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# DESICCATION TOLERANCE IN *ROMANOMERMIS CULICIVORAX*

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The mermithid nematode *Romanomermis culicivorax* Ross *et* Smith has been used successfully for the biological control of mosquitoes (Petersen and Willis 1972, 1976; Brown *et al.*, 1977; Levy and Miller, 1977). To assess the potential use of this species in Pakistan, investigations were made on its response to moisture stress.

## *Materials and Methods*

Sand cultures of *R. culicivorax* were obtained from the Gulf Coast Mosquito Research Laboratory of the U.S. Department of Agriculture, Lake Charles, Louisiana in 1983 and were stored in a refrigerator at about 6°C until required. Individually handpicked nematodes were subjected to moisture stress in four different dehydration treatments. Each experiment was repeated twice.

Twelve watch glasses were filled with coarse sand that had been thoroughly washed with distilled water. In six of them, six male *R. culicivorax* were placed in each while in each of the others, six female nematodes were embedded carefully by making small depressions in the sand and later filling them with moist sand. Then the watch glasses were covered with a large inverted funnel, the opening of which was loosely plugged with cotton wool in order to allow only slow evaporation of the water. Every 24 hr, nematodes in two of the watch glasses — one with males, the other only females — were observed. To assess their survival,

the nematodes were picked up with a needle and immediately transferred to a drop of distilled water on a glass slide placed on the stage of a stereoscopic binocular microscope. The posture of nematodes, the estimated rate of water uptake and their survival were noted.

Two male or two female *R. culicivorax* were each placed in separate drops of distilled water on twelve plain glass slides. They were left uncovered on the laboratory bench and periodically observed under a stereoscopic binocular microscope. Dehydration was reckoned to have started from the time water completely disappeared from around the nematode's body and movement had ceased. Two minutes after this stage was reached, water was poured over the nematode and the rehydration was repeated five times at 2 min intervals.

Nematodes were transferred to a drop of paraffin oil on plain glass slides in the manner described by Saeed and Roessner (1984). Two males or two females were placed in separate drops on the same slide. Twenty four slides were prepared and they were placed in a closed chamber for protection from dust. Observations were made after 1, 2, 4, 6, 8 and 10 days. Each time, four males and four females (2 slides of each sex) were observed. After noting their posture, the paraffin oil was blotted away and the nematodes were rehydrated by pouring water over them. The reactivation of nematodes, if any, was observed and the rate of water uptake recorded. Photographs were taken with a camera attached to an inverted light microscope.

To test the anhydrobiotic capability of nematodes, specimens were immersed in paraffin oil and placed for 96 hr in a chamber with 94-98% RH. The nematodes were then transferred to a desiccator containing phosphorus pentoxide to provide an atmosphere of 0% RH. Observations of the nematodes were made on three rehydrated individuals of each sex at intervals of 4, 8, 12 and 24 hr.

## *Results*

In sand, females survived for 3 days and males for 4 days. After 5 days, when only 3.4% moisture remained in the sand, none of the nematodes could be revived by immersion in water for up to 24 hr and subjecting them to stimuli as described earlier. There was no definite posture of the inactive nematodes but water uptake was immediate. In water drops on glass slides, females survived for only 2 min but males could be reactivated after 8 min. The bodies of both sexes were only partly coiled but anterior-

posterior body shrinking was quite visible. The nematodes also left impressions of their body lipid on the glass slides (Fig. 1A). Frequently only the anterior of the nematode body remained active for some time until the nematodes completely relaxed and died.

In paraffin oil drops, all males but only two females survived for 6 days after cessation of activity. On the eighth day, only two males reactivated and after 10 days neither males nor females could be rescued. The nematodes were shrunken but not coiled in the characteristic posture of anhydrobiotic nematodes. Males tended to coil at their posterior end (Fig. 1B). Anterior-posterior contraction, coupled with transverse and longitudinal folding of the body cuticle, was discernible under the light microscope (Fig. 1C). Upon rehydration, water uptake was immediate and nematodes soon started progressively decoiling (Fig. 1D). When nematodes were treated for longer periods, 2-3 min were required for surviving nematodes to regain turgidity and resume body movements.

In the desiccation chamber, none of the females survived to the first observation at 4 hr and only one male of the 12 subjected to treatment survived for 8 hr. It required only 2 min immersion in water to regain turgidity.

## *Discussion*

Results indicate that males of *R. culicivorax* are slightly less susceptible to water loss stress than females. Males also seem to be more tolerant to other unfavourable conditions. This is evident from the fact that in cultures prepared in June, 1983, viable males were recoverable in the begining of the year 1986 while females had died in early 1984 when this experiment was done. In fact, it was only with considerable effort that we were able to find the required number of females from the sand tray received from the USDA. Although the effect of shipment cannot be ruled out (to which there could be some sex-linked differential susceptibility), there is no doubt that males of this nematode species are better survivors. Longer survival of males of *R. culicivorax* in varying oxygen concentrations has already been reported (Imbriani and Platzer, 1981).

