Developmental Retardation and Morphological Abnormalities Observed in Moulting Larvae of the Reniform Nematode, *Rotylenchulus reniformis*[1]

During the course of studies on the larval development of a parthenogenetic strain of the reniform nematode, *Rotylenchulus reniformis*, originally collected in Asahi, Chiba Pref., some larvae were frequently delayed in moulting and developing, or failed to complete their moults under some conditions for microscopic observation. These phenomena and experimental conditions are briefly described as follows.

The first evidence was observed in case of ciné-camera photography of the moulting process of the second stage larvae, in which nematodes for examination were put in a drop of deionized water or root diffusate of sweet potato seedlings maintained on the bottom glass slip inside a small cell container. The container consisted of a plastic ring (20 mm in diameter and 15 mm deep), attached to a thin cover slip with high vacuum silicone grease (DOW CORNING®), and covered with another slip. The root diffusate was prepared from 500 ml of deionized water in which two cuttings of sweet potato seedlings, var. Nôrin No. 2, were rooted for ten days. Time-lapse photographs of moulting larvae were taken with a Bolex H16M camera, attached to a Nikon inverted microscope, MD type, illuminated by electric flash with a 30 W tungsten bulb, in a dark room under 23.5–26.5°C at various intervals of 8 sec to 30 min. A Nikon ND 8X filter was placed between the light source and nematodes to cut off infrared light. In these experiments, three nematode larvae, incubated in a drop of deionized water, moved actively for the first 24 hr, then gradually became inactive or motionless, and finally indicated no sign of moulting even after 26 days, moulting is generally recognized by the disappearance of the stylet knobs (Nakasono, 1964). Another group of five nematodes, put with root diffusate, moved

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vigorously for the first two days and then progressively became less active on the third day, at which time the stylet knobs of three larvae had disappeared. Only one of these three larvae continued the moulting process but did not complete the third moult during the next six days. The other two remained unchanged for nine days.

Time-lapse photography in the latter experiment was interrupted on the ninth day and the observation cell containing the larvae was maintained under the same conditions, except that it was not exposed to the flash light. After 50 days, all five nematodes in the cell completed moulting and developed into young females. This suggests that the nematodes in the cell had undergone three moults under the dark condition, where no flash light was applied after the interruption of photographing, although how long a period was needed for the completion of moults is not known. It may be added that nematodes avoided light in the medium throughout the experiments.

It has been reported that the second stage larvae of this nematode start with the second moult within three to six days after hatching and terminate their final (the fourth) moult in 9–13 days in tap or distilled water without feeding at 25°C and become young females in 13 to 16 days (Nakasono, 1964, 1966). The facts that no initiation of moulting took place during the period of 26 days, and that the time-lapse of more than five days in the second moult as well as four days in the third stage development were observed are considered to be uncommon for this nematode.

Another is the morphological abnormality in the moulting larvae. This was encountered during other experiments where freshly hatched second stage larvae were separately incubated in a hanging drop of deionized water or root diffusate, as described above, within cavity glass slides under 25°C dark conditions. Morphological changes were followed by photographing and sketching with a 35 mm camera attached to a Nikon S type microscope, and with a camera lucida, illuminated by similar electric light passing through a green glass filter and a infrared cut-off filter at various intervals of 30 min to 24 hr. Ten larvae were incubated in deionized water and eight in root diffusate.

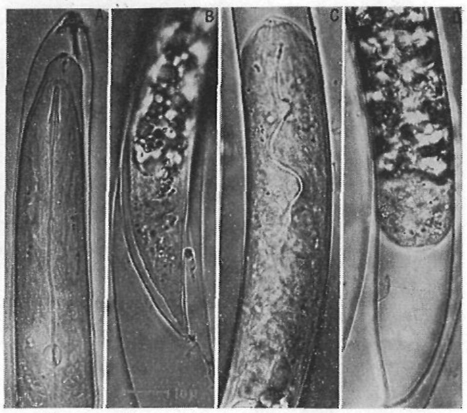

Fig. 1 Photomicrographs of moulting larvae. A: Anterior region of a larva, normally undergoing the fimal moult, and newly reforming the stylet; B: Posterior region of a larva, normally completed the final moult; C and D: Abnormalities in anterior and posterior regions of a larva, failing to reform the stylet and to complete the final moult, being shortened and swollen, with shortened and rounded tail.

In these experiments, about 40% of the larvae examined died due to the failure of normal moults after a great retardation. Two larvae, incubated with root diffusate, showed characteristic abnormalities in reforming of the stylet and in body shape during the final moult and the remaining larvae completed three normal moults to become young females but one female showed an exceptional shape, bluntly rounded in tail like that of spiral nematodes (Fig. 1).

What is the causal factor for the observed retardation and abnormalities on the moulting larvae is not precisely known. Temperature would not be concerned here. From the fact that the electric illumination of the photomicroscope was applied to nematode larvae more frequently and more intensively than in the similar experiments conducted before, illuminating light was presumed as a probable factor having adversely influenced the larvae. Checking the spectral characteristics of glass filters and the energy curves of illuminating lights used here showed that nematodes were exposed to light, including near ultraviolet to a small extent, at an intensity of about 20000 erg $cm^{-2}sec^{-1}$ with lapse times of 8 sec to 30 min in the first case, and to light