A Method of Distinguishing Between Living and Dead Nematodes by Enzymatically Induced Fluorescence

ALAN F. BIRD

Since it is sometimes difficult to distinguish between living and dead nematodes, dyes are used, such as New Blue R (7), Chrysoidin (5), Eosin-Y (1), and several fluorochromes (3), with varied success.

A method is described here that is rapid (results in 15 min) and has a mechanism of staining that is understood. The technique was described first by Rotman and Papermaster (6), working with living mammalian cells, and later by Heslop-Harrison and Heslop-Harrison (2), working with plant material. It takes advantage of the presence of esterases which hydrolyse nonfluorescent fatty acid esters of fluorescein to yield fluorescein, which accumulates and is detectable by its fluorescence. Since esterases are known to be present in quantity in nematodes, concentrated principally in the nervous system, male spicules, and gut (4), this technique seemed worth testing as a rapid means of distinguishing between living and dead nematodes.

The nematodes tested were freshly hatched second-stage larvae of *Meloidogyne javanica* and adult females of *Caenorhabditis elegans*.

A stock solution of fluorescein diacetate (FDA) was made up in acetone (5 mg/ml) and stored at −10 C. A 1:25 dilution of this stock was made up in 0.067M phosphate buffer (pH 7.3) and a drop of this was added to a drop of buffer containing living or dead nematodes, giving a final concentration of 0.01% FDA. A coverslip was placed over this drop and sealed. The slide was examined under ultra-violet (UV) light with a Zeiss interference band pass filter with excitation from 450 to 490 nm and a barrier filter at 520 nm and above. Death was induced by heating for 1 min at 60 C, by fixation in 4% phosphate-buffered (pH 7.3) paraformaldehyde, or by starvation.

Living nematodes exhibited fluorescence in discrete particles in the gut (Figs. 1B, 2B), whereas dead specimens showed a general diffuse fluorescence throughout the whole animal (Figs. 1D, 2D). The membranes surrounding the particles containing esterase in the intestine apparently leak at death, so that the fluorescein is distributed randomly within the nematode and retained within the cuticle. That diffusion is less when killing is by fixation in paraformaldehyde than by heating or starvation.

The method provides a rapid means of assessing the viability of nematodes. Either tungsten or halogen light sources can be used just as effectively as UV, in which case the only special apparatus required would be the filters.

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C.S.I.R.O. Division of Horticultural Research, G.P.O. Box 350, Adelaide, South Australia, 5001. Acknowledgment: I am most grateful to Miss S. D. Harris of this laboratory for technical assistance.

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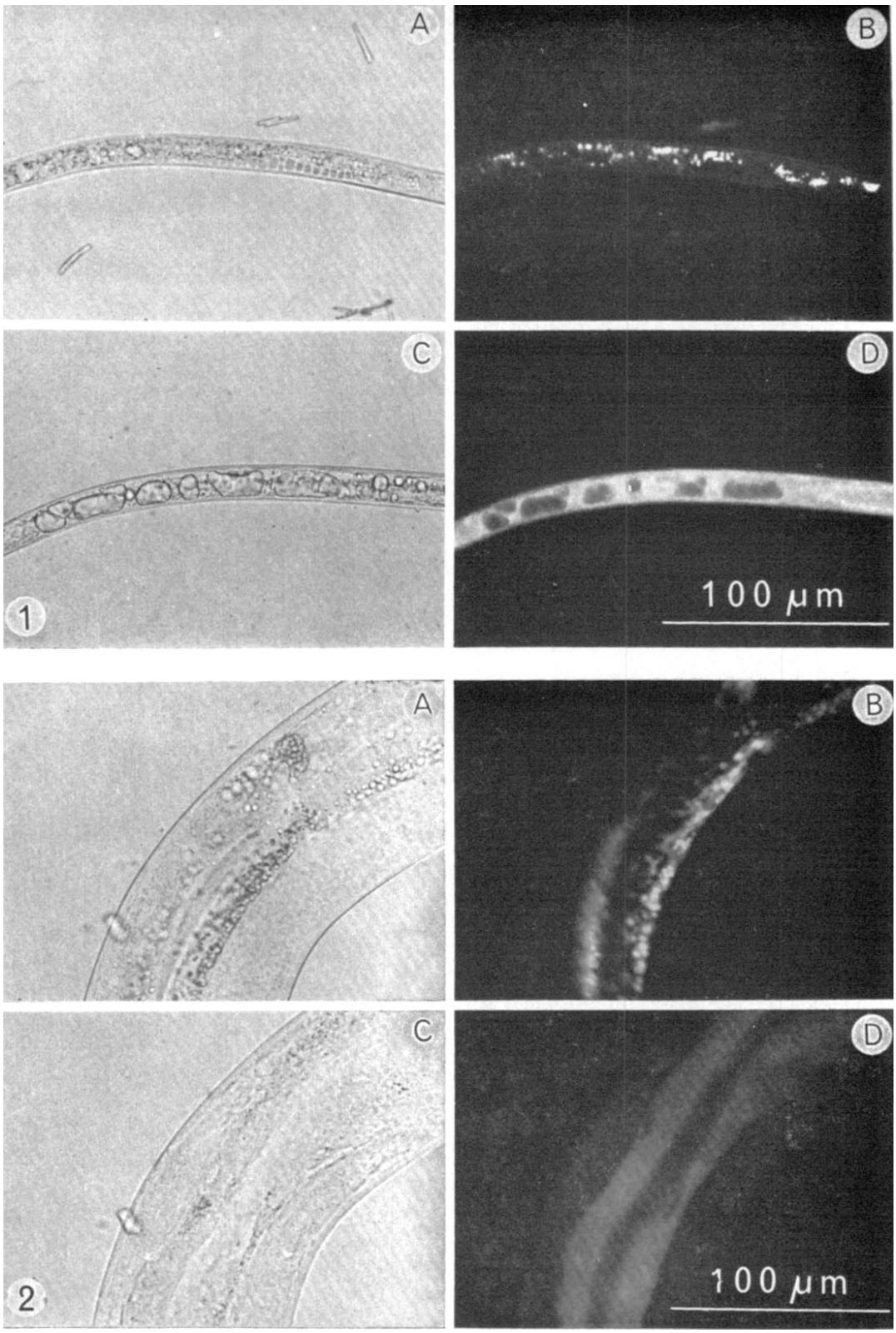

FIG. 1. Second-stage larvae of *Meloidogyne javanica*. A) Living specimen under normal transmitted light. B) Same specimen exhibiting fluorescence. C) Dead specimen (heat-treated) under normal transmitted light. D) Same specimen exhibiting fluorescence.

FIG. 2. Females of *Caenorhabditis elegans*. A) Living specimen under normal transmitted light. B) Same specimen exhibiting fluorescence. C) Same specimen but dead following heat treatment, under normal transmitted light. D) Same as C but exhibiting fluorescence.

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