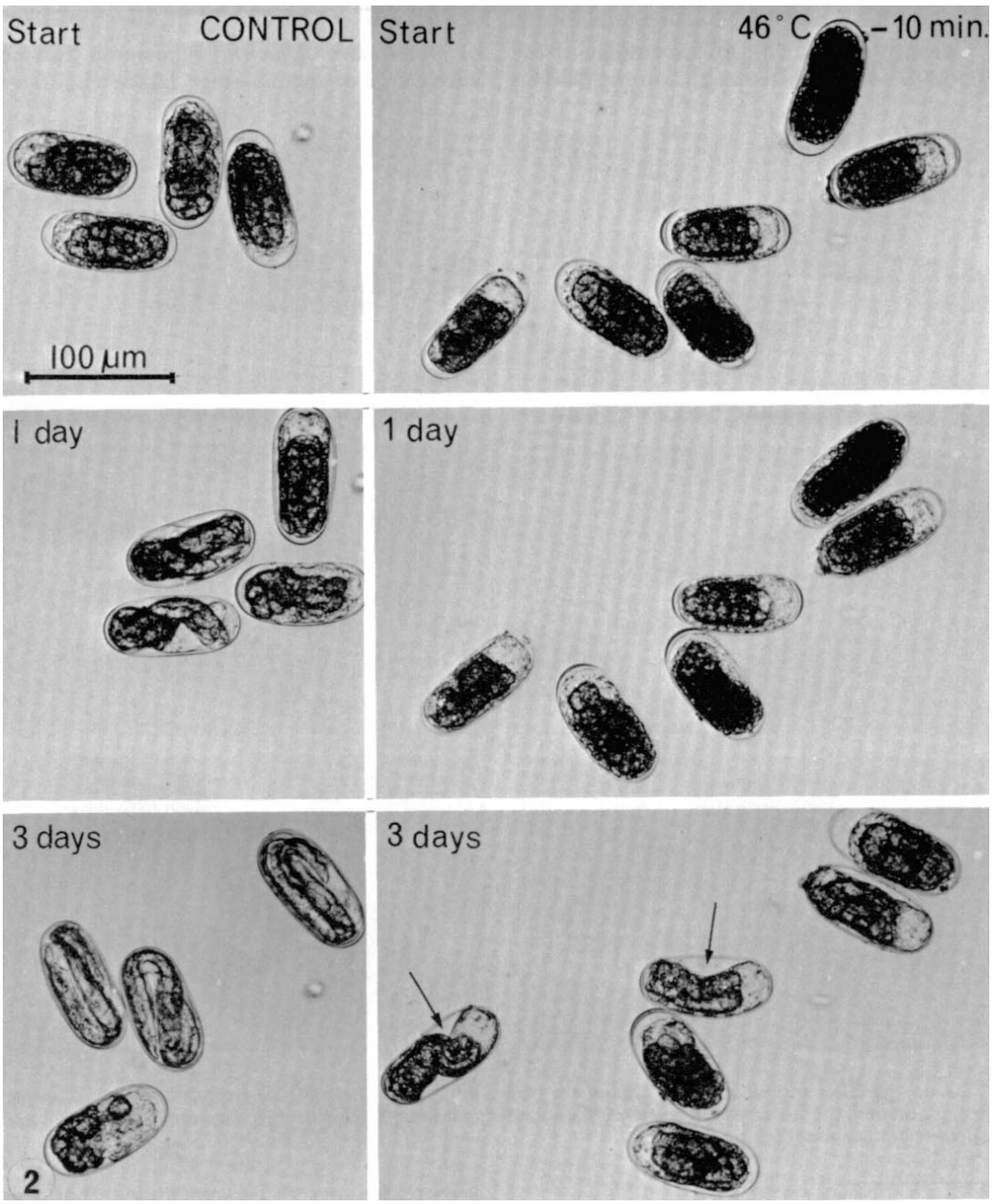

FIG. 2. The effect of heat stress on eggs in the gastrula stage is to retard further development until the third day when the tadpole stage starts to form (see arrows) and normal development continues. The same group of eggs was photographed each time.

