

Restoration of Morphological Features of Globodera pallida After Storage in Formalin

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A main criterion in identifying different nematode species is their various morphological features. Used frequently in nematode taxonomy is stylet length. In preserved specimens, however, the tip and knobs of the stylet often become indistinct

with time, preventing accurate measurement of stylet length. Toward solving that problem the effect of adding dyes to material stored in 4% formalin was investigated.

To see whether dyes would restore the clarity of the stylet tip and knobs of preserved specimens, we compared a number of dyes (New Blue R, Meldola Blue, Nile Blue A, Cotton Blue in lactophenol, potassium permanganate, and potassium dichromate) at different concentrations and

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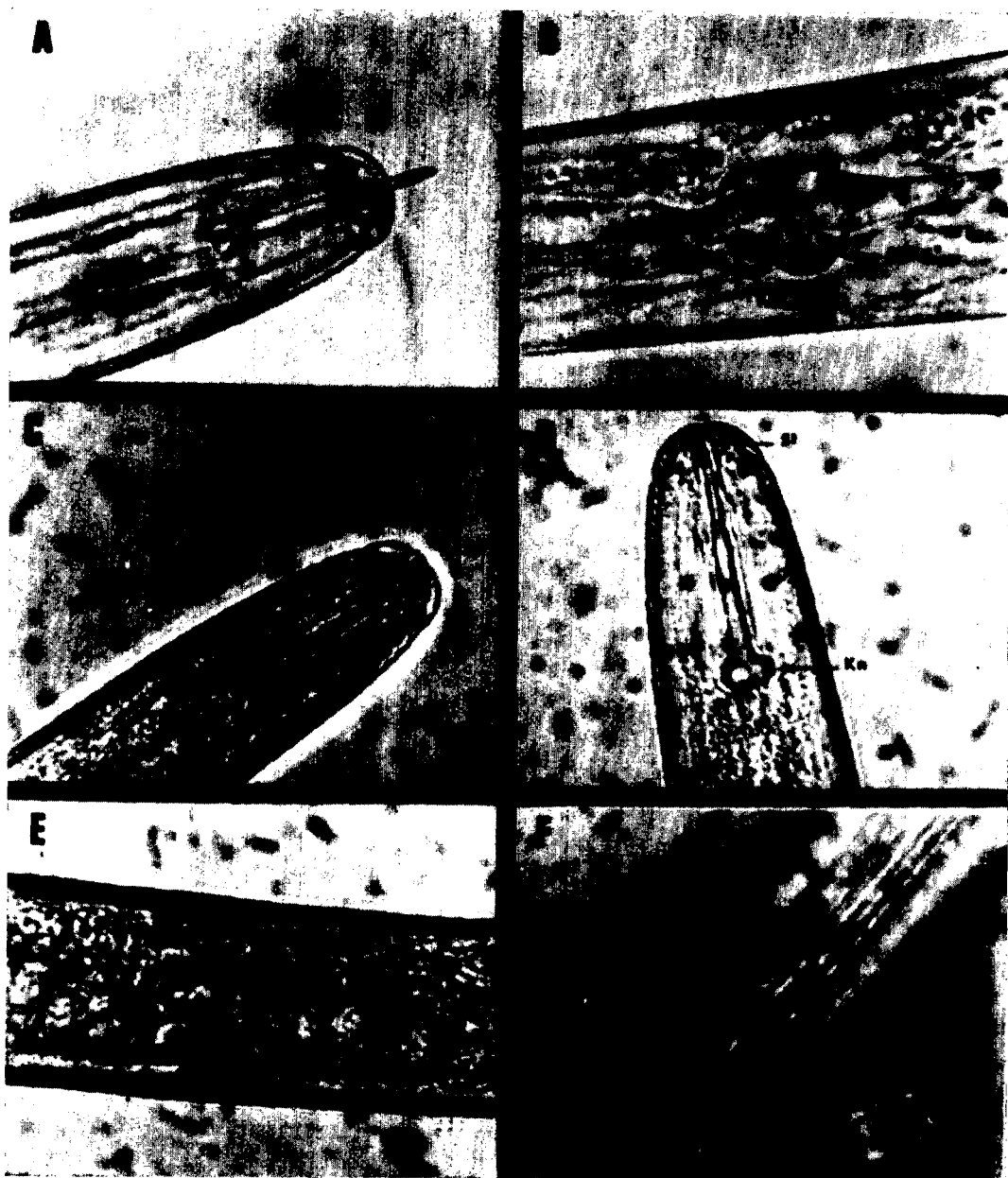


FIG. 1 A-F. Effect of different processing techniques on second-stage juveniles of potato cyst nematode, *Globodera pallida*; A, B) Stylet protrusion and "shrinkage" of median bulb (MB), effects of ammonia treatment on live specimens. C) Poor stylet definition of juveniles stored in 4% formalin for one year. D) Clear definition of stylet tip (St) and knobs (Kn) as a result of potassium permanganate treatment on materials similarly preserved. E, F) Restoration of other morphological features by the same treatment.

temperatures. The dyes were tested on hatched second-stage juveniles of potato cyst nematode (*Globodera pallida*) killed with Stone's techniques (2) to avoid changes in the measurement of morphological features, and stored in 4% formalin for 12-15 months.

The most effective dye, potassium permanganate, was compared with a method developed by Hooper (1) that makes 80% of the stylet tips visible by causing them to protrude.

The dye treatment that improved stylet definition most was addition of a drop (ca.

TABLE 1. Dimensions in microns of second stage juveniles of *G. pallida* after potassium permanganate or ammonia treatments.^a

	Total length ^b	Stylet length ^b	Headtip to metacorporeal valve	Metacorporeal valve to excretory pore	Headtip to excretory pore ^b	Anus to tail tip ^b length
KMnO ₄	436.4	24.0	71.7	35.0	106.7	55.3
NH ₃	444.0	23.8	64.5	42.5	107.0	54.3
SED	5.2	0.2	0.7	0.7	0.9	1.1
LSD 5%			1.4	1.4		
1%			1.9	1.9		

^aAverages for 30 nematodes.

^bAverages for these dimensions do not differ significantly.

35 μ l) of 0.5% aqueous solution of KMnO₄ to 4 ml of nematode suspension and incubation overnight at room temperature (ca. 20 C). This treatment improved cuticular details (Fig. 1F) and definition of the glandular region of the esophagus (Fig. 1E). The visibility of the stylet tip and knobs was also improved (Fig. 1D) over that for untreated juveniles (Fig. 1C).

Hooper's ammonia treatment also increased visibility of the stylet tip (Fig. 1A), but it caused shrinkage of the median bulb (Fig. 1B). The distance between the metacorporeal valve and the head tip or the excretory pore differed between specimens treated with KMnO₄ and those treated with ammonia (Table 1).

These differences seem to be caused by displacement of the median bulb as an ammonia effect in the latest techniques.

Since the distance from headtip to excretory pore did not differ significantly, however, this feature would stand as a morphological character, together with stylet length, to identify populations of fresh-hatched second-stage juveniles of potato cyst nematodes.

The results suggest that both techniques will aid stylet measurement of potato cyst nematodes and that KMnO₄ is useful for restoring specimens that have been stored in formalin for long periods.

LITERATURE CITED

1. HOOPER, D. J. 1977. Spicule and stylet protrusion induced by ammonia solution in some plant and soil nematodes. *Nematologica* 23:126-127.
2. STONE, A. R. 1971. Effect of processing on measurements of *Heterodera rostochiensis* larvae. *Nematologica* 17:167-171.