*Romanomermis culicivorax* is not a typical soil-inhabiting nematode. Because its juvenile stage is parasitic on mosquito larvae, its natural habitats are the thoroughly moist places which are the usual breeding sites of mosquitoes. It is therefore understandable that this nematode does not have the inherent property of desiccation tolerance. Normally it is not

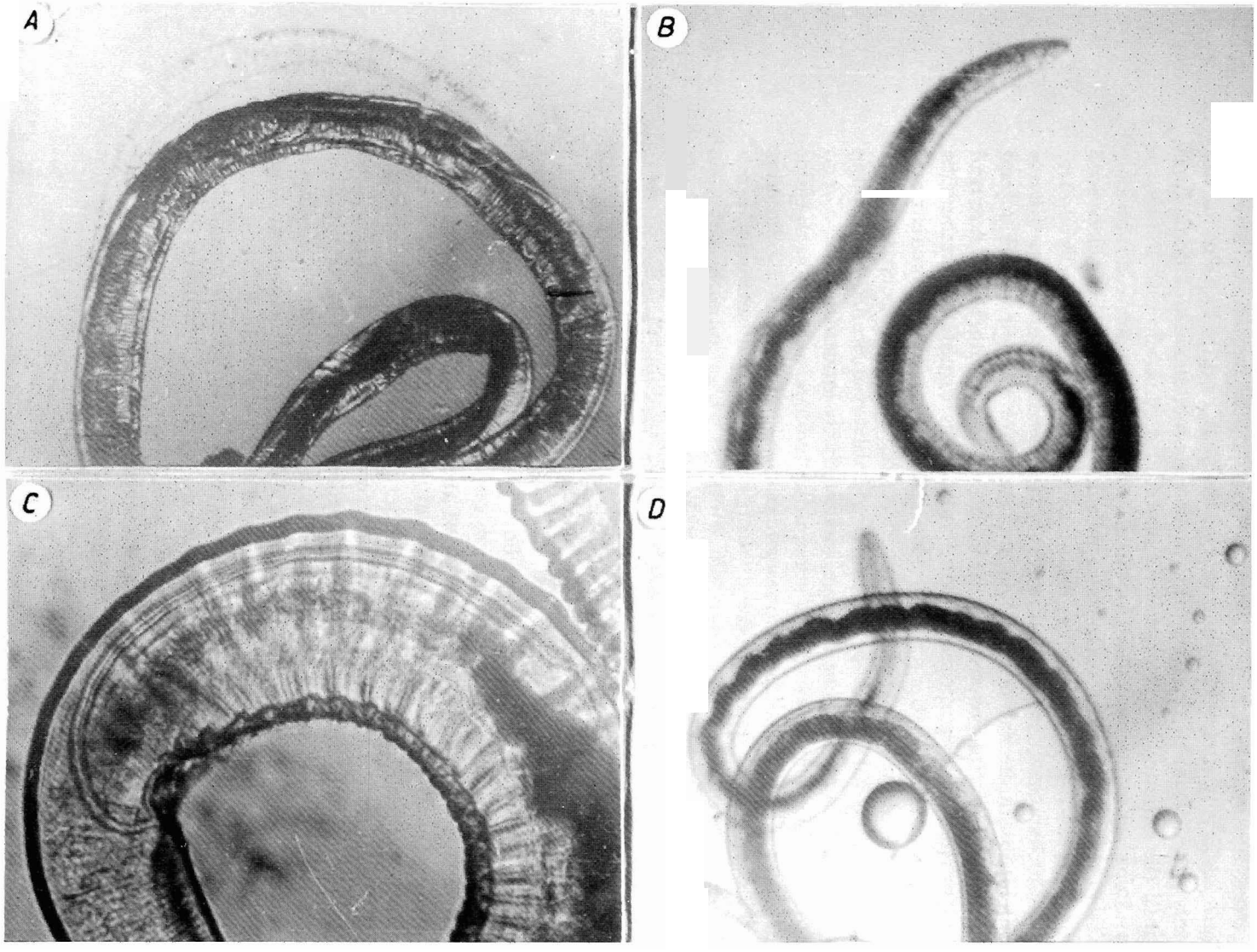


Fig. 1 - *Romanomermis culicivorax*: A, showing impression of body coverings when the nematode was dried on a glass slide; B, dehydrated nematode in paraffin oil; C, male nematode showing transverse and longitudinal folding of body cuticle; D, decoiling of the nematode after rehydration.

exposed to dryness. In the case of plant parasitic nematodes, only those forms which frequently have to remain exposed to adverse environmental conditions are known to survive anhydrobiotically. Among them, generally, the nematodes parasitic on aerial plant parts, or those which dwell in the upper strata of soil are included. Investigations will continue on research that may provide ways of improving the transportation, and possibly preservation of *R. culicivorax*.

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## SUMMARY

Males and females of *Romanomermis culicivorax* were subjected to desiccation in four different ways viz. in sand-filled watch glasses, in water drops on glass slides, slow dehydration in paraffin oil, and in 0% RH atmosphere in a desiccator. Of the two sexes, males showed greater tolerance to water stress than females. Males tended to coil up at their posterior end. Besides anterior-posterior body contraction, transverse and longitudinal folding of body cuticle also occurred. The species cannot be regarded as an anhydrobiotic nematode. In moist sand, males were found to survive for as long as 31 months.

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