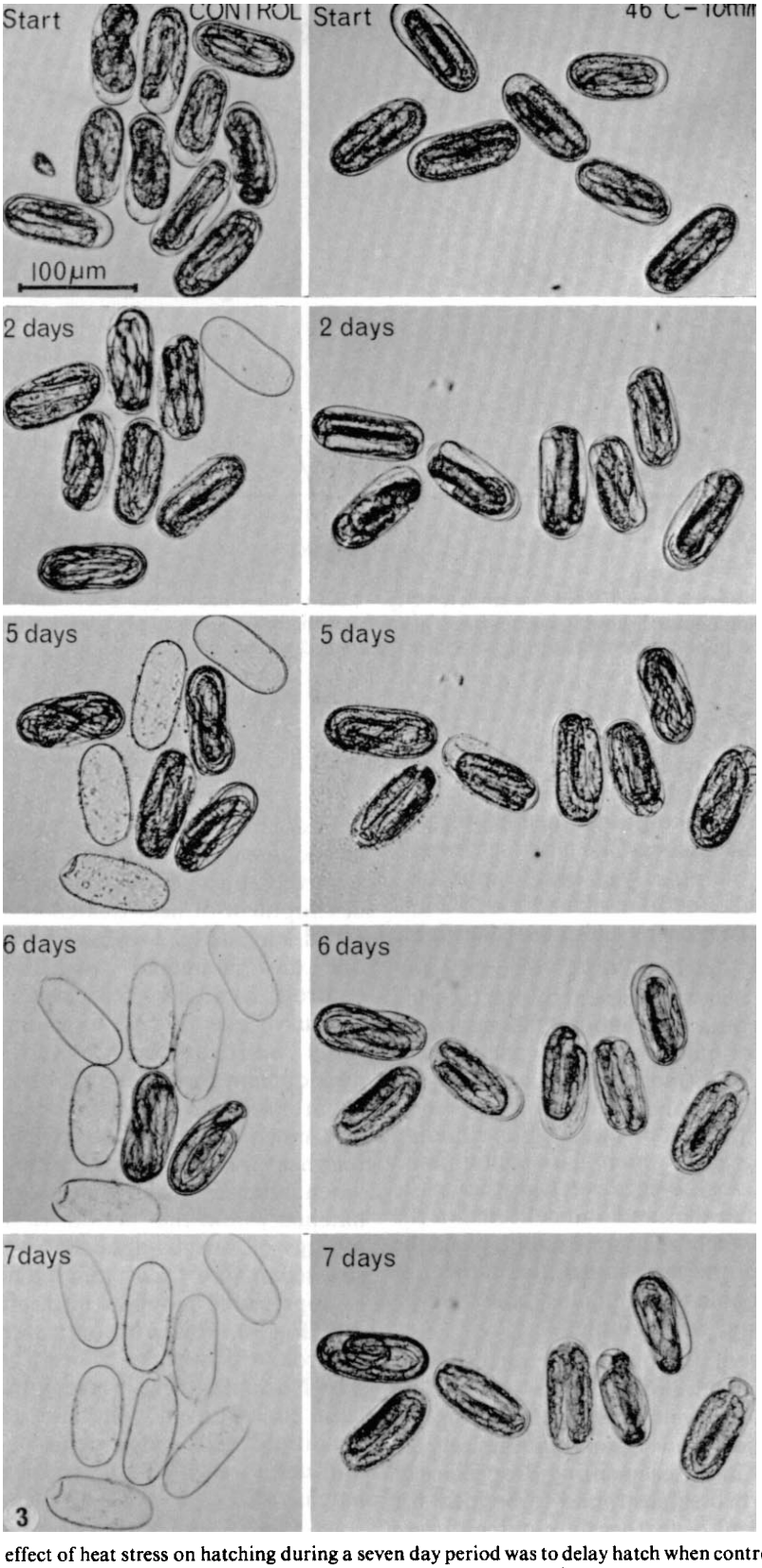

FIG. 3. The effect of heat stress on hatching during a seven day period was to delay hatch when control eggs that had not been stressed hatched. The same group of eggs was photographed each time.

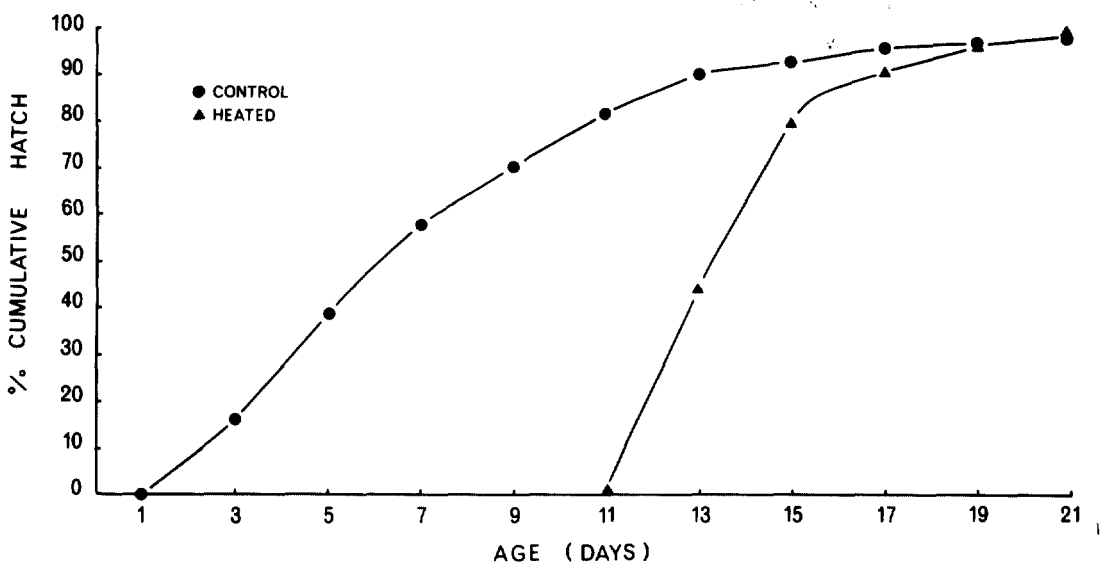

FIG. 4. Graph showing the effect of heat stress (46 C for 10 min) on hatching of eggs of *Meloidogyne javanica*. The eggs (selected at random and showing various stages of development from two-celled to L_2) were maintained at 26.5 C in saturated sand in polythene tubes throughout the experimental period.

from the gelatinous matrix by methods described previously (1). These eggs were suspended by shaking and then segregated into two equal batches. One batch served as a control and the other was heated in a thin-walled test tube for 10 min at 46 ± 0.1 C in a water bath. The test was replicated four times.

The rate of hatch at 26.5 C of control and heat-treated eggs was assessed by pipetting 0.2 ml aliquots containing about 3,000 eggs onto the surface of saturated sand (particle size range, 150-250 μm) contained in 2-cm-long, 5-mm inside diam polythene tubing covered at one end with nylon mesh and standing vertically in a solid watch glass containing 2 ml of water. As the eggs hatched, the larvae moved through the sand and collected in the watch glass, where they were counted. At the completion of the experiment, the number of unhatched eggs in the sand plus the few larvae still in the sand were counted.

The effect of thermal stress on different stages of egg development was studied by removing each developmental stage from the egg suspension and observing the rate of development in water in sitting-drop slides at 26.5 C. The two stages found to be ideal for easy assessment of further development were the two-celled stage and the gastrula stage just prior to the tadpole stage.

RESULTS

Heat treatment in the earlier stages of development retarded egg development for relatively short periods of time only. Thus, the development of heat-treated eggs in the two-celled stage (Fig. 1) was suppressed by only one day compared with the nonheated controls. Similarly, heat-treated eggs in the gastrula stage (Fig. 2) were only about two days behind the controls. Normal development took place after these periods of suppression. However, the effect on hatching was much more pronounced. Fig. 3 shows that heat-treated eggs containing L_2's did not hatch after a week whilst all the controls hatched within that period. In fact, hatching of eggs of various ages that were thermally stressed at 46 C for 10 min did not take place in appreciable numbers until after the tenth day (Fig. 4). This is in contrast to hatching in controls which began to occur after the first day. Normally there is an egg mortality of at least 20-30% even under the most suitable conditions. The batch depicted in Fig. 3 is unusual in this regard; it showed a 100% hatch.

No significant difference ($P<0.05$) was detected between the overall total number of unhatched eggs remaining in the columns of

sand in either treatment at the completion of the experiment.

DISCUSSION

The L_2 in the egg responds to heat stress somewhat differently from the developing egg. This mechanism may have survival value since it delays entry into an environment from which stress has come.

The manner in which normal growth and development are regulated in nematodes is little understood. Situations in which growth is arrested or suspended, further complicate this state of affairs. Arrested development is not uncommon in parasitic nematodes and has been recorded in nematodes parasitic in sheep, plants, and humans (2, 3, 4).

It is possible that a simple technique for inducing short-term arrested development such as I have described may be of use in experimental studies on these survival mechanisms.

LITERATURE CITED

1. BIRD, A. F. 1972. Influence of temperature on embryogenesis in Meloidogyne javanica. J. Nematol. 4:206-213.
2. BLITZ, N. M., and H. C. GIBBS. 1972. Studies on the arrested development of Haemonchus contortus in sheep. I. The induction of arrested development. Int. J. Parasitol. 2:5-12.
3. CROWE, J. H. 1971. Anhydrobiosis: an unsolved problem. Am. Nat. 105:563-573.
4. SCHAD, G. A., A. B. CHOWDHURY, C. G. DEAN, V. K. KOCHAR, T. A. NAWALINSKI, J. THOMAS, and J. A. TONASCIA. 1973. Arrested development in human hookworm infections: an adaptation to a seasonally unfavourable external environment. Science 180:502-504.
5. STEELE, A. E. 1973. The effects of hot water treatments on survival of Heterodera schachtii. J. Nematol. 5:81-